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Reproductive Biology of *Capoeta tinca* Inhabiting Kayaboğazı Dam Lake (North-West Anatolia, Turkey)

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Summary

This study investigates the reproduction biology of *Capoeta tinca* population in Kayaboğazı Dam Lake in the region of Northwest Anatolia in Turkey (Kütahya). The investigation was carried out between March and December 2003. During the study, breeding and sexual maturation properties of *Capoeta tinca* were studied. The sexual maturity position for both sexes were II age. The spawning period was from beginning of May to the end of June. The minimum fishing size was found as 214 mm in terms of fork length. According to age, the reproduction period was changing between different populations depending on ecological conditions in Turkey's areas.

Keywords: *Capoeta tinca*, Reproduction biology, Kayaboğazı Dam Lake, Turkey

Kayaboğazı Baraj Gölü (Kuzey Batı Anadolu -Türkiye)'nde Yaşayan *Capoeta tinca*'nın Üreme Biyolojisi

Özet

Bu çalışmada Kuzey Batı Anadolu Bölgesi(Türkiye)'nde yer alan Kayaboğazı Baraj Gölü'ndeki *Capoeta tinca* popülasyonunun üreme biyolojisi incelenmiştir. Araştırma Mart ile Kasım 2003 tarihleri arasında gerçekleştirilmiştir. Çalışma süresince *Capoeta tinca*'nın eşeyssel olgunluk ve yumurta bırakma dönemleri incelenmiştir. Eşeyssel olgunluğa ulaşma yaşı, erkek ve dişi cinsiyetler için II olarak belirlenmiştir. Üreme periyodu Mayıs başlangıcından Haziran sonuna kadar olan dönemi kapsamaktadır. En küçük av büyüklüğünün (çatal boy olarak) 214 mm olduğu tespit edilmiştir. Türkiye'nin farklı bölgelerinde değişen ekolojik şartlar nedeniyle, üreme periyodunun yaşa bağlı olarak popülasyonlar arasında farklılıklar gösterdiği anlaşılmıştır.

Anahtar sözcükler: *Capoeta tinca*, Üreme biyolojisi, Kayaboğazı Baraj Gölü, Türkiye

INTRODUCTION

Capoeta tinca has a wide distribution in North and Northwest Anatolia of Turkey and lives in systems that are hydrologically connected to the Black Sea ^{1,2}. Because of its delicious flesh, people prefer this species to consume as food and it is so important commercially in Turkey ². *Capoeta tinca* can easily adapt to the changing conditions in any aquatic system, it occurs in lotic and lentic habitats both, caught as a commercial fish from natural and man-made lakes ³. There are some limited studies concerning *C. tinca* by some researchers in Turkey ⁴⁻¹¹.

Present study, basically undertakes the reproduction of *Capoeta tinca* population in Kayaboğazı Dam Lake on Kocasu (Adranos) stream in the Susurluk basin in Kütahya province in the North-West Anatolia.

MATERIAL and METHODS

The Kayaboğazı Dam Lake is located on Tavşanlı plain (39°21' and 39°35'N- 29°23' and 29°37'E) (Fig 1). This study was made between March and December 2003 in Kayaboğazı Dam Lake. Water sampling and its evaluation

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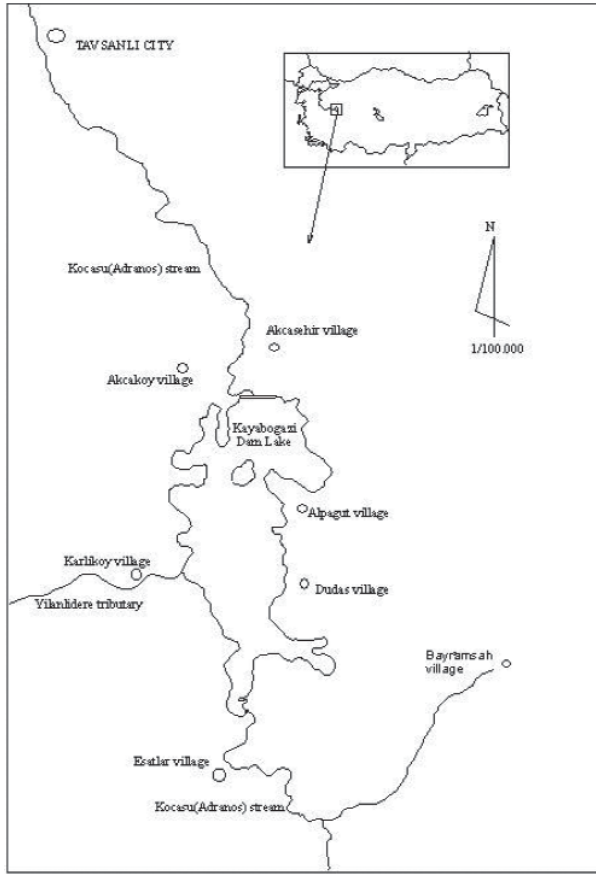


Fig 1. Map of Kayaboğazı Dam Lake

Şekil 1. Kayaboğazı Baraj Gölü'nün haritası

was made in situ using WTW model oxygen meter, and conductimeter in every stations as unit of °C, mg/L and µmho/cm for each parameters, conveniently.

Sampling was performed with gill nets of various mesh sizes (18, 24, 36, 44 and 60 mm knot to knot). In

Table 1. The means with the standard error of gonadosomatic index values, mean egg diameter and number of *Capoeta tinca* living in Kayaboğazı dam lake

Tablo 1. Kayaboğazı Baraj Gölü'nde yaşayan *Capoeta tinca*'nın gonadosomatik indeksi, ortalama yumurta çapı ve sayıları ile standart hataları

Months	GSI X±Sx	Egg diameter (mm) X±Sx	Egg number/1 g X±Sx
March	2.85±0.64	0.88±0.078	1420±398.62
April	3.93±0.70	0.95±0.064	1352±242.36
May	5.55±0.99	1.387±0.095	1014±172.87
June	3.50±0.67	1.065±0.074	1076±221.27
July	1.14±0.07	0.56±0.063	1120±120.00
August	1.41±0.04	0.58±0.059	1150±135.68
September	1.42±0.13	0.65±0.076	1170±270.64
October	1.54±0.05	0.75±0.089	1260±83.33
November	2.30±0.57	0.77±0.042	1340±119.22
December	2.40±0.20	0.84±0.034	1380±91.39

the laboratory, body weight and fork length of each individuals were measured with a precision of 0.01 g and 0.1 cm, respectively. Scales taking from under dorsal fin were used for age determination¹². Sexual characteristics in this species were determined on gonadal tissues naked eye or using a lens (x10)¹³. Males differ from females morphologically by the presence of breeding tubercles on the head during spawning period. The sexual maturity was determined in gonads under binocular microscope. Gonadosomatic indexes (GSI) were calculated as: $GSI = \text{Gonad weight} \times 100 / \text{total body weight}$.

Seasonal changes in GSI, egg diameter and number of eggs per gram of ovary were used to determine the reproduction biology of this species. The diameter of each ripening ovum was measured by means of compasses to the nearest 0.01 mm in compass.

Minimum of 25 ova selected from different parts of the ovary (proximal, middle and distal) were measured. The number of eggs was estimated by gravimetric methods¹⁴. In this study, regression analyse was used to determine the relationship between fecundity (F) and body weight (W) or Length (L)¹⁵. Maturity age was determined on gonads by opening the ventral cavity between April and July term¹⁴.

RESULTS

Water temperature and dissolved oxygen and electrical conductivity ranged from 4.7°C to 20.6°C, 6.48 mg/l to 9.23 mg/l and 374.10 µmho/cm to 510.24 µmho/cm in this lake, respectively.

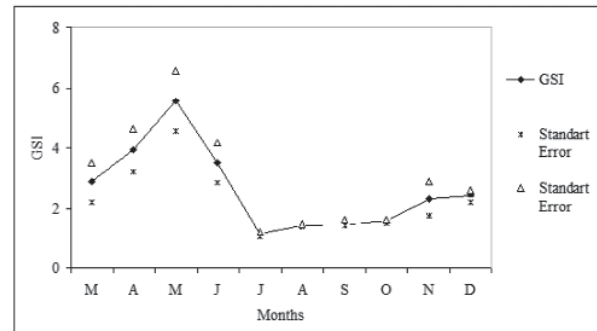


Fig 2. Seasonal changes in GSI of *Capoeta tinca* in Kayaboğazı Dam Lake

Şekil 2. Kayaboğazı Baraj Gölü'ndeki *Capoeta tinca*'nın GSI değerlerinin mevsimsel değişimi

Gonadosomatic index values, mean egg diameter and number of *Capoeta tinca* in Kayaboğazı Dam Lake were given in Table 1. Some findings for reproduction biology of *C. tinca* from different locations in Turkey were given in Table 2.

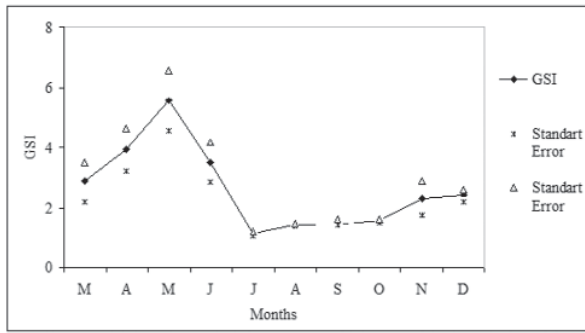


Fig 3. Seasonal changes in egg diameter and number of *Capoeta tinca* in Kayaboğazı Dam Lake

Şekil 3. Kayaboğazı Baraj Gölü'ndeki *Capoeta tinca*'nın yumurta çapı ve sayılarının mevsimsel değişimi

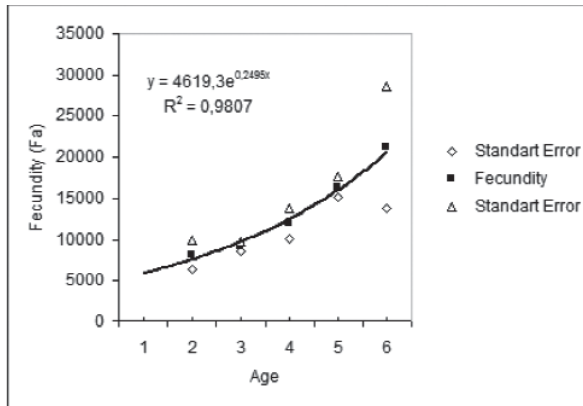


Fig 4. Age-fecundity relation of *Capoeta tinca* in Kayaboğazı Dam Lake

Şekil 4. Kayaboğazı Baraj Gölü'ndeki *Capoeta tinca*'nın yaş-fecundite ilişkisi

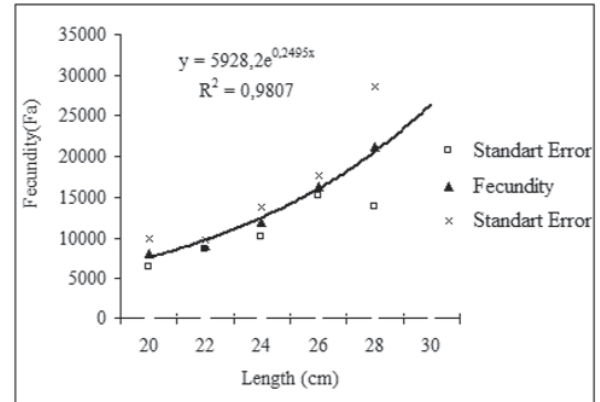


Fig 5. Length-fecundity relation of *Capoeta tinca* in Kayaboğazı Dam Lake

Şekil 5. Kayaboğazı Baraj Gölü'ndeki *Capoeta tinca*'nın boy-fecundite ilişkisi

Both sexes attained sexual maturity in the second year and spawning took place from the beginning of May to the end of June in Kayaboğazı Dam Lake (Fig 2 and 3).

A high correlation was found between age-fecundity and length-fecundity (Fig 4 and 5).

DISCUSSION

In this study, both sexes of *Capoeta tinca* attained sexual maturity at second year was determined in Kayaboğazı Dam Lake. This result was similar with those of Akgül's results in Kelkit stream in vicinity of eastern Black Sea and Bircan and Aral's results in Altınkaya Dam Lake in Bafra-Samsun (Table 2)^{5,16}.

Table 2. Some findings for reproductive biology of *Capoeta tinca* from different regions in Turkey

Tablo 2. Türkiye'nin farklı bölgelerinden *Capoeta tinca*'nın üreme biyolojisi ile ilgili bazı bulgular

Spawning Period	Temperature (°C)	Maturity Age (Male/Female)	Study Area	References
June-July	-	III/II	Kızılırmak river (Central Anatolia)	4
July-September	-	II/II	Kelkit Stream (Vicinity of Eastern Black Sea)	5
April-June	-	II/II	Altınkaya Dam (Bafra-Samsun)	16
July to September	-	II/III	Kızılırmak basin (Central Anatolia)	18
May-July	15.3, 22.5	III-IV/IV-V	Sarıyar Dam (Central Anatolia)	6
May and June	19.0	II/III	Gelingüllü Dam (Central Anatolia)	10
May-June	-	II/III	Çoruh Basin (Eastern Anatolia)	20
May to June	-	III/IV	Kapulukaya Dam (Kırıkkale)	19
May-July	16.0	II/III	Oltu Stream (Eastern Anatolia)	9
May-July	-	II/III	Kirmir Stream (Central Anatolia)	8
Beginning of May, end of June	15.0, 21.0	II/II	Kayaboğazı Dam (NorthwestAnatolia)	present study

The result of sexual maturity in our study was different from findings of some authors ([Table 2](#)). Many factors such as physicochemical, bio-ecological and climatical factors may have effect as direct and indirect, attaining sexual maturity of fish ¹⁷. The differences of findings of some authors can be taken root from temperature and feeding conditions in different water systems ^{4,6,10}.

With purpose of determining the spawning time was used GSI values, egg diameter and direct observation of gonad maturity of *C. tinca* in the dam lake. Spawning phenomenon took place from beginning of May to the end of June in this dam. Climatical discrepancies have shown different table in different area of Turkey. Hence, same species can appear different biological behavior from area to area in Turkey. For example: As seen [Table 2](#), findings of Akgül ^{4,5}, Ekmekçi ⁶, Yılmaz and Gül ⁷, Yıldırım and Aras ⁹, Ekmekçi and Özeren ¹⁰, Bircan and Aral ¹⁶, Erk'akan and Akgül ¹⁸, Yılmaz ¹⁹, about spawning period for *C. tinca* populations were different from our results. But, findings of Solak ²⁰, were similar to our study's results. Briefly, we can express as follow in [Table 2](#).

Water temperature results during the spawning period of *C. tinca* in Kayaboğazi Dam Lake were similar to the results of Yıldırım and Aras ⁹, Ekmekçi and Özeren's ¹⁰, findings ([Table 2](#)).

We have some suggestions concerning fishing legality in area:

1- Minimum fork length for fishing must be 214 mm (both sexes).

2- Fishing period must be limited to be forbidden between out of May beginning and up to end of June for *C. tinca* population in this lake.

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Hsp-70 Gene Expression Analyses in the Different Ages of Rainbow Trout

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[1] This work was the entire of master thesis entitled the expression analyses of Hsp-70 gene in the different ages rainbow trouts

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Summary

The purpose of this study was to determinate the association between heat shock protein 70 (Hsp-70) expression and the age in rainbow trout. Total RNA was extracted with TRIzol Reagent (Life Technologies) and the first strand cDNA was synthesized using Super Script III Reverse Transcriptase. Gene amplification was done by PCR method and quantitative determination of gene expression was using beta actin gene as control. Consequently, we demonstrated that Hsp70 expression significantly increased the age.

Keywords: Rainbow trouts (*Oncorhynchus mykiss*), Age, Gene expression, Heat shock protein, Hsp70

Farklı Yaşlardaki Gökkuşığı Alabalıklarında (*Oncorhynchus mykiss*) Hsp-70 Geninin Ekspresyon Analizi

Özet

Bu çalışmanın amacı gökkuşığı alabalıklarında (*Oncorhynchus mykiss*) Hsp-70 geni ekspresyonu ile yaş arasındaki ilişkiyi belirlemektir. TRIzol Reagent (Life Technologies) ile toplam RNA, Super Script III Reverse Transcriptase ile de cDNA kütüphanesi hazırlandı. Gen amplifikasyonu PCR kullanılarak, ekspresyonun miktar belirlenmesi ise beta aktin kullanılarak yapıldı. Sonuçta Hsp-70 ekspresyonunun yaşla birlikte önemli oranda arttığı gözlemlendi.

Anahtar sözcükler: Gökkuşığı alabalığı (*Oncorhynchus mykiss*), Yaş, Gen ekspresyonu, Isı şok protein, Hsp70

INTRODUCTION

Heat shock proteins (Hsps) are a group of intracellular proteins that have an unusually high degree of identity at the amino acid level, among diverse organisms. As this family of proteins is induced by stressors other than heat, they are also commonly referred to as 'stress proteins' in the literature. The term stress proteins also may refer to several other groups of proteins that respond to stressors ¹.

The naming of Hsps are generally based on their molecular mass (kilodaltons, kDa). Heat shock proteins are also grouped according to function (eg chaperonin), DNA sequence, and antibody cross-reactivity. The

commonly used categories are: 100 kDa; 90 kDa; 70 kDa; 60 kDa; and the 16±30 kDa group, and are usually referred to as Hsp-100, Hsp-90, Hsp-70, Hsp-60, and the low molecular weight (LMW) class of proteins, respectively ².

In the last three decades, there has been an exponential increase in the interest and research activity concerning the description, classification and functional significance of these proteins. Heat shock proteins are constitutively expressed in cells to maintain a number of critical cellular processes relating to protein folding, fidelity and translocation. These proteins also are induced

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in cells in response to a variety of stressors and enhance survival by protecting vital cellular functions¹.

Research in this area continues to grow at an ever-increasing rate and applications to problems and opportunities in human health and environmental monitoring are developing rapidly. Heat shock protein studies in fish are still in the early stages compared with those in bacteria, yeast and mammals. Studies in fish are still in the descriptive stages of documenting novel proteins that are produced in various tissues in response to a variety of biological and abiotic stressors. For example, Hsp-70, which are expressed in response to trace metal exposure, organic pollutants changes in temperature, hypoxia, exposure to ultraviolet radiation, exercise, hyperthermia, oxidative stress, metabolic challenge, ethanol, free radicals, amino acid analogs, heavy metals and aging^{3,4}.

A characteristic feature of aging is a progressive impairment in the ability to maintain homeostasis in the face of environmental challenges. Age-related decline in Hsp expression may contribute to this dysfunctional homeostasis. Specifically, Hsp mRNA and protein production in response to heat shock decreases with age in a variety of species from flies to rats⁵. However, the age-related changes in Hsp response in rainbow trout, a heavily utilized research animal, have not been reported to date. Fish have long been used as an experimental model for aging⁶⁻⁸. The adaptability of the rainbow trout⁹ to mutagenesis studies¹⁰ makes it an attractive vertebrate model to study the aging process¹.

In this paper, we have evaluated effect of age on expression of Hsp-70 in muscle of rainbow trout in different age.

There are many studies involving Hsp-70 expression but none only aging¹¹. Nor is there any aging study relation to Hsp-70 expression in fish. In this study, there was a relationship Hsp-70 expression and age. Thus, in this study it was seen that if fish have aging some genes can be expressed in their body such as Hsp-70. Therefore this study can become a reference for future research on relation between Hsp expression and age or plan of rearing density. And it provides information on age-related cellular and molecular changes that occur in fish species.

MATERIAL and METHODS

Fish Material

The study used different ages (8, 18 and 36 months) rainbow trout (*Oncorhynchus mykiss*), obtained from

Ataturk University, Agricultural Faculty, Fishery Department Research and Extension Center Faculty of Agriculture. They were stabulated, with inconsistent mortality, at biomass density (250 kg/m³) in a 10*2*1.3 m³ pond connected to a water recycling system served with about 24 water refilling per day. Water parameters were strictly controlled: temperature 10±1°C, total hardness 102 mg as CaCO₃, free O₂ 8±0.5 ppm, pH 7.8. At the average size of 8 months 150 g, 18 months 500 g and 36 months 1500 g. Food was automatically distributed daily ensured as a 1.5% body mass ratio, with extruded pellet for marine fish (Bleacksea Food). Temperature, O₂ and pH were continuously monitored, while other parameters were analyzed at a weekly rate. After 15 days, three fish for each group was randomly sampled, immediately stunned and slaughter. Skeletal muscle were removed from dorsal region, frozen in liquid N₂ and stored at 80°C until molecular biology analyses.

RNA Preperation and cDNAs Synthesis

Total RNA was isolated using TRIzol® Reagent according to the manufacturer's recommendation (Invitrogen), and the RNA pellets were resuspended in TE buffer (Tris-EDTA, pH 8.0). To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I (Sigma) by the manufacturer's instructions. The total RNA was quantified by measuring the absorbance at 260 nm using a UV-VIS Spektrofotometre (Aquamate), and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All samples had 260/280 nm ratios <1.6. Additionally, the integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 1.0% agarose gels. Total RNA was reverse transcribed using a commercially available cDNA synthesis kit SuperScript™ III Reverse Transcriptase (Invitrogen). cDNA was stored at -20°C.

Quantitative RT-PCR

For PCR reaction ~100 ng template, 1X PCR buffer (1.5 mM MgCl₂), 200 µL of each dNTP, 0.2 µL gene-specific hsp-forward (5'- TGCACCTAGGTTTTCATAGAAT - 3') and hsp-reverse (5'- ATGGAGGTGTAGAAGTCGATGC- 3') primers, and 2.5 units of Taq DNA polymerase were mixed for a total reaction volume of 15 µL. Thermal cycling conditions were as follows: initial activation at 80°C for 1 min, at 94°C for 3 min, 40-PCR cycles at 94°C for 45s, 58°C for 45s, 72°C for 45s, and a final extension at 72°C for 5 min. For actin gene, Primers actinF (5'TGGGGCAGTATGGCTTGTATG3') and actinR (5' CTCTGGCACCCCT AATCACCTCT-3') were used as the internal control¹¹. PCR cycles for actin gene was in initial

activation at 80°C for 1 min, at 94°C for 3 min, 35-PCR cycles at 94°C for 20s, 54°C for 20s, 72°C for 20s, and a final extension at 72°C for 5 min. The PCR products were loaded into 1% agarose gel and run in TAE 1X buffer at 100 mV for 30 min. A distinct band estimated at ~954bp nucleotides (Hsp) and ~165bp (actin) nucleotides were generated and amplified products quantified by using ImageJ 1.37c (<http://rsb.info.nih.gov/ij/>).

Statistical Analysis

The Student's t-test and standard deviation were used for data analysis. Significance of differences was defined as $P < 0.01$ ¹².

RESULTS

In the present study, we have obtained for Hsp-70 (Gen Bank accession number AB062281), and we have completed a cDNA of 3746 bp by SuperScript™ III Reverse Transcriptase (Invitrogen). We amplified a section of 954 bp of Hsp-70 gene and comprehensively compared their mRNA amplified products quantified by using ImageJ

1.37c. β -actin was amplified as 165 bp and used as a control ¹³, Fig. 1.

Quantitative mRNA levels of Hsp-70 were plotted by using gel density in 40th PCR cycle. β -actin was used as control (Fig. 2). As far as quantitative mRNA is concerned, the results show that age fairly effect on fish. If it is compared with all age, quantitative mRNA levels more than ten times as high in 36 months as other age.

DISCUSSION

There are many experiments about Hsp on organisms. The expression of Hsp-70 has been studied in relation to various stressors such as heat shock ¹⁴⁻¹⁹, aquatic toxicants ²⁰⁻²⁵ and psychological stress ^{26,27}. In all of this studies, Hsp expression has been increased dramatically similar our results. However, the expression of various types of Hsp protein and mRNA levels in response to heat stress has been shown to decrease with age ^{18,28-33}. Pahlavani ³⁴, showed that the induction of Hsp-70 expression by heat shock decreases 40-50% with age. There are only a few reports on the effects of aging on

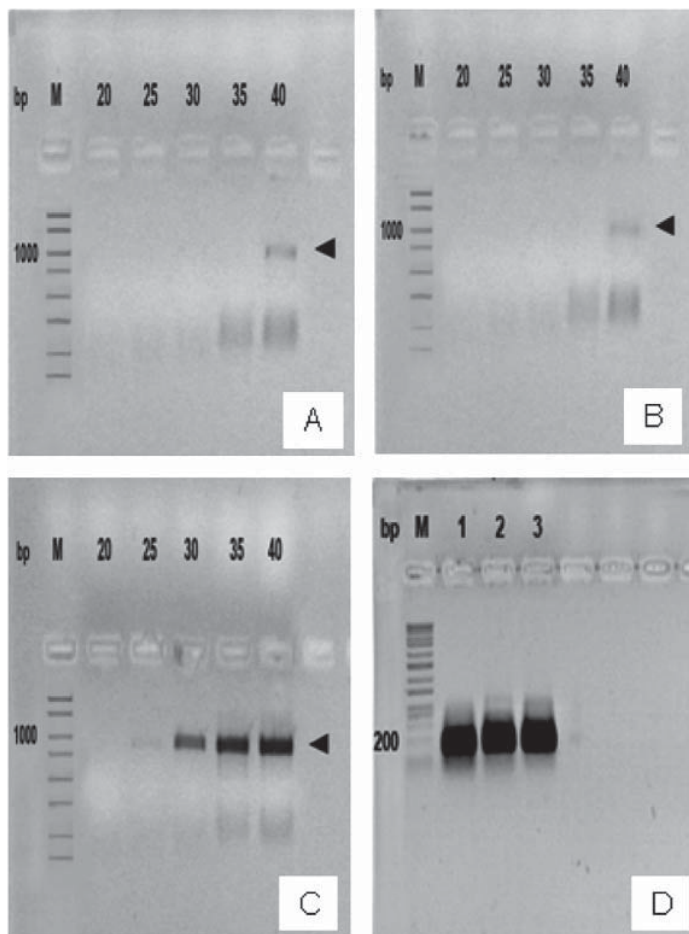


Fig 1. HSP-70 gene agarose gel patterns, 8 months (A), 18 months (B), 36 months (C), 20-40: PCR cycling (A,B,C); β -actin control: Line 1: 8 months, Line 2: 18 months, Line 3: 36 months (D)

Şekil 1. HSP-70 geninin agaroz jel görüntüleri; 8 aylık (A), 18 aylık (B), 36 aylık (C), 20-40 cycle (A,B,C); beta aktin kontrol: 1. Sıra: 8 aylık, 2. Sıra: 18 aylık, 3. Sıra : 36 aylık (D)

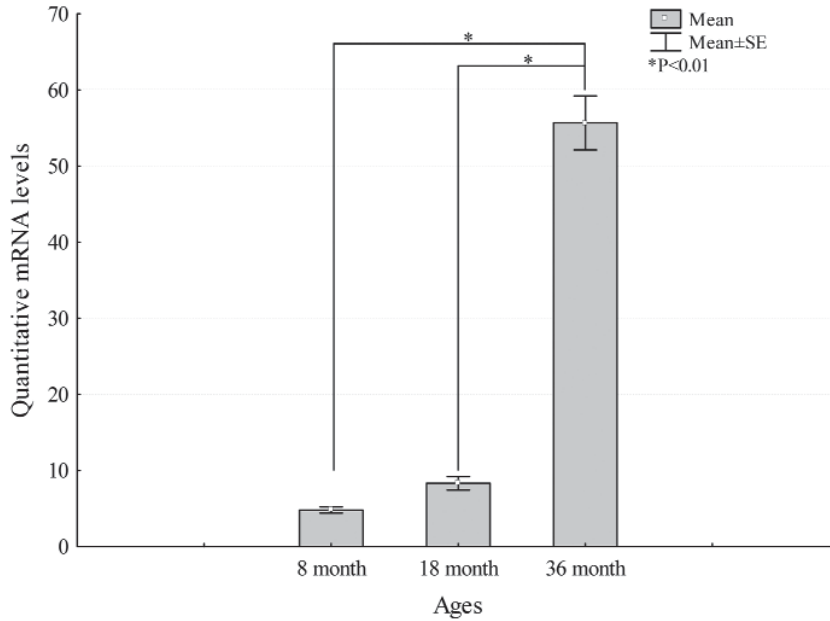


Fig 2. Relatives expression of Hsp-70 in skeletal muscle of different ages groups. Asterisks denotes significant difference from age groups ($P < 0.01$). $n=7$

Şekil 2. Hsp-70 geninin farklı yaş gruplarında ekspresyon ilişkisi. Şekilde yaş grupları arasında önemli farklılıklar belirlenmiştir ($P < 0.01$). Örnek sayısı=7

fish⁶⁻⁸. These studies provide only limited information on age-related cellular and molecular changes that occur in fish species.

There are many studies involving Hsp expression. But, there isn't any experiment related with heat shock protein and age. Thus, the purpose of the present study was to isolate Hsp genes from rainbow trout and to analyze their expression profiles at the mRNA levels. In this regard, we quantitatively compared the mRNA levels between different ages under rearing the same condition using PCR analysis. The present work demonstrated the expression of Hsp-70 gene was increased with fish age.

In this study the effect of age on Hsp-70 gene expression in different age fish was investigated. Consequently, Hsp-70 gene expression increase with fish age.

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İzmit Körfezi'nden Avlanan Derin Su Pembe Karidesi'nin (*Parapenaeus longirostris* Lucas, 1846) Biyokimyasal Kompozisyonu ve Mineral İçeriklerinin Belirlenmesi

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Özet

Bu çalışmanın amacı, İzmit körfezinden avlanan derin su pembe karideslerinin (*Parapenaeus longirostris* Lucas, 1846) mevsimsel değişimler göz önüne alınarak hepatopankreas ve abdominal kas dokularındaki glikojen, total lipid, trigliserit, kolesterol ve mineral (Ca, Na, K, Fe, Mg) içeriklerini incelemektir. Derin su pembe karidesi İzmit Körfezi'nde en bol bulunan penaeid türü karidedir ve mevsimsel olarak toplanmışlardır. Karideslerin abdominal kas dokuları ve hepatopankreasları laboratuvar da serbest glikoz, glikojen, total lipid, trigliserit ve total kolesterol ve mineral içeriklerinin tayinleri için çıkartılmıştır. Ayrıca bu çalışmada dişi ve erkek karideslerin abdominal kas dokularında K, Na, Ca, Fe, Mg elementlerinin seviyeleri belirlenmiştir. Elde edilen verilere göre hem erkek hem de dişi karideslerin metabolik enerji ve fizyolojik aktiviteler için kullandığı temel kaynaklar total lipid ve glikojendir.

Anahtar sözcükler: *Parapenaeus longirostris*, Derin su pembe karidesi, Biyokimyasal kompozisyon, Mineral içeriği, İzmit Körfezi

Determination of Biochemical Composition and Mineral Contents of Deep Sea Pink Shrimp (*Parapenaeus longirostris* Lucas, 1846) Caught from the Gulf of İzmit, Turkey

Summary

The aim of this study is to identify seasonal variation of biochemical composition and mineral content (K, Na, Ca, Fe, Mg) in abdominal muscle tissues and hepatopankreas of the Deep sea pink shrimp (*Parapenaeus longirostris*) caught from of the Gulf of İzmit, Turkey. Deep sea pink shrimps is an abundant penaeid species which inhabits the Gulf İzmit and they were collected seasonally. In the laboratory, abdominal muscles and hepatopankreas were removed for determination of glycogen, free glucose, total lipid, triglyceride, total cholesterol levels and mineral content. Furthermore, K, Na, Ca, Fe, Mg levels have determined in abdominal muscle tissues of females and males. Our findings in *Parapenaeus longirostris* suggest that lipids and carbohydrates are main reserve of metabolic energy used during physiological activities in both males and female shrimps.

Keywords: *Parapenaeus longirostris*, Deep sea pink shrimp, Biochemical composition, Mineral content, Gulf of İzmit

GİRİŞ

Deniz ürünleri, sağlıklı ve dengeli bir beslenme için tüketilmesi gereken önemli bir besin kaynağıdır.^{1,2} Kabuklular (Crustacea) yenilebilir et kalitesi nedeniyle

ekonomik öneme sahip olan ve tüm dünyada tüketilen deniz ürünleridir. Karides, yengeç ve istakoz gibi kabuklu türleri lezzetlerinin yanı sıra aminoasit, peptid, protein,

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doymamış yağ asitleri, mineral ve diğer besleyici maddeleri ihtiva ederler ³⁻⁵. Kabuklular yaşadıkları coğrafyada mevsim değişikliği, tuzluluk, oksijen azlığı/yokluğu, kabuk değişimi, seksüel olgunluk, besin bulma ve beslenme gibi yıllık ve günlük bazı değişkenlere maruz kalırlar. Bu değişkenlere bağlı olarak kabukluların et verimi ve kalitesi de etkilenmektedir ⁶. Kabuklular da diğer canlılara benzer şekilde hareket, solunum, sindirim, dolaşım, boşaltım ve üreme gibi yaşamsal faaliyetlerini sürdürmek için gerekli enerjiyi protein, karbonhidrat ve yağlardan elde ederler. Kabukluların kan sıvısında bulunan monosakkarit glikozdur ve bazı temel gereksinimler için kullanılır. Bunlar sırasıyla mukopolisakkarit, kitin ve glikojen sentezleri, piruvat oluşumu ve temel enerji kaynağı olarak gözlemlenmiştir.

Glikoz kabukluların hepatopankreas ve kas dokularında glikojen halinde depolanmakla birlikte türlere göre farklılıklar gösterebilmektedir ⁷. Kabukluların hemolimflerindeki glikoz düzeyleri kas, üreme ve sinir sistemlerinin normal işlevlerini görebilmesi için gereklidir ^{8,9}. Kabuklularda adipoz dokunun bulunmaması nedeniyle hepatopankreas ana lipid deposu görevini yerine getirmektedir. Hepatopankreas kabukluların lipid deposu olarak kabul edilmekle birlikte kas doku ve ovaryum da lipid deposu işlevi görebilir ^{10,11}. Gonadal maturasyon periyodunda hemolimf ve dokuların biyokimyasal kompozisyonu ile mevsimsel değişim etkilerinin analizi kabukluların biyolojisi açısından çok önemlidir ¹². Kabuklular etlerinde çok miktarda doymamış yağ asiti de içerirler ¹³.

Ülkemizde kabukluların biyokimyasal kompozisyonları ve mineral içerikleri hakkında çok fazla bilgi bulunmamaktadır. Derin su pembe karidesi özellikle Avrupa'da yüksek ticari öneme sahip bir tür olup, Doğu Atlantik ve tüm Akdeniz'de kabuklu balıkçılığında çok önemli bir yere sahiptir ¹⁴⁻¹⁷. Türkiye'de de özellikle Marmara, Ege ve Akdeniz kıyılarında derin su pembe karidesinin avcılığı yüksek potansiyele sahiptir ¹⁸⁻²⁰. Bu çalışmada doğal çevrelerinden avlanan derin su pembe karideslerinin hepatopankreas ve abdominal kas dokularına ait bazı biyokimyasal kompozisyon analizleri ve mineral içerikleri mevsimsel değişikliklerin etkileri de göz önüne alınarak incelenmiştir.

MATERYAL ve METOT

Örneklerin Hazırlanması

Çalışmada kullanılan 120 adet karides örneği, İzmit Körfezi'nde ticari balıkçı teknelerinden yararlanılarak, Mayıs 2007-Nisan 2008 tarihleri arasında mevsimsel olarak sırasıyla 50-100 m ve 100 m'den daha derin

derinlik katmanlarında 2.5 mil/saat hızla dip trolü çekimlerinden elde edilmiştir. Derin su pembe karidesi, yaşam evresini tamamen derin sularda geçiren denizel nekto-bentik bir türdür ve yaşam alanı olarak katı zemin haricinde her tip zemini ve daha çok siltli-killi çamurlu bölgeleri tercih etmektedir. Örnekler Marmara Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Zooloji Araştırma Laboratuvarına özel kaplarda +4°C'de taşınarak getirilmiştir. Laboratuvara getirilen karidesler önce cinsiyetlerine göre ayrılmış, elektronik tartı ile tartılmış (0.001 g) ve numaralandırılmıştır. Karideslerin hepatopankreas ve abdominal kas dokuları ayrılmış ve yaş ağırlıkları tartılarak, derin dondurucuda saklanmıştır.

Hepatopankreas ve Abdominal Kas Dokularının Metabolik Analizleri

- Glikojen ve Serbest Glikoz Miktarı Tayini

Dokulardaki glikojen ve glikoz miktarlarının tayini ticari kitler (Randox Laboratories Ltd, UK) kullanılarak ve spektrofotometrik metodlar ile gerçekleştirilmiştir. Sonuçlar dokularda mg/g olarak belirtilmiştir.

- Total Lipid, Trigliserit ve Total Kolesterol Miktarlarının Tayini

Trigliserit tayininde GPO-PAP yöntemine göre geliştirilen ticari kit (Randox Laboratories Ltd, UK) kullanılmıştır. Bu yöntemde trigliseritler lipaz enzimi ile enzimatik hidrolize uğratılır ve peroksidaz enziminin katalitik etkisiyle indikatör madde oluşturulur. Oluşan renkli bileşiğin konsantrasyonu 520 nm'de tayin edilir. Total kolesterol tayininde ise end point yöntemine göre geliştirilen ticari kit (Randox Laboratories Ltd, UK) kullanılarak gerçekleştirilmiştir. Oluşan renkli bileşiğin konsantrasyonu 540 nm'de tayin edilir. Sonuçlar dokularda mg/g olarak belirtilmiştir.

- Mineral Analizleri

Mineral analizleri için karideslerin kas dokuları, metal temasını önlemek amacıyla plastik bıçak kullanılarak ayrılmıştır. Karideslerin kas dokuları tartılarak plastik torbalarda -20°C'de derin dondurucuda saklanmıştır. Dokular tartım işlemlerinden hemen sonra dondurularak kurutma yolu ile kurutulmuş ve tartılmıştır. Örnekler teflon kaplara alınarak derişik sülfirik asit (H₂SO₄) ve nitrik asit-hidrojen peroksit (1:1) karışımı eklenmiş ve ısıtma tablası üzerinde asit uçurulmuştur. Teflon kabın dibinde kalan koyu renkli çökelti üzerine 50 ml %2.5'luk nitrik asit ilave edilmiştir. Elde edilen karışım analiz zamanına kadar buzdolabında plastik şişelerde +4°C'de saklanmıştır. Tüm kurutma ve asitleme işlemleri kontaminasyonu önlemek ve kayıpları engellemek amacıyla kapalı bir sistem içinde yapılmıştır. Dokulardaki potas-

yum (K), sodyum (Na), kalsiyum (Ca) ve demir (Fe) değerleri standart stok çözeltilerle kalibre edilmiş Perkin-Elmer AA 700 modeli Atomik Absorbsiyon Spektrofotometresi ile belirlenmiştir²¹⁻²³.

İstatistiksel Hesaplamalar

Bütün metabolik parametreler homojen ve normal dağılım göstermiştir. İstatistiksel analizler için Bonferroni testini takiben "One way ANOVA" testi kullanılmıştır. Farklı grupların karşılaştırılmasında "Two way ANOVA" testi uygulanmıştır. Önem seviyesi %5 olarak kabul edilmiştir ve tüm verilerin istatistiksel hesaplaması SPSS (SPSS, 11.5) bilgisayar programında yapılmıştır²⁴.

BULGULAR

Bu araştırmadan elde edilen verilere göre, *Parapenaeus longirostris* türü karideslerin hepatopankreaslarında bulunan glikojen ve serbest glikoz düzeyleri ilkbahar ve yaz aylarında yüksek değerlerde bulunurken,

sonbahar ve kış aylarında azaldığı görülmektedir. Verilerin istatistiksel analizlerinde anlamlı bir fark bulunamamıştır ($P \geq 0.05$) (Tablo 1). Karideslerin abdominal kas dokularında bulunan glikojen ve serbest glikoz düzeyleri ise kış ve ilkbahar aylarında yüksek değerlerde bulunurken, sonbahar ve erken kış dönemlerinde azalmaya başladığı izlenmiştir (Tablo 2). Bu değerlerin ilkbahar ve yaz aylarında yüksek değerlerde bulunması karbonhidratların temel enerji kaynağı olarak kullanıldığı ve bu aylarda hayvanların yoğun bir aktivite yaşadıklarını düşündürmektedir (Şekil 1).

Total lipid düzeyleri hepatopankreasta sonbahar, kış ve ilkbahar aylarında artış gösterirken (Tablo 1), yaz aylarında azalmaya başladığı gözlemlenmiştir. Kas dokuya ait total lipid değerleri ise, en yüksek sonbahar ve kış, en düşük ilkbahar ve yaz aylarında gözlenmiştir (Tablo 2). Trigliserit düzeyleri hepatopankreasta Sonbahar en yüksek değerlerde bulunurken (Tablo 1), diğer mevsimlerde daha düşük fakat kendi aralarında hemen hemen eşit oranlarda olduğu belirlenmiştir (Tablo 2).

Tablo 1. Hepatopankreasta ait glikojen, serbest glikoz, total lipid, trigliserit ve total kolesterol düzeyleri (mg/g)

Table 1. Glycogen, free glucose, total lipid, triglyceride and total cholesterol levels in hepatopancreas (mg/g)

Parametre	İlkbahar		Yaz		Sonbahar		Kış	
	♂	♀	♂	♀	♂	♀	♂	♀
Glikojen (mg/g)	2.7	2.7	1.7	2.1	0.9	0.4	1.3	0.6
Serbest glikoz (mg/g)	0.05	0.06	0.04	0.03	0.03	0.05	0.04	0.07
Total lipid (mg/g)	15	22	10	10	33	20	25	12
Trigliserit (mg/g)	2.0	1.3	1.5	0.8	2.3	1.6	1.0	1.0
Total kolesterol (mg/g)	0.3	0.3	0.1	0.1	0.5	0.4	0.5	0.3

Tablo 2. Abdominal kas dokularına ait glikojen, serbest glikoz, total lipid, trigliserit ve total kolesterol düzeyleri (mg/g)

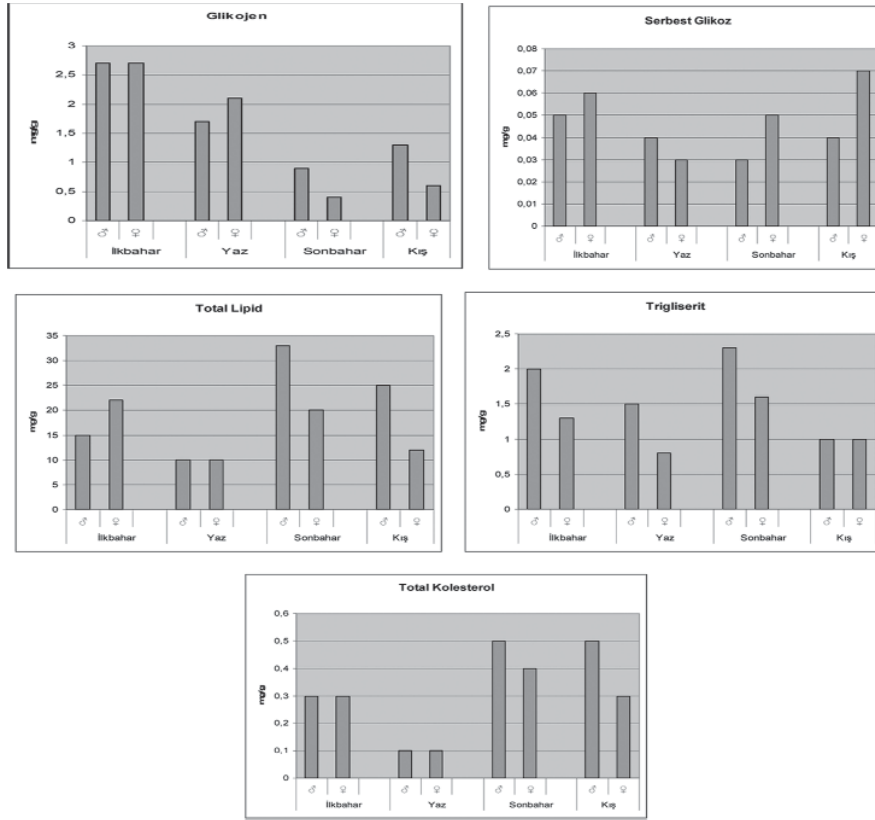
Table 2. Glycogen, free glucose, total lipid, triglyceride and total cholesterol levels in abdominal muscle tissue (mg/g)

Parametre	İlkbahar		Yaz		Sonbahar		Kış	
	♂	♀	♂	♀	♂	♀	♂	♀
Glikojen (mg/g)	7.0	10.1	4.3	6.0	0.5	0.3	8.7	9.0
Serbest glikoz (mg/g)	0.04	0.05	0.02	0.02	0.02	0.03	0.02	0.02
Total lipid (mg/g)	2.8	2.0	2.5	2.3	3.3	1.5	2.5	1.2
Trigliserit (mg/g)	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.3
Total kolesterol (mg/g)	0.4	0.8	0.2	0.2	0.7	0.7	0.7	0.4

Tablo 3. Abdominal kas dokularına ait mineral içerikleri (K, Na, Ca, Fe, Mg) (mg/100 g)

Table 3. Mineral contents in abdominal muscle tissue (K, Na, Ca, Fe, Mg) (mg/100 g)

Parametre	İlkbahar		Yaz		Sonbahar		Kış	
	♂	♀	♂	♀	♂	♀	♂	♀
Potasyum (K)	200.1±0.7	200.1±0.2	229.1±0.5	237.1±0.7	210.1±0.4	200.1±0.1	206.1±0.6	210.1±0.7
Sodyum (Na)	160.5±0.3	154.5±1.1	135.9±0.3	123.3±0.2	145.6±0.8	144.4±0.1	153.2±0.5	150.1±0.1
Kalsiyum (Ca)	50.5±0.5	55.1±0.1	61.3±0.1	60.2±0.1	62.2±0.3	60.2±0.1	55.1±0.9	50.5±0.1
Demir (Fe)	1.59±0.1	1.57±0.1	1.55±0.1	1.55±0.1	1.41±0.1	1.45±0.2	1.51±0.1	1.58±0.1
Magnezyum (Mg)	32.5±0.1	34.0±0.3	35.1±1	36.2±1	33.0±1	32.5±0.2	32.5±0.1	33.1±0.2



Şekil 1. Hepatopankreasa ait glikojen, serbest glukoz, total lipid, trigliserit ve total kolesterol düzeylerinin mevsimsel değişiklikleri (mg/g)

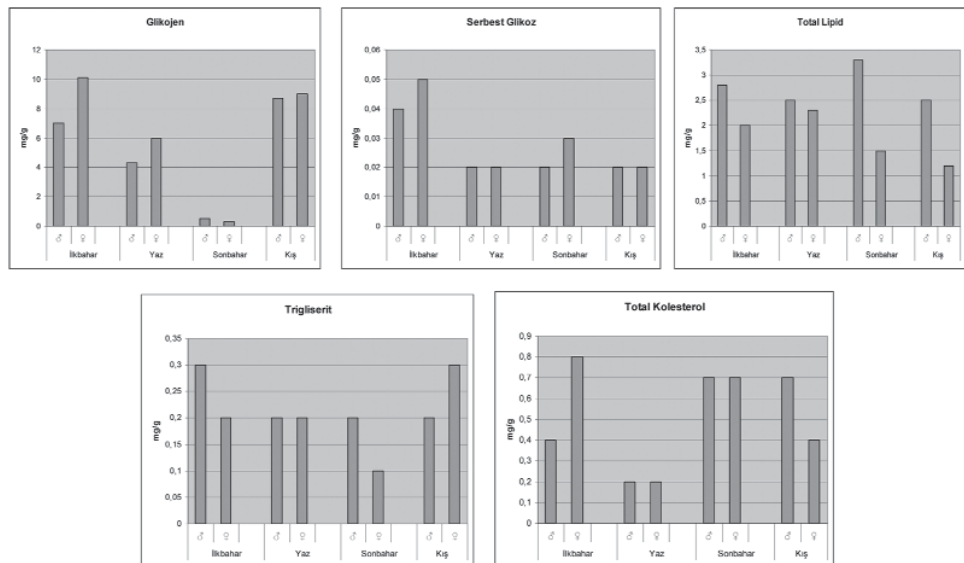
Fig 1. Seasonal variations of glycogen, free glucose, total lipid, triglyceride and total cholesterol levels in hepato-pancreas (mg/g)

Kas dokuya ait trigliserit değerlerinin ise tüm mevsimlerde benzer oranlarda olduğu gözlenmiştir. Verilerin istatistiksel analizlerinde anlamlı bir fark bulunamamıştır ($P \geq 0.05$). (Tablo 2). Hepatopankreasa ait total kolesterol değerleri en yüksek Sonbahar aylarında, en düşük Yaz aylarında belirlenmiştir (Tablo 1). Kas dokuya ait total kolesterol değerleri ise birbirleri ile çok fazla değişiklik göstermezken en düşük değerler yaz aylarında gözlenmiştir (Tablo 2).

Karideslerin abdominal kas dokularında bulunan metal düzeyleri, karideslerin aşırı bir metal birikimine maruz kalmadığını, normal değerlere sahip oldukları görülmektedir. Buna göre, potasyum en yüksek Yaz aylarında gözlenmiş olup diğer mevsimlerde fazla bir farklılık bulunmamaktadır (Şekil 2). Sodyum en düşük olarak Yaz aylarında ölçülmüştür. Diğer mevsimler arasında fazla farklılık gözlenmemiştir. Kalsiyum en düşük olarak İlkbahar aylarında gözlenmiştir. Demir ise en

Şekil 2. Abdominal kas dokuya ait glikojen, serbest glukoz, total lipid, trigliserit ve total kolesterol düzeylerinin mevsimsel değişiklikleri (mg/g)

Fig 2. Seasonal variations of glycogen, free glucose, total lipid, triglyceride and total cholesterol levels in abdominal muscle tissue (mg/g)



düşük Sonbaharda gözlenmiş olup diğer mevsimlere ait veriler arasında farklılık gözlenmemiştir. Magnezyum ise tüm mevsimler boyunca hemen hemen hiç değişmeden kalmıştır. Verilerin istatistiksel analizlerinde anlamlı bir fark bulunamamıştır ($P \geq 0.05$). (Tablo 3).

TARTIŞMA ve SONUÇ

Protein, karbonhidrat ve yağ gibi temel hücre bileşenlerinin canlılarda bulunma düzeyleri bir bakıma bu hayvanların doğaya adaptasyon stratejilerinin bir ifadesi olarak kabul edilebilir. Kabuklu türleri sahip oldukları glikojen depolarını açlık durumlarında, kabuk değiştirme sürecinde, osmoregülasyonda, oksijen azlığında veya yokluğunda, büyüme ve üremenin farklı evrelerinde tüketirler²⁵⁻²⁷. Rosa ve Nunes¹² *Aristeus antennatus*, *Parapenaeus longirostris* ve *Nephrops norvegicus* gibi derin su kabuklu türleri ile yaptıkları çalışmalarında, glikojenin esas olarak hepatopankreasta, az bir kısmının ise kaslarda depolandığını belirtmişlerdir. Bizim çalışmamızda hepatopankreas ve abdominal kas dokularının glikojen düzeylerinin hem erkek hem de dişi bireylerde hemen hemen eşit miktarlarda bulunduğu ve sonbahar-kış aylarında azalmaya başladığı gözlenmiştir. Marmara Denizi'ndeki derin su pembe karideslerinin balıkçılığının geliştirilmesi üzerine yapılan bir araştırmada, olgunlaşmış ve yumurtalarını bırakan dişi karidesler daha çok Mart, Nisan, Mayıs, Haziran ve Ekim, Kasım aylarında gözlenmiştir. Karideslerin boyu Marmara Denizi'nde mevsimlere ve derinliğe bağlı olarak değişmektedir. Bu durum büyük olasılıkla üreme dönemleri ile ilgilidir. Karideslerde besinler yumurtlama döneminden önce depolanmaktadır²⁸. Bizim çalışmamızda *P. longirostris* türü karideslerin hepatopankreaslarında bulunan glikojen ve serbest glikoz düzeyleri ilkbahar ve Yaz aylarında yüksek değerlerde bulunurken, Sonbahar ve Kış aylarında azaldığı görülmektedir. Gonadosomatik indeksin ise üç ayrı dönemde; Mart, Haziran ve Ekim aylarında en yüksek değere ulaştığı gözlemlenmiştir ve bu aylardaki glikojen düzeylerindeki azalma hayvanların yüksek aktivite içerisinde olduklarını göstermektedir. Bu sonuçlar, bizim çalışmamızdan elde edilen değerler ile uygunluk göstermektedir.

Bueno ve Bond-Buckup²⁹ *Parastacus defossus* türü ile yaptıkları çalışmalarında sonbahar aylarında hepatopankreas ve kas dokularında glikojen miktarlarının azaldığını rapor etmişlerdir. Bu sonuçlar bizim verilerimizle uyumlu görünmektedir. Bizim çalışmamızda Sonbahar ve Kış aylarında hepatopankreas ve kas dokusundaki glikojen düzeylerinde azalma gözlenmiştir. Elde edilen verilere göre hayvanların bu aylarda yoğun bir fizyolojik aktivite yaşadıkları ve karbonhidratların temel enerji kaynağı olarak kullanıldığı düşünülmektedir.

Castiglioni ve ark.¹¹ *Parastacus varicosus* türü kerevitlerin hem erkek hem de dişi bireylerinin abdominal kas dokularında glikojenin ilkbahar ve Yaz aylarında azaldığını gözlemlemişlerdir. Bizim çalışmamızda Kış aylarında oldukça yükselen kas glikojen düzeyleri ilkbahar, Yaz ve Sonbahar aylarında azalmaya başladığı görülmüştür. Bu veriler bizim çalışmamız ile farklı sonuçlar içermekle beraber karidesler açısından bazı çevre koşullarının bu azalmaya neden olabileceğini düşündürmektedir. Elde edilen veriler, glikojenin hem dişi hem de erkek bireylerde yüksek aktivite sırasında enerji kaynağı olarak tüketildiğini göstermektedir. Glikojenin depolanma bölgesi de türlere göre farklılıklar gösterebilmektedir. Dolayısıyla türlerin çevre faktörlerine adaptasyonları da farklı gelişebilir.

Yapılan bir araştırmaya göre kabukluların hepatopankreaslarında bulunan glikojen seviyeleri dişilerde mevsimsel dalgalanmalar gösterir. Dişilerin hepatopankreaslarında glikojen konsantrasyonları Sonbaharda yükselirken Yaz aylarında düşüş göstermiştir. Kaslardaki glikojen düzeyleri ise yıl boyunca pek fazla değişiklik göstermemiştir. Erkeklerin kas dokularındaki glikojen seviyelerinde dalgalanma görülmemiştir. Bu sonuçlara göre dişilerin hepatopankreaslarındaki glikojen seviyelerinin özellikle Sonbaharda üreme dönemlerinde düşmesi, bu dönemde kullanılmaya başlandığını göstermektedir³⁰. Bizim çalışmamızda da hem dişi hem de erkek bireylerin hepatopankreas ve kaslarında glikojen seviyeleri açısından bir dalgalanma gözlemlendiği söylenebilir.

Nektobentik türlerin, bentik/endobentik türlere göre daha fazla protein, lipid, kolesterol ve glikojen içerdiği belirtilen bir araştırmada derin su türlerinin enerji içeriklerinin de yüksek olması gerektiği düşünülmektedir³¹. Üç karides türü ile yapılan bir araştırmada, karideslerin kolesterol seviyelerinin ergin ve ergin olmayan dişilerde önemli farklılıklara sahip olduğu rapor edilmiştir. Aynı zamanda ovaryumlarda ve hepatopankreasta erginleşme süresince kolesterol içeriği oldukça artış göstermiştir¹². Bazı araştırmalarda, kabukluların hepatopankreas ve kas dokularının lipid deposu olarak işlev gördüğü rapor edilmiştir⁷. Buna karşın, kabuklularda yüksek enerji gerektiren yumurtlama ve gametogenez gibi fizyolojik dönemler sırasında lipidlerin ana depo olan hepatopankreastan, gonadlara aktarılabildiği rapor edilmiştir. Erkek ve dişilerin kas dokularındaki lipid düzeyleri birbirlerinden fizyolojik nedenlerle farklıdır²⁹⁻³².

Kolesterol, diğer birçok canlıda olduğu gibi, kabuklu türlerinde de hücre membranlarının ve cinsiyet hormonlarının önemli bir yapısal bileşenidir^{33,34}. Yapılan bir araştırmada erkek ve dişi karideslerin farklı dokularında trigliseritlerin, total lipid düzeylerine oranı incelenmiştir. Erkek bireylerde dişilere oranla daha düşük trigliserit dü-

zeyleri belirlenmiştir. Araştırmacılar trigliseritlerin erkek kabuklu türlerinde ana lipid kaynağı olarak kullanılmadığını belirtmişlerdir. Dişilerin total lipidlerinin %44 kadarını trigliseritler oluşturmaktadır. Benzer oran kolesterol açısından da söylenebileceği gibi trigliseritlerin dişiler için daha önemli bir depo maddesi olduğu anlaşılmaktadır. Bu çalışmada Yaz aylarında, total kolesterol düzeyleri hepatopankreas ve abdominal kas dokularında her iki cinste de azalmıştır. Trigliserit düzeyleri Yaz aylarında sadece dişilerin hepatopankreaslarında azalmıştır. Bu durum lipidlerin Yaz ayları süresince dişiler tarafından enerji kaynağı olarak kullanıldığını göstermektedir ³⁵. Bizim çalışmamızda ise Kış aylarında total kolesterol düzeyleri tüm dokularda azalmıştır. Bu durum Sonbahar-Kış aylarında üremenin yüksek olması şeklinde açıklanabilir. Bu çalışmadan elde edilen verilere göre, *P. longirostris* türü için lipidlerin özellikle üreme dönemlerinde önemli bir enerji kaynağı olduğu gözlenmiştir. *Haliporoides sibogae* türünün büyüme ve üreme özelliklerinin araştırıldığı bir çalışmada, türün yumurtlama periyodunun Eylül'den Nisan'a kadar devam ettiğini, Ocak-Mart sonundaki dönemde pik oluşturduğunu ve aynı yaş grubundaki dişilerin erkeklere göre daha erken büyüdüklerini gözlenmiştir ³⁶. Bizim çalışmamızdan da benzer sonuçlar elde edilmiştir.

Sucul canlılarda mineral birikimi, besin ve solungaçlar yoluyla gerçekleşmektedir. Bu alım yaş, cinsiyet gibi endojen veya elementlerin dokularda kullanılabilirliği ve dağılımı, mevsim, lokasyon, yağış miktarı, derinlik, tuzluluk, ısı ve antropojenik etkiler gibi eksojen kaynaklara göre değişiklikler gösterebilir ³⁷⁻³⁹. Tüm sucul canlılar, elementleri solungaç ve barsakları ile absorbe ederler ve kan yolu ile de vücudun diğer kısımlarına taşırlar ⁴⁰. Dekapodlar kalsiyum translokasyonunu hem eksojen hem de endojen kaynaklardan gerçekleştirirler. Hemo-limf ve hepatopankreasta depolanan kalsiyum kabuk değiştirme zamanlarında endojen kaynak olarak kullanılmaktadır. Bazı deniz dekapodlarında hepatopankreasta kalsiyum ve fosfat birikebilmektedir. Depolanan kalsiyum iskelet yapımında, fosfat ise kitin sentezinde kullanılır. Esansiyel minerallerin ana fonksiyonları iskelet yapımı, koloidal sistemin sürdürülmesi ve asit-baz dengesinin düzenlenmesidir. Mineraller ayrıca hormonların, enzimlerin ve enzim aktivatörlerinin önemli bileşenleridir ⁴¹. Mg, Al, Ca, Fe, Co, Cu ve Zn gibi bazı mineraller sucul canlıların da sağlıklı yaşam sürebilmeleri için gereklidir ⁴². Bu çalışmadan elde edilen sonuçlara göre, *P. longirostris* türü karideslerin etinde en fazla bulunan mineral potasyum ve sodyumdur. Kalsiyum kabukluların dış iskeletlerinde, kas kasılmasında, osmoregülasyonda ve bazı enzimatik reaksiyonlarda koenzim olarak rol oynar. Demir ise oksidasyon olaylarında katalizör görevi gören bir mineraldir ^{43,44}. Çalışmamızda, *P. longirostris* türü kari-

deslerin abdominal kas dokularında bulunan metal düzeylerinin normal değerler arasında olduğu gözlenmiştir.

Marmara Denizi'nde dağılım gösteren derin su pembe karidesi stokları denizel kaynaklar içerisinde döviz girdisi açısından ülkemizin hatırı sayılır ticari kaynaklarından birisidir. Karides eti ideal gıda özelliklerine sahip bir protein sahip olup gelecekte de besin kaliteli protein arayışında olan ülkemiz ve dünya için önemli bir besin maddesidir. Türkiye denizleri karides türleri bakımından zengin bir popülasyona sahip olmasına karşın bu türlerin biyokimyasal ve mineral içerikleri hakkında çok fazla araştırma bulunduğu söylenemez. Yapılan araştırmaların çoğu sistematik ve biyolojik özelliklerinin tanınmasına yöneliktir. Denizlerimizde yaşayan türlerin maruz kaldığı çevre koşullarının (oksijen azlığı/yokluğu, besin bulma, pH, ısı, ışık, kirlenme gibi) bu türlerin fizyolojilerinde nasıl bir adaptasyon stratejisi izlediğini bilmek çok önemlidir. Bu çalışmada ölçülen biyokimyasal kompozisyon analizleri aynı zamanda karideslerin enerjiyi depolama ve harcama stratejileri hakkında da fikir vermektedir. Bu çalışmada, İzmit körfezinden avlanan karideslerin mevsimsel değişimler göz önüne alınarak hepatopankreas ve abdominal kas dokularındaki serbest glikoz, glikojen, total lipid, trigliserit, total kolesterol ve mineral (Ca, Na, K, Fe, Mg) içerikleri incelenmiştir. Sonuç olarak, bizim çalışmamızda hepatopankreas ve abdominal kas dokularının serbest glikoz ve glikojen düzeylerinin hem erkek hem de dişi bireylerde hemen hemen eşit miktarlarda bulunduğu ve Sonbahar-Kış aylarında azalmaya başladığı gözlenmiştir. Ayrıca *P. longirostris* türü için kolesterol ve lipidler özellikle üreme dönemlerinde önemli bir enerji kaynağı olarak belirlenmiştir. Mineral içerikleri ise tüm örneklerin abdominal kas dokularında normal değerler arasında bulunmuştur.

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Dioktil Adipat'ın (DOA) *Labidochromis caeruleus* Fryer, 1956 (Cichlidae, Teleostei) Karaciğer Histolojisi Üzerindeki Etkileri ^[1]

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Özet

Polimerizasyon süreçlerinde en çok kullanılan plastikleştiricilerden biri olmasına rağmen, dioktil adipat'ın (DOA, C₂₂H₄₂O₄) ekotoksikolojik etkileri çok iyi bilinmemektedir. Bu noktadan hareketle sunulan araştırmanın amacı, 30 gün süreyle 0.75 ppm DOA uygulamasına maruz bırakılan *Labidochromis caeruleus* (sarı prenses) karaciğerlerindeki histopatolojik değişimlere ilişkin ışık mikroskobu bulgularının değerlendirilmesine yöneliktir. Kontrol gruplarıyla karşılaştırıldığında, DOA'ya maruz kalan grupta karaciğerde oluşan en belirgin değişimler çarpıcı bir steatoz (yağlanma), bazı fibröz yapılaşmalar ve genişleyen nekrotik alanlar olarak not edilmiş, ayrıca sinüzoidlerde ve merkezi vende dilatasyon, poikilositoz ve kanama gözlenmiştir. Çalışmada sunulan veriler, DOA'nın, en azından *Labidochromis caeruleus* için hepatotoksik olduğunu göstermektedir.

Anahtar sözcükler: Dioktil adipat, DOA, Karaciğer, Histopatoloji, *Labidochromis caeruleus*

The Effects of Dioctyl Adipate (DOA) on The Liver Histology of *Labidochromis caeruleus* Fryer, 1956 (Cichlidae, Teleostei)

Summary

Although dioctyl adipate (DOA, C₂₂H₄₂O₄) is one of the most common plasticizers that are used for polymerization, the ecotoxicological effects of it is not well known. From this point of view, aim of this preliminary investigation was to analyze the histopathological changes in the liver of *Labidochromis caeruleus* (yellow princess), exposed to 0.75 ppm DOA for 30 days. When compared to controls, the most prominent changes occurred in the DOA exposed group were noted as a significant steatosis (fat depositions), fibrosis and expanding necrotic areas. The dilatation of sinusoids and central veins, poikilocytosis, and hemorrhage were also observed. The data presented in this study shows that, DOA is a hepatotoxic chemical at least for *Labidochromis caeruleus*.

Keywords: Dioctyl adipate, DOA, Liver, Histopathology, *Labidochromis caeruleus*

GİRİŞ

İnşaat sektörü başta olmak kaydıyla ambalaj, kablo ve boru üretimi, kozmetik ve sağlık sektörlerinde, ayrıca kompozit yakıtlarda yaygın kullanım alanına sahip bir polimer olan polivinil klorürün (PVC) üretiminde, ikincil plastikleştirici olarak en çok tercih edilen bağlayıcı maddelerden biri, fitalat grubuna giren dioktil adipattır (DOA).

Fitalatlar kararlı bileşiklerdir, ısı ve sürtünmeyle serbest hale geçerek canlı vücuduna girebilirler. Bu maddelerin insan ve çevre sağlığı üzerindeki etkileriyle ilgili veriler sınırlıdır. *Daphnia magna* üzerinde yapılan araştırmalarda akut ve kronik toksisite değerleri ortaya konmuş olmasına karşın, DOA'nın çevreye verebileceği zarar konu-



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sunda, algler ve balıklar için akut toksisiteden bahsedilemeyeceği ve güvenli bir kullanım alanına sahip olduğu şeklinde, gerçekten iyimser denilebilecek bir yaklaşım sergilenmektedir ^{1,2}. Söz konusu maddenin metanol, dietil eter, n-oktanol ve asetonunda çözünabilirken suda çözünmediğini öne süren ticari raporlara karşın, US EPA kayıtlarında suda 0.78 mg/L gibi oldukça düşük bir çözünürlük değeri verilmektedir, Log K_{ow}≥6.11 olarak verilen oktanol/su bölünme katsayısı, toprakta mobilizasyonun olmayışı ve balıklarda 27 olarak bildirilen biyokonsantrasyon faktörü de bu verilere eklendiğinde, aynı kaynaklar DOA'nın çevreye ancak çok az hasar verebileceğini öne sürmekte ve hatta biyokonsantrasyon faktörü uzantısında balıklar açısından akut toksik olmak bir yana, herhangi bir çevresel öneminin bulunmayacağı, ancak adipatı metabolize edemeyen sucul organizmalar için önemli olabileceği not etmektedirler. US EPA raporlarına göre sıçanlar için verilen LD₅₀ değeri 5600-45000 mg/kg olarak, yüzey sularında bulunabilecek değer olan 0.5 µg/L'nin çok üzerindedir. Genotoksik olmamakla birlikte memelilerde fertilité, üreme ve gelişimde bazı olumsuz etkiler gösterebileceği kaydedilen DOA, IARC ölçütlerinde Grup 3 içerisinde, yani insanlar için kanserojenik ve/veya mutajenik değildir. Fakat kullanımı sırasında solunmasından, gözle ve deriyle temasından kaçınılması gerektiği yolunda ve ayrıca kronik maruziyette kilo kaybına, kemik yoğunluğunda azalmaya, karaciğer ve testis hasarları oluşturabileceğine dair uyarılar vardır ².

Sunulan araştırma, sucul toksisite çalışmaları için uygun bir kemikli balık türü olarak belirlenen sarı prenses (*Labidochromis caeruleus*) örnekleri üzerinden DOA'nın, dönüşüm ve metabolizma bağlamında zehirsizleştirme mekanizmalarının en önemli organı olan karaciğer histolojisindeki olası etkilerinin, histolojik yöntemlerle belirlenmesi hedeflenerek gerçekleştirilmiştir.

MATERYAL ve METOT

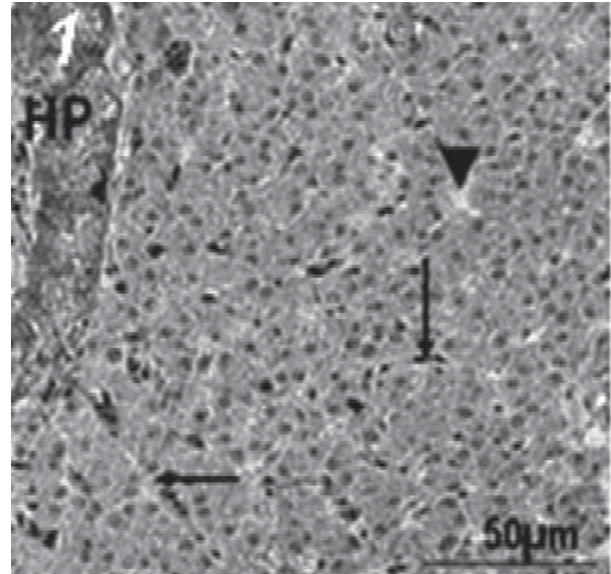
Araştırmamız Ege Üniversitesi Eczacılık Fakültesi tarafından hazırlanan Deney Hayvanları İçin Etik Kurul Kararlarına göre ve Ege Üniversitesi Tıp Fakültesi Hayvan Etik Kurulu 2008-49 Sayılı Raporu uyarınca (EÜHADYEK Sertifika No: 000018) yürütülmüştür. Dişi bireylerde strese bağlı oluşabilecek hormonal değişikliklerden kaçınılması amacıyla çalışmada sadece erkek balık örnekleri kullanılmıştır. Ticari akvaryumculardan sağlanan onbeş adet erkek *L. caeruleus* örneği (ağırlık 3±0.5 gr; total boy 8±2 cm) dört hafta süreyle 20 L hacmindeki cam akvaryumlarda, 25±1°C su sıcaklığında, 7.7-8.5 pH'da ve doğal fotoperiyotta tutulduktan sonra beşer örnekten oluşan iki kontrol ve bir deneme grubuna ayrılmışlardır. Balıklar iki günde bir ticari yemlerle (Sera-San) beslenmişlerdir. DOA örneği (CAS Number 103-23-1) Plastifay AŞ (İstanbul) tarafından sağlanmıştır.

İlk gruba negatif kontrol olarak hiçbir kimyasal verilmemiştir. İkinci kontrol grubunun bulunduğu akvaryuma ise DOA'nın çözülmesi amacıyla, bu tip çalışmalarda en sık kullanılan çözücü olarak aseton eklenip, bu grup aseton kontrol grubu olarak tanımlanmıştır. Deneme grubunun bulunduğu akvaryuma 0.75 ppm DOA aseton içinde çözülerek bir defada uygulanmıştır. Otuz günlük deneme süresi bittiğinde MS222 ile anesteziyen sonra kontrol ve deneme gruplarından alınan karaciğer dokuları Bouin sıvısında tesbit edilip parafine gömülmüştür. Baird-Tatlock marka mikrotomla alınan 5-7 µm kalınlığındaki kesitler hematoxilen-eozin (H&E) ve Mallory Trikróm (MT) boyama yöntemleriyle boyanıp, Olympus CX 51 ışık mikroskopunda incelenerek fotoğrafları çekilmiştir.

BULGULAR

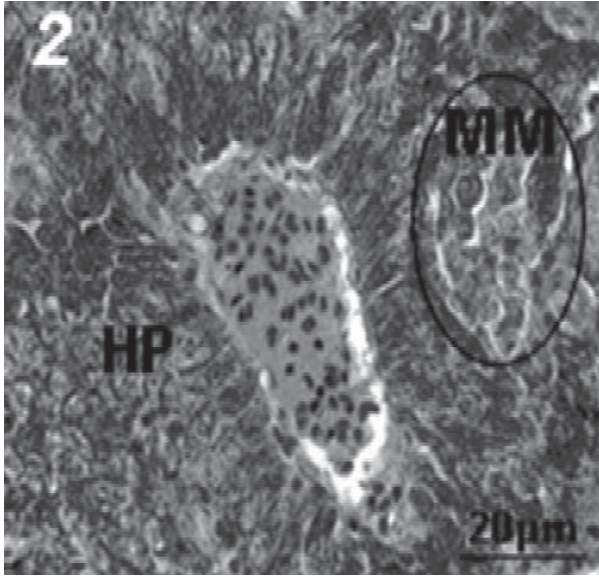
Deneme ve kontrol gruplarında hiçbir ölüm vakası olmamış ve örneklerde herhangi bir davranış değişimi gözlenmemiştir. Negatif kontrol grubundaki gözlemlere göre, merkezi ven etrafında lobüler bir düzenleme izlenmeyen *L. caeruleus* karaciğer parankimasında hepatopankreas yapısını oluşturan ekzokrin pankreas asinusları (*Şekil 1*) çevresinde daha sık olmak kaydıyla melanomakrofaj yığınları yer alır (*Şekil 2*).

Parankimanın esas hücreleri olan çokgen şekilli ve iri nükleuslu hepatositler (*Şekil 3*) oldukça düzgün kordonlar oluştururlar, aralarında çok ince sinüzoidler ve



Şekil 1. Negatif kontrol grubunda karaciğer parankimasında hepatopankreas (HP), merkezi ven (MV) ve sinüzoidler (ok), çok hafif steatoz (ok başı), H&E

Fig 1. Hepatopankreas (HP), central vein (MV), sinusoids (arrow) and a slight steatosis (arrowhead) in the liver parenchyma of negative control group, H&E

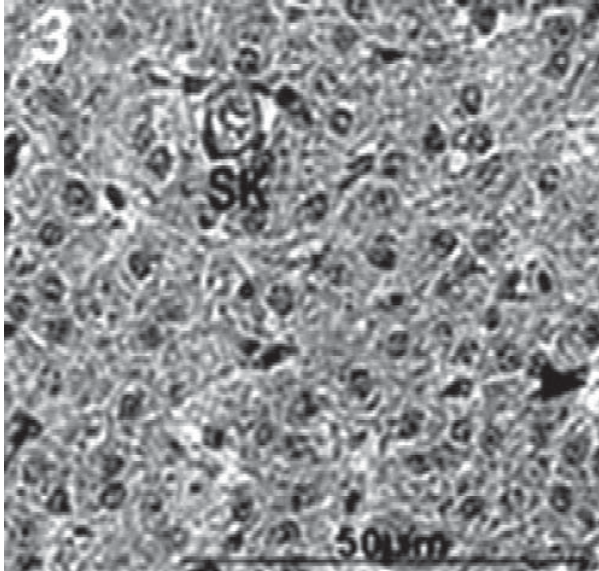


Şekil 2. Negatif kontrol grubunda hepatopankreas yakınında melanomakrofaj merkezi (MM, daire içerisinde), MT

Fig 2. Melanomacrophage center (MM, encircled) nearby the hepatopancreas (HP) of negative control group, MT

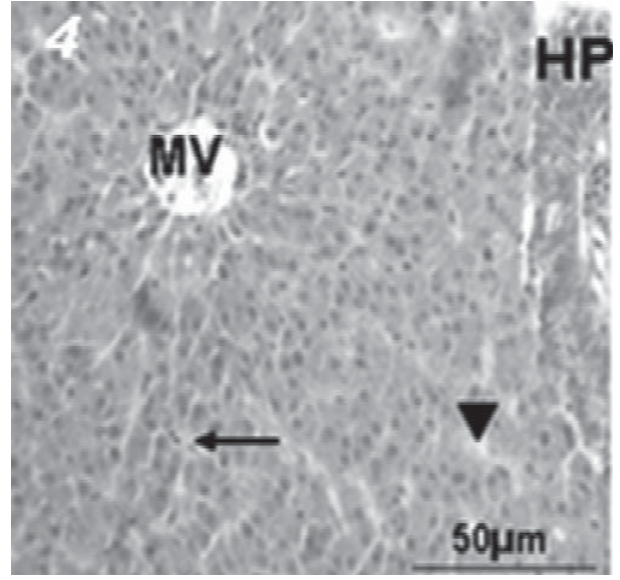
safrı kanalları bulunur. Örneklerde ayrıca az sayıda vakuolle karakterize edilen çok hafif bir steatozis not edilmiştir.

Karaciğer parankima yapıları kontrol grubu örneklerine çok büyük oranda benzerlik gösteren aseton kontrol grubu örneklerinde (Şekil 4-6) belirgin bir histopatoloji saptanmamıştır.



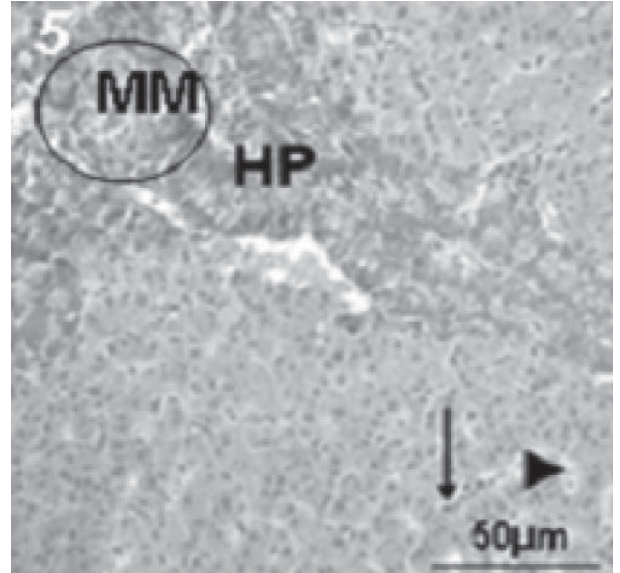
Şekil 3. Negatif kontrol grubunda hepatositlerden ayrıntı, safra kanalı (SK) ve çok hafif steatozis, H&E

Fig 3. Details of hepatocytes, bile duct (SK) and also a slight steatosis (arrowhead) at negative control group. H&E



Şekil 4. Aseton kontrol grubu karaciğer parankimasında merkezi ven (MV), hepatopankreas (HP), sinüzoidler (ok) ve hafif steatozis (ok başı), H&E

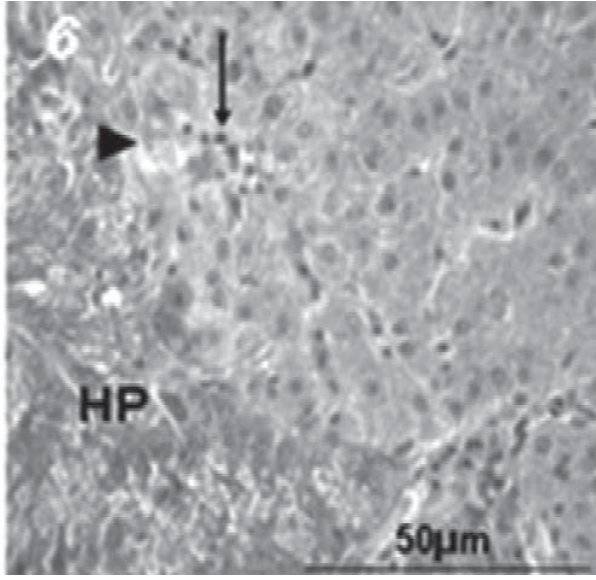
Fig 4. Central vein (MV), hepatopancreas (HP), sinusoids (arrow) and a slight steatosis (arrowhead) in the liver parenchyma of acetone control group, H&E



Şekil 5. Aseton kontrol grubunda hepatopankreastan ayrıntı, melanomakrofaj merkezi (daire içerisinde, MM) ve sinüzoidler (ok), H&E

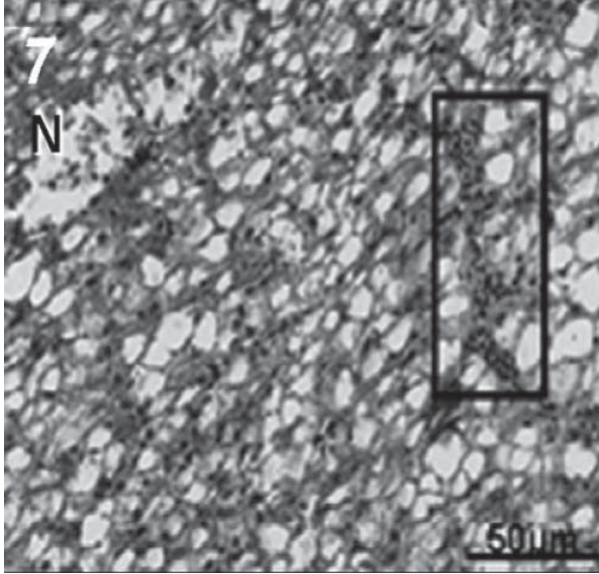
Fig 5. Details of hepatopancreas, melanomacrophage center (MM, encircled,) and sinusoids (arrow) at acetone control group. H&E

Merkezi ven, sinüzoidler, hepatopankreas ve melanomakrofaj yığınları (Şekil 4, 5) önceki gruptaki gibi kolayca seçilebilmektedir. Hepatositlerde herhangi bir şekil bozukluğu yoktur ve bu grupta da çok hafif steatozis izlenmektedir (Şekil 4, 6).



Şekil 6. Aseton kontrol grubunda hepatosit ve sinüzoidlerden (ok) ayrıntı, ayrıca çok hafif steatoz (ok başı), MT

Fig 6. Details of hepatocyte, sinusoids (arrow) and also a slight steatosis (arrowhead) at acetone control group, MT

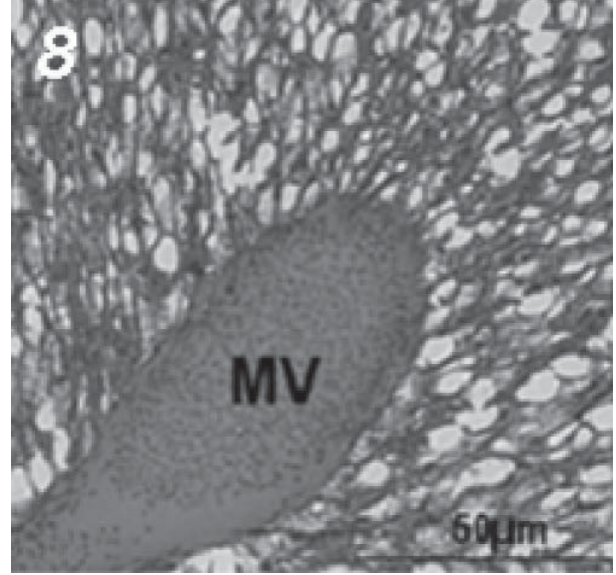


Şekil 7. DOA uygulanan grupta karaciğer parankimasında çok yaygın vakuoller halinde steatoz, nekrotik alan (N) ve sinüzoidlerde genişleme (dikdörtgen içerisinde), H&E

Fig 7. Steatosis as widened vacuoles, necrotic area (N) and dilatation of sinusoids (rectangled) in the liver parenchyma of DOA exposed group, H&E

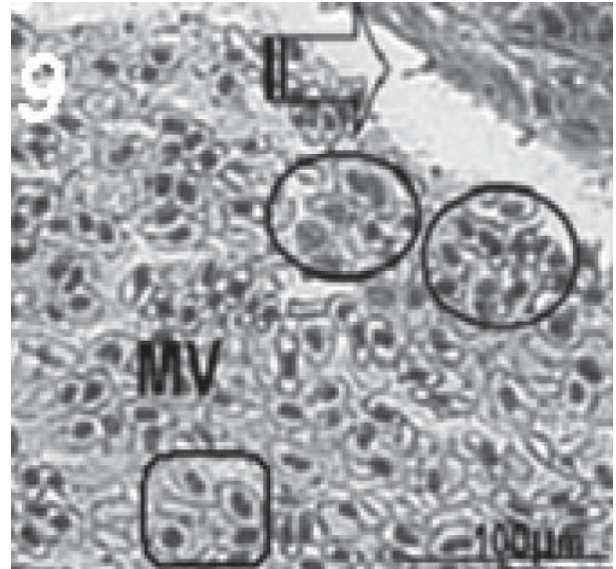
DOA uygulaması sonucunda karaciğer dokusunda göze hemen çarpan değişim, çok yaygın vakuoller oluşumlarla belirginleşen steatozdur, ayrıca çok geniş olmayan nekrotik alanlara rastlanmakta, sinüzoidlerdeki genişleme ve eritrosit miktarında artma dikkat çekmektedir (*Şekil 7*).

Çevresinde çok tipik steatozis gözlenen, çeperleri yer yer parankimadan ayrılmış gibi duran ve içerdiği kan hücrelerinin miktarı artan merkezi vendeki (*Şekil 8*) eritrositlerden bazılarında çeşitli şekil bozuklukları (poikilositozis) belirlenmiştir. Bazı eritrositlerin çok şişkin görü-



Şekil 8. DOA uygulanan grupta merkezi ven (MV) çevresinde çok çarpıcı steatoz, H&E

Fig 8. Striking steatosis all around of the central vein (MV) of DOA exposed group, H&E

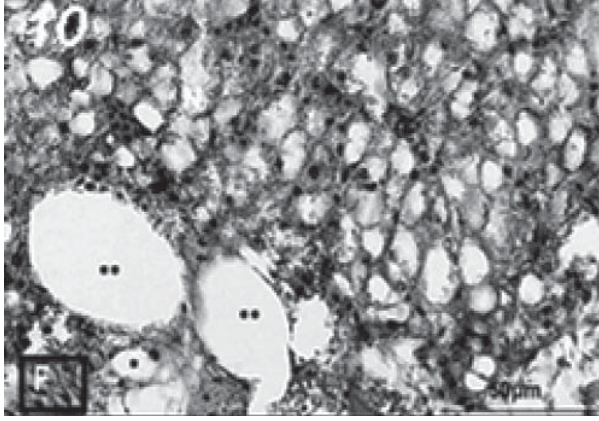


Şekil 9. DOA uygulanan grupta merkezi ven çeperinde ayrılmalar (ok); bazı eritrositlerde çok şişkin görünüm (yuvarlatılmış dikdörtgen içerisinde) ve şekli bozulmuş nükleuslar (daire içerisinde), H&E

Fig 9. Separations (arrow) in the wall of central vein, swollen appearance (rounded rectangle), and deformed nuclei (encircled) in some erythrocytes at DOA exposed group, H&E

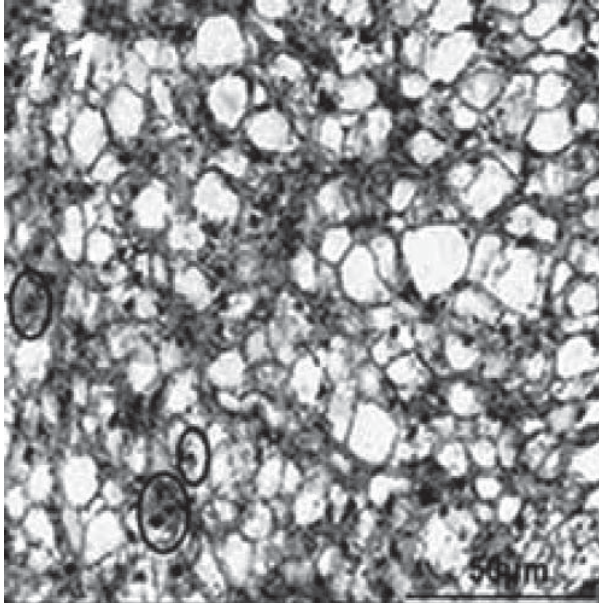
nümü yanında diğer bazılarında nukleus deformasyonları izlenmektedir (Şekil 9). Parankimada çok geniş boşluklar halinde izlenen steatozise ek olarak yer yer fibriller oluşumlara (fibrozis) rastlanmaktadır (Şekil 10).

Steatoz, hepatosit nukleuslarında şekil bozukluklarına sebep olacak kadar çarpıcıdır (Şekil 11), bu tip hepatositlerde hücre zarı bütünlüğü de bozulmuştur. Bütün bu değişimlere ayrıca doku genelinde çok yaygın nekroz eşlik etmektedir (Şekil 10, 11). Nekrotik alanların bazı preparatlarında izlenen çok genişlemiş durumu (Şekil 12), dokunun boş-



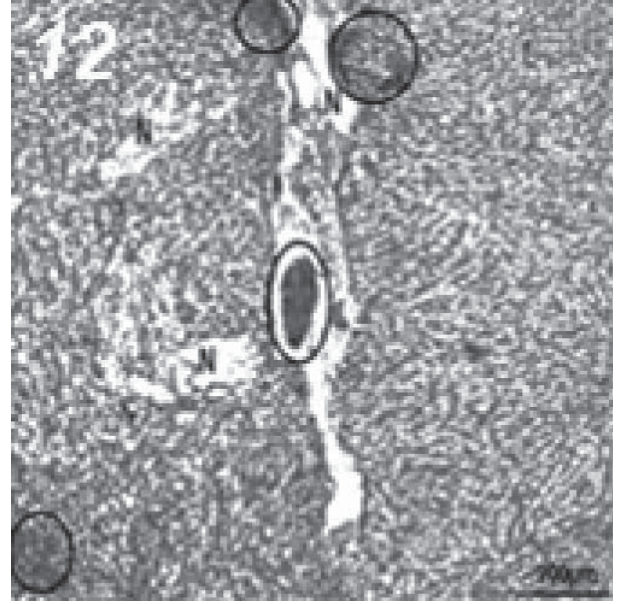
Şekil 10. DOA uygulanan grupta çok geniş vakuoller halinde steatoz (**) ve nekrotik alanlar ve (N) fibroz (dikdörtgen içerisinde, F), MT

Fig 10. Steatosis with large dilated vacuoles (**), necrotic areas (N) and fibrosis (F, rectangled) at DOA exposed group, MT



Şekil 11. DOA uygulanan grupta hepatosit nukleuslarında bozulmalar (daire içerisinde), MT

Fig 11. Deformations of hepatocyte nuclei (encircled) in DOA exposed group. MT



Şekil 12. DOA uygulanan grupta çok geniş nekrotik alanlarda (N) ve parankimada yaygın hemoraji (daire içerisinde), H&E

Fig 12. Widespread hemorrhage (encircled) in widely necrotic areas (N) and parenchyma in DOA exposed group, H&E

luklu bir görünüm almasına neden olarak karaciğerdeki deformasyonu çarpıcı biçimde ortaya koyan bir bulgudur.

Bu tip alanlarda kanama (hemoraji) odaklarında artışa rastlanmıştır, hemoraji ayrıca doku genelinde de izlenmektedir.

TARTIŞMA ve SONUÇ

Labidochromis caeruleus karaciğerinin genel yapısı diğer teleostlarla benzerlik göstermekteyse de, hepatositlerin merkezi ven etrafında düzgün kordonlar halinde izlenmesi birçok teleostta görülmez ^{3,4}.

Metabolik işlevlerin ve detoksifikasyon süreçlerinin temel organı olarak karaciğer, açlık gibi stres oluşturan değişimlerden ve toksik maddelere maruziyetten en çok etkilenen organlardan biridir ^{4,6}. Birçok toksik madde ve toksin ⁷, karaciğerde biyolojik olarak daha az zehirli maddelere dönüştürülür ve safra kesesi yardımıyla dışarı atılır. Bazı toksik maddeler ise depolanır, hatta daha da zehirli kimyasallar haline getirilir ⁸.

Omurgalıların en alt grubunu oluşturan teleostlarda bu organ sucul ekosistem kirliliğinin duyarlı bir göstergesi olarak pek çok araştırmaya konu olmaktadır. Çeşitli kimyasal maddelerin ve çevresel kirliliğin etkileri sonucunda balık karaciğerinde ortaya çıkan değişimler geniş ölçüde araştırılarak; birçok ağır metalin amonyum, klorin ve fenoller gibi endüstriyel atıkların ve petrol hidro-

karbonlarının, ayrıca çok sayıda organofosfatlı ve organoklorinli pestisitlerin etkileri değerlendirilmiştir ^{9,10}. Bu çerçevede karaciğerde izlenen başlıca histopatolojik bulgular hepatosit morfolojisinde değişimler ve vakuolizasyon artışı, nekroz, iskemik ve steatoz olarak belirtilmektedir ^{6,10}.

Özellikle aşırı endüstriyel atık boşaltımının sözkonusu olduğu sucul sistemlerde balık karaciğerleri üzerinde yapılan histopatolojik değerlendirmeler geniş ölçüde ilgi çekmektedir. Örneğin Almanya'da, özellikle ağır metal bağlamında çevresel kirliliğin yoğun görüldüğü Elbe Nehrinden toplanan *Platichthys flesus*, *Gymnocephalus cernua* ve *Osmerus eperlanus* türlerinde ¹¹ karaciğer histopatolojisi, hepatositomegali, sinüzoidlerde kanlanma, nekroz, fibroz, steatoz ve melanomakrofaj birikimleri olarak not edilmiştir. *Anguilla anguilla* üzerinde yapılan bir çalışmada ise ¹² olağanın çok üstünde vakuolizasyonla belirlenen, sudaki kontaminasyondan kaynaklanabileceği ve karaciğerde metabolizmanın bozulması sonucu fazla miktarda trigliserit birikimi sonucu gerçekleştiği bildirilmiştir. Bu görüş çerçevesinde steatozis, DOA uygulamasının metabolizasyonu ciddi ölçüde etkileyebileceği düşüncesini kuvvetlendirecek bir bulgu olarak değerlendirilmiştir.

Balıklarda kimyasal maddelerin etkisiyle oluşan karaciğer histopatolojisine yönelik olarak ülkemizde yayınlanmış birçok araştırma raporu vardır. Örneğin Atamanalp ¹³, geniş çaplı araştırmasında gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) sentetik insektisid olan cypermethrin uygulamasıyla vakuoler dejenerasyon izlenen hepatositler uzantısında karaciğerde yağlanma, hemoraji, nekroz, yangı işareti olarak hücre infiltrasyonu ve fibrotik alanlarda genişlemeler olduğunu; Cengiz ve Unlu ¹⁴ ise *Gambusia affinis* karaciğerinde deltamethrine uygulamasının hipertrofi, nekroz ve yağ dejenerasyonlarına yol açtığını bildirilmiştir.

Ayrıca, sucul ortamlardaki kirliliğin belirlenmesi amacıyla karaciğer dokusunun incelendiği çalışmalar da bulunmaktadır. Seyhan Baraj Gölünde yaşayan *Cyprinidae* türleri üzerinde histolojik ve biyokimyasal yöntemleri kullanılarak yapılan bir çalışmada Gül ve ark. ¹⁵; karaciğerin sinüzoidlere çok yakın bazı bölgelerindeki proliferatif hepatositlerde yaygın dejenerasyon gözlediklerini, özellikle bir istasyondan toplanan örneklerde karaciğerde mikro ve makrovesiküller halinde yağlanma izlendiğini not ederek, tüm bu değişimleri baraj gölündeki kirlilikle ilişkilendirmişlerdir. Çine dere-sinin su kirliliğini belirlemeye yönelik araştırmalarında; Koca ve ark. ¹⁶ ise *Lepomis gibbosus*'un karaciğerlerinde çeşitli histopatolojik bulgular gözlemlendiğini bildirmektedirler.

Çalışmamızda deneme grubunda izlenen histopatolojik değişimler, yukarıda örneklenen raporlarda sunulan bulgularla büyük ölçüde uyum göstermektedir.

Sinüzoidlerde izlenen kanlanma olgusu, birçok farklı çalışmada hepatosellüler hasarın göstergesi olarak vurgulanmaktadır ^{11,17,18}. Organofosfat kirleticilerin uygulanması sonucunda *Corydoras paleatus* ¹⁹ ve *Prochilodus lineatus* ²⁰ hepatositlerinde de benzer bozulmalar olduğu bildirilmiştir. Çalışmamızda adeta parankimadan ayrılmış şekilde izlenen merkezi venlerde ve eritrosit şekillerinde saptanan değişimler, DOA uygulaması ile organofosfatların oluşturduğundan daha farklı ve büyük olasılıkla daha belirleyici patolojik bir bulgudur. Merkezi venlerde yer yer belirgin biçimde izlenen seperasyon, doku bütünlüğünün bozulması, dokunun dağılmaya yüz tutması anlamında, DOA etkisini belirleyici bir bulgu olarak değerlendirilmiştir. DOA'nın, *Labidochromis caeruleus*'ta, ilk maruziyet yüzeyi olan solungaçlar üzerinde de çarpıcı etkiler oluşturduğu bilinmektedir ²¹.

Uluslararası Kanser Araştırma Ajansının (IARC) çalışma gruplarının, DOA'nın insanlardaki toksik etkilerine ilişkin veri elde edemedikleri bildirilmektedir ²². Aynı raporda, fare ve sıçanlarda DOA maruziyetinin karaciğerde büyümeye, peroksizomlarda proliferasyona yol açtığı belirtilmektedir. Maruziyet sonucunda fare karaciğerinde gözlenen adenom ve karsinomlar sıçan karaciğerinde oluşmamıştır. Hem bu hayvanlarda, hem de kobaylarda bazı karaciğer enzimlerinde aktivasyon değişimleri not edilmekle beraber, DOA, insanlar için kanserojenik olarak sınıflandırılmamıştır. IARC, DOA'nın genotoksik olmadığını bildirmekte, ancak konuyla ilgili çalışmaların yetersiz olduğuna da vurgu yapmaktadır. Bu durumda, ekotoksikolojik etkileri konusunda çok az veri bulunan DOA'nın, çok daha dikkatle değerlendirilmesi gereken bir kimyasal olduğu açıktır.

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Comparison of Hatching Performances and Yolk Sac Absorptions of Black Sea Trout (*Salmo trutta labrax* Pallas, 1811), Brook Trout (*Salvelinus fontinalis* Mitchill, 1814) and Their Hybrid

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Summary

The aims of this study were to determine hatching performance, body and yolk weight at hatching, the growth rate during the yolk absorption and yolk conversion efficiency of Black Sea trout, brook trout and their hybrid larvae, and to compare the relationships between length, total wet weight, dry yolk body weights of alevins and degree-days. The Black Sea trout egg has shorter incubation duration than brook trout and their hybrid. Survival from fertilization to swim-up of brook trout, Black Sea trout, and hybrid were 45.8%, 54.3% and 30.7%, respectively. Length, total wet weight, dry larva weights, and dry yolk weights of brook trout, Black Sea trout and hybrid exhibited a linear relationship with degree-days; dry yolk weight decreased, while length, total wet weight and dry body weight increased with degree-days. The linear regression parameters excluding length slope were significantly different. Length and weight increase as well as growth rates, daily yolk sac consumption and yolk sac efficiency values were calculated as 0.21 mm/day, 0.63 mg/day, 0.30 mg/day, and 0.61 for brook trout; 0.19 mm/day, 0.87 mg/day, 0.60 mg/day, and 0.76 for Black Sea trout, and 0.20 mm/day, 0.45 mg/day, 0.28 mg/day, and 0.46 for hybrid, respectively.

Keywords: Brook trout, Black Sea trout, Hybrid, Hatching performances, Yolk sac absorption

Karadeniz Alabalığı (*Salmo trutta labrax* Pallas, 1811), Kaynak Alabalığı (*Salvelinus fontinalis* Mitchill, 1814) ve Hibridlerinin Kuluçka Performansı ve Besin Kesesi Absorpsiyonlarının Karşılaştırılması

Özet

Bu araştırmanın amacı, Karadeniz alabalığı, kaynak alabalığı ve Hibridlerinin kuluçka performansı ve besin kesesi absorpsiyonu ve değerlendirme randımanlarının ortaya konması ve çıkış süresi (gün-derece) ile boy, toplam yaş ağırlık, kuru vücut ve kese ağırlıkları arasındaki ilişkileri karşılaştırmaktır. Araştırma sonucunda Karadeniz alabalığının, kaynak alabalığı ve hibrid bireylere göre daha kısa bir kuluçka süresine sahip olduğu, yumurtaların yaşama oranının Karadeniz alabalığında %45.8, kaynak alabalığında %54.3 ve hibrid bireylerde ise %30.7 olduğu belirlendi. Karadeniz alabalığı, kaynak alabalığı ve hibrid bireylerde, gün-derece ile boy, toplam yaş ağırlık, kuru vücut ve kese ağırlıkları arasındaki lineer ilişkiler, besin kesesi için azalan, diğerlerinde ise artan olarak ortaya konmuştur. Boy ilişkisinin eğimi hariç, diğer ilişkilerin regresyon parametrelerinde önemli farklılıklar belirlendi. Boy ve ağırlıkça büyümedeki artış, günlük besin kesesi tüketimi ve besin kesesi değerlendirme randımanları, sırasıyla Karadeniz alabalığında 0.19 mm/gün, 0.87 mg/gün, 0.60 mg/gün ve 0.76; kaynak alabalığında 0.21 mm/gün, 0.63 mg/gün, 0.30 mg/gün ve 0.61 ve hibrid bireylerde ise 0.20 mm/gün, 0.45 mg/gün, 0.28 mg/gün ve 0.46 olarak hesaplandı.

Anahtar sözcükler: Karadeniz alabalığı, Kaynak alabalığı, Hibrid, Kuluçka performansı, Besin kesesi tüketimi



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INTRODUCTION

Hybridization is the mating of different individuals or crosses between separate species. The desired aim is to produce individuals that are sterile and with better growth performance, flesh quality, disease resistance, etc ¹.

The Black Sea trout, *Salmo trutta labrax*, is a native finfish species of the Eastern Black Sea coast and rivers, is a new species for intensive aquaculture ² while the brook trout *Salvelinus fontinalis*, is not a very popular culture species. However, it has been cultured commercially in the North America, Europe and in the Eastern Black Sea region of Turkey in some trout farms. A few studies were done about hatching ^{2,3} and yolk sac absorption on the Black Sea trout ⁴, and brook trout ⁵.

The first external feeding activities of the fish larvae start when the larvae swim-up. The observations demonstrated that, larva has a remnant of yolk at the swimming up stage. In trout hatcheries the first external feeding starts when over 30% percent larvae begin swimming up. As in other finfish these development and transition stages are very critical due to susceptibility to diseases and pathogens, environmental fluctuations and starvation. It is nevertheless possible to increase survival and growth rates through proper hatchery management skills during these critical stages. Although several studies have been performed on larval development of salmonid species, namely, sea trout ⁶, Black Sea trout ⁴, Atlantic salmon ^{7,8}, rainbow trout ⁹, brook trout ⁵, brook trout and Arctic charr and their hybrids ¹⁰, this study is the first on Black Sea trout and brook trout hybrid.

Aims of this study are to determine hatching performance, body and yolk weight at hatching, the growth rate during the yolk absorption and yolk conversion efficiency of Black Sea trout, brook trout and their hybrid larvae, and to compare the relationships between length, total wet weight, dry yolk and body weights of alevins and degree-days under pilot hatchery conditions in the Eastern Black Sea in Turkey.

MATERIAL and METHODS

The eggs were collected from eight brook trout and five Black Sea trout broods, 3-year-old with a mean weight of 432.6±99.69 g, 639.2±212.34 g, respectively, and they are fertilized with milt obtained from two males at trout hatchery in Faculty of Marine Sciences, Karadeniz Technical University. Four cross-types were produced: brook trout (Sf), Black Sea trout (St), and the hybrids between brook trout and Black Sea trout (Sf x St, St x Sf; the first letter indicates the female parent species).

The all Sf x St hybrid eggs could not survive in eyed-stage, so this group are not included in this study. The eggs (2143 brook trout, 2164 Black Sea trout and 2220 hybrid) were divided into three parts and placed in an egg tray of 5 liter in vertical incubator, and hatched in well spring water at a temperature of 9.9±1.3°C.

Observations of the eggs and alevins were made together with the temperature readings. The eyed-egg stage was defined as when the eyes were clearly visible as black spots. The 50% hatch time was defined as when 50% of the embryos were swim-up larvae. The swim-up stage was considered to begin when approximately 50% of the alevins were starting to feed and were actively swimming up in the water for food intake. The dead eggs and larvae were removed and counted periodically.

The larvae sampling was commenced at 5-day intervals from hatching to 764.8 degree-days post-hatch. The first larvae were sampled at 443.5 degree-days (50% eggs hatched) and then at 489.0, 537.0, 583.5, 628.8, 670.5, 717.8 and 764.8 degree-days (after swim-up stage started), respectively. Ten larvae were randomly sampled at each sampling period (8 times), i.e. a total of 80 larvae were used during the study at each cross-types. They were anesthetized in 20 ppm benzocaine, and then preserved in 10% formalin until further analysis. After a minimum interval of 3 weeks, fixed larvae were dissected to separate the yolk from the body. The body and yolk were dried separately at 60°C for 48 h and weighed (±0.1 mg) after 48 h ⁶.

Egg and larvae survival rates ($S=(N_2/N_1) \times 100$), growth performance (increase in weight) and growth ($G=[(Wt_2-Wt_1)/t]$) were determined periodically (N_1 and N_2 : number of eggs or larvae time 1 and time 2; W : weight, mg; t : time, day). Yolk conversion efficiency (E) was calculated from the formula; $E=(L_t-L_0)/(Y_0-Y_t)$, where, L is larval weight at any time t and time zero, and Y is yolk weight at any time t and time zero ⁹.

To determine statistical significance ($P<0.05$) of differences in length, wet weight, dry body and yolk sac weights among the group, analysis of variance and Tukey tests were used. The significance of all slopes ($H_0: b=0$) and regressions were tested at the 0.05 probability level ^{9,11}, and statistical significance of differences among regression evaluations and slopes values were determined with analysis of covariance ¹².

RESULTS

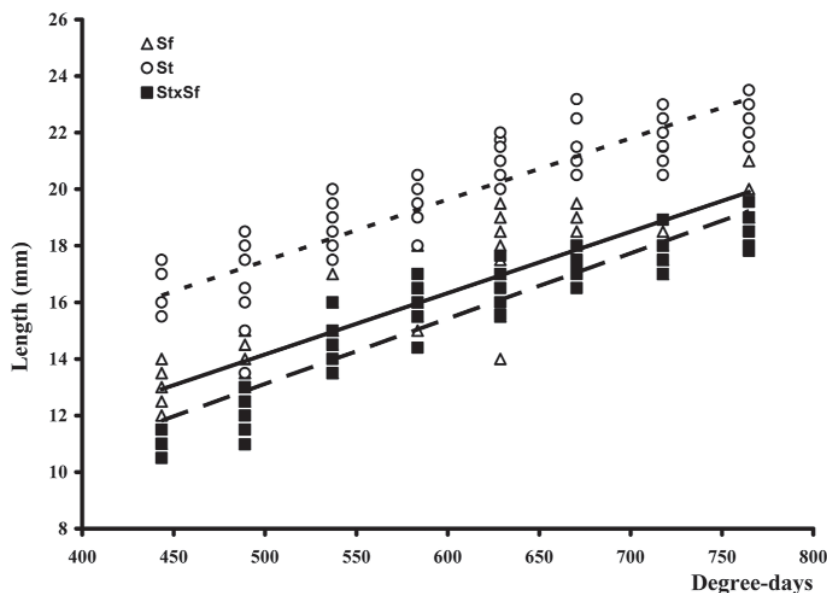
Egg size of brook trout and Black Sea trout were 4.6±0.03 mm (4.3-4.9) and 4.6±0.02 mm (4.5-4.7), respectively. Incubation water temperature was varied

Table 1. Duration (day) of eyed-egg, hatch and swim-up phases (degree-days values in parenthesis, letters showed differences)**Tablo 1.** Gözlenme, çıkış ve serbest yüzme süresi (gün) (parantez içleri gün-derece, harfler istatistiksel farklılıkları göstermektedir)

Parameter	Brook Trout	Black Sea Trout	Hybrid	ANOVA
Eyed-egg Stage	18-24 (212-283)	17-26 (201-306)	18-25 (212-295)	P>0.05
Hatching	38-48 ^a (410-518)	35-41 ^b (388-443)	38-48 ^a (410-518)	P<0.05
Swim-up	75-80 ^a (743-792)	71-76 ^b (703-752)	76-81 ^a (752-802)	P<0.05

between 7.5 and 12.6°C (9.89±1.26). The Black Sea trout egg has shorter incubation duration than brook trout and hybrid. Survival from fertilization to swim-up of brook trout, Black Sea trout, and hybrid were determined as 45.8%, 54.3% and 30.7%, respectively (Table 1).

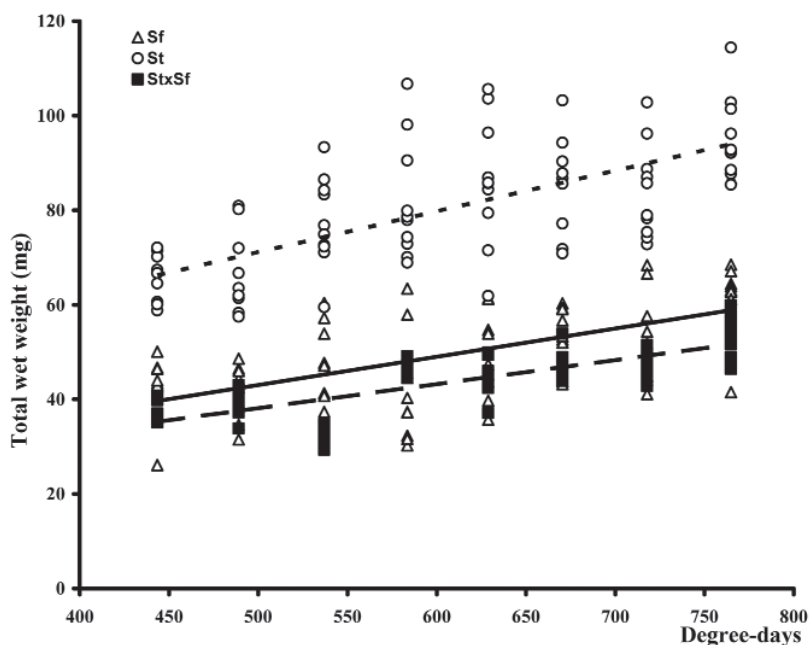
Length (Fig. 1), total wet weight (Fig. 2), dry larva weights (Fig. 3), and dry yolk weights (Fig. 4) of brook trout (Sf), Black Sea trout (St) and hybrid (StxSf) exhibited linear relationship with degree-days (ranged 443.5-764.8); dry yolk weight decreased, while length, total wet weight and dry body weight increased. Among the linear regression parameters were significantly

**Fig 1.** Relationship between total length (y) and degree-days (x) [in brook trout (Sf; Δ, —): $y = 3.3072 + 0.0217 x$, $r^2 = 0.82$; Black Sea trout (St; ○, ...): $y = 6.5831 + 0.0218 x$, $r^2 = 0.79$; and hybrid (StxSf; ■, --): $y = 2.0300 + 0.0223 x$, $r^2 = 0.82$]. Among the groups, constant (a) showed similar, and slope (b) differed significantly ($P < 0.001$)

Şekil 1. Toplam boy (y) ile gün-derece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): $y = 3.3072 + 0.0217 x$, $r^2 = 0.82$; Karadeniz alabalığı (St; ○, ...) : $y = 6.5831 + 0.0218 x$, $r^2 = 0.79$; hibrid (StxSf; ■, --) : $y = 2.0300 + 0.0223 x$, $r^2 = 0.82$]. Gruplar arasında sabit değer (a) benzer, eğim (b) ise istatistiksel olarak farklı ($P < 0.001$) bulunmuştur

Fig 2. Relationship between total wet weights (y) and degree-days (x) [in brook trout (Sf; Δ, —): $y = 13.1346 + 0.0598 x$, $r^2 = 0.33$; Black Sea trout (St; ○, ...): $y = 28.8957 + 0.0854 x$, $r^2 = 0.39$; and hybrid (StxSf; ■, --): $y = 12.7089 + 0.0509 x$, $r^2 = 0.57$]. Constant (a) and slope (b) values of St differed significantly than the others ($P < 0.001$)

Şekil 2. Toplam yaş ağırlık (y) ile gün-derece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ, —): $y = 13.1346 + 0.0598 x$, $r^2 = 0.33$; Karadeniz alabalığı (St; ○, ...) : $y = 28.8957 + 0.0854 x$, $r^2 = 0.39$; hibrid (StxSf; ■, --) : $y = 12.7089 + 0.0509 x$, $r^2 = 0.57$]. Karadeniz alabalığına ait sabit değer (a) ve eğim (b) diğer gruplardan istatistiksel olarak farklı ($P < 0.001$) bulunmuştur



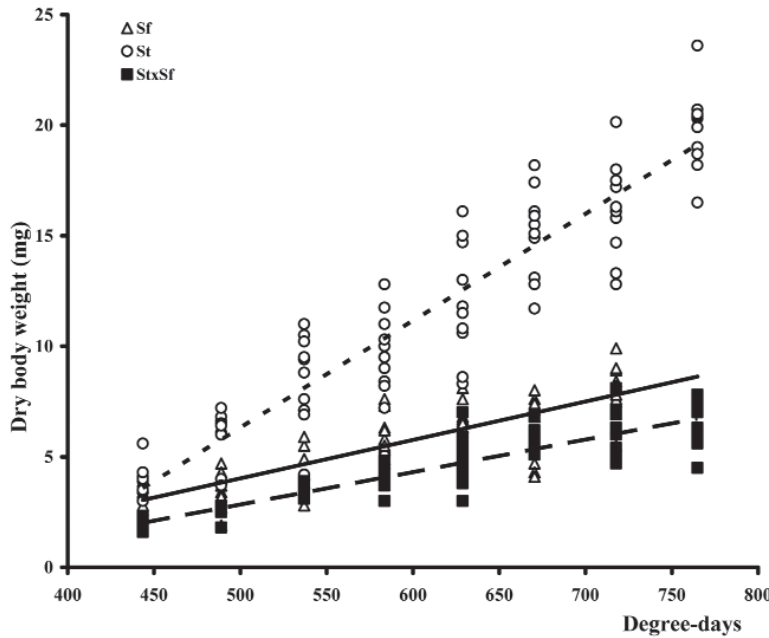
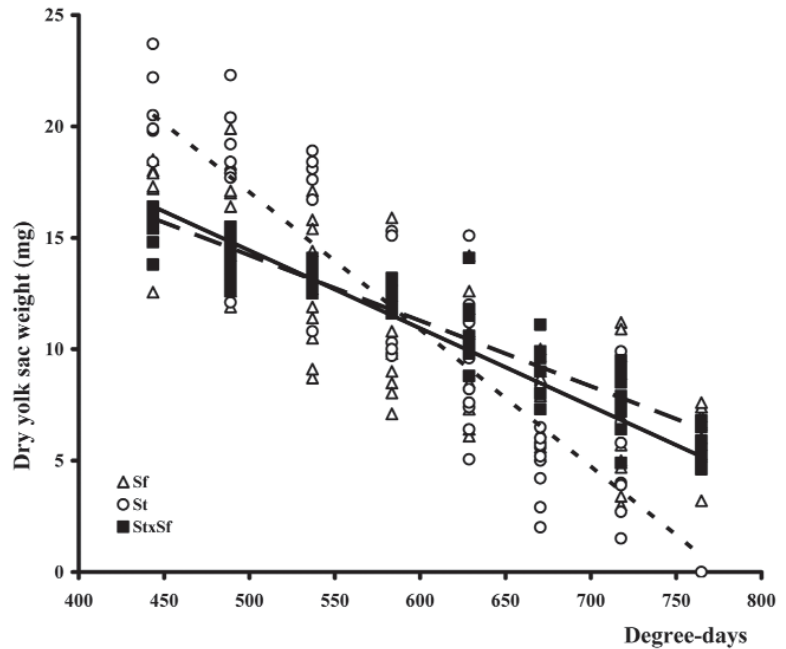


Fig 3. Relationship between dry body weights (y) and degree-days (x) [in brook trout (Sf; Δ , —): $y = -6.0905 + 0.0183 x$, $r^2 = 0.79$; Black Sea trout (St; $\circ$, ...): $y = -17.7872 + 1.4737 x$, $r^2 = 0.84$; and hybrid (StxSf; $\blacksquare$, - -): $y = -4.4839 + 0.4841 x$, $r^2 = 0.81$]. Among the groups, all constant (a) and slope (b) values differed significantly ($P < 0.001$)

Şekil 3. Toplam kuru ağırlık (y) ile gündece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ , —): $y = 13.1346 + 0.0598 x$, $r^2 = 0.33$; Karadeniz alabalığı (St; $\circ$, ...): $y = 28.8957 + 0.0854 x$, $r^2 = 0.39$; hibrid (StxSf; $\blacksquare$, - -): $y = 12.7089 + 0.0509 x$, $r^2 = 0.57$]. Tüm gruplarda sabit değer (a) ve eğimler (b) birbirinden istatistiksel olarak farklı ($P < 0.001$) bulunmuştur

Fig 4. Relationship between dry yolk sac weights (y) and degree-days (x) [in brook trout (Sf; Δ , —): $y = 31.8916 - 0.0349 x$, $r^2 = 0.71$; Black Sea trout (St; $\circ$, ...): $y = 47.8207 - 0.0616 x$, $r^2 = 0.87$; and hybrid (StxSf; $\blacksquare$, - -): $y = 28.9151 - 0.0294 x$, $r^2 = 0.90$]. Constant (a) and slope (b) values of St differed significantly than the others ($P < 0.001$)

Şekil 4. Kuru besin kesesi ağırlığı (y) ile gündece (x) arasındaki ilişki [kaynak alabalığı (Sf; Δ , —): $y = 13.1346 + 0.0598 x$, $r^2 = 0.33$; Karadeniz alabalığı (St; $\circ$, ...): $y = 28.8957 + 0.0854 x$, $r^2 = 0.39$; hibrid (StxSf; $\blacksquare$, - -): $y = 12.7089 + 0.0509 x$, $r^2 = 0.57$]. Karadeniz alabalığına ait sabit değer (a) ve eğim (b) diğer gruplardan istatistiksel olarak farklı ($P < 0.001$) bulunmuştur.



differences, excluding length slope.

Length and weight increasing and growth rates, daily yolk sac consumption and yolk sac efficiency values were calculated as 0.21 mm/day, 0.63 mg/day, 0.30 mg/day, and 0.61 for brook trout; 0.19 mm/day, 0.87 mg/day, 0.60 mg/day, and 0.76 for Black Sea trout, and 0.20 mm/day, 0.45 mg/day, 0.28 mg/day, and 0.46 for hybrid, respectively.

DISCUSSION

Durations of incubation periods differed among the three cross-types, and Black Sea trout has shorter period than the others. Egg survival rate of hybrid was low as expected¹³ and survival rates of brook trout³ and Black Sea trout² eggs and larvae were normal. Although Black Sea trout and hybrid have the same egg size, the

variation observed in net body weight at hatching was significantly related to paternal and maternal species ¹⁰, Black sea trout showed larger larvae ($P<0.01$).

Dry yolk weight of brook trout, Black Sea trout, and hybrid larvae at hatching were calculated as 16.43 ± 0.59 mg, 20.83 ± 2.00 mg and 15.56 ± 0.73 , respectively in the present study. Brook trout and hybrid values are higher than the value of 12.2 mg reported by Dumas et al.¹⁰, whereas it is smaller than that presented by Hodson and Blunt ⁹ for rainbow trout which was estimated as 31 mg from figures, and Black Sea trout value is smaller than that presented by Peterson and Martin-Robichoud ⁸ for Atlantic salmon (27.8 mg). These differences in yolk size within and between species might arise from egg and larva size, incubation temperature and thus period, and nutrition and husbandry of brood fish. There are contrasting views on relation between size of larva and amount of yolk at hatching. For example, as cited by Dumas et al. (1995), Rana (1990) observed an inverse relationship between the size of larva and the yolk. In contrast, Dumas et al.¹⁰ found that hybrids of Arctic charr (female) and brook trout (male) had higher net body weights at hatching than pure Arctic charr larva, but they both had the same amount of yolk. The authors concluded that the amount of yolk hatching is mainly determined by the maternal species and larvae of brook trout females had more yolk reserves than larvae from Arctic charr females.

The observed value of YCE on a dry weight basis approached 0.7, although the theoretical value was 0.82. Blaxter (cited Hodson and Blunt ⁹) reviewed estimates of YCE for several species and the range was 0.4-0.8. The values of YCE for *Salmo* species were from 0.41 to 0.70 at 10°C. Dumas et al.¹⁰ and Başçınar et al.⁵ reported that YCE values were 0.65 at 8-13°C, and 0.50 for brook trout at 4.5-13°C, respectively, and Hansen ⁶ reported values ranging from 0.46 to 0.68 at 7.0-8.5°C for *Salmo trutta*. All these values can be compared well with the present findings. A high value of YCE for Black sea trout suggests that yolk sac consumption results the earlier than the others (Fig. 4), moreover brook trout and hybrid larvae start free swimming at about half yolk sac consumption. Regression slope (b values) differed significantly, and it supported that yolk consumption rate of hybrid was affected from maternal species (brook trout).

This study was undertaken to evaluate hatching performance and early development (from hatching to free swimming stage) of brook trout, Black Sea trout and their hybrid larvae and has provided data on the growth rate during the yolk absorption, yolk conversion efficiency and dry weight. These can be used both for

further comparative studies and developing efficient hatchery management programs.

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Oral Vaccination Against Lactococcosis in Rainbow Trout (*Oncorhynchus mykiss*) Using Sodium Alginate and Poly (lactide-co-glycolide) Carrier ^[1]

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Summary

As an alternative immunization procedure against lactococcosis in rainbow trout, encapsulated antigens immobilized in sodium alginate (SA) and poly lactide-co-glycolide (PLGA) polymers were administered as a feed additive. Positive controls were intraperitoneally (IP) administered with aqueous-based bacterin (vaccine) while negative controls were not vaccinated. Positive control groups were immunized intraperitoneally by using the aqueous-based bacterin. Relative percentage survival (RPS) values of groups immunized orally with SA and (PLGA) encapsulated vaccines were not significantly different and was 53.48% and 62.79% respectively (on the 30th day). No statistically significant difference was determined between the SA and (PLGA) vaccine groups and relative percentage survival (RPS) values of the two groups determined to be 53.48% and 62.79%, respectively. To determine the effect of the booster immunization by using encapsulated immobilized vaccines, booster immunization was performed on the 61st day after oral administration of the same vaccines and RPS value was more than 60% on the 90th 120th days. After an aqueous-based vaccine followed by an immobilized vaccine for booster, the RPS value has increased over more than 80% indicating that booster application has increased the protection of rainbow trout against lactococcosis. It can be suggested that both SA and PLGA oral vaccines can be effectively used in rainbow trout against lactococcosis and there was no significant differences between the protection levels, however, since the preparation costs of SA oral vaccines are relatively lower compared to PLGA ones its usage for vaccination appears more appropriate.

Keywords: *Lactococcus garvieae*, Rainbow trout, Oral immunization, Sodium alginate, Poly (lactide-co-glycolide)

Taşıyıcı olarak poli (laktid-ko-glikolid) ve Sodium Alginate Kullanılarak Gökkuşığı Alabalıkları (*Oncorhynchus mykiss*)'nın *Lactococcus garvieae*'ye Karşı Oral İmmünizasyonu

Özet

Sodium aljinat (SA) ve poli laktid-ko-glikolid (PLGA) polimerleriyle immobilize edilmiş antijenler, gökkuşığı alabalıklarında lactococcosise karşı alternatif bir immunizasyon yöntemi olarak kullanıldı. Su bazlı bakterinin periton içi enjeksiyonla verildiği grup pozitif kontrol olarak, aşı uygulanmayan grup ise negatif kontrol olarak belirlendi. SA ve PLGA ile immobilize edilmiş oral aşılarla immunize edilen grupların korunma oranları arasında önemli bir fark bulunmadı ve RPS (30. gün) değerleri sırasıyla % 53.48 ile % 62.79 olarak tespit edildi. Balıklarda immobilize edilmiş oral aşılarla ilk aşılamadan 61 gün sonra booster yapıldığında ise RPS değerlerinin her iki grupta da 90 ve 120. günlerde % 60'ın üzerinde olduğu belirlendi. İlk olarak IP enjeksiyonla aşılanan balıklara daha sonra immobilize edilmiş oral aşılarla (61. gün) booster yapıldığında RPS değerlerinin %80'in üzerinde çıktığı ve gökkuşığı alabalıklarında laktokokkozise karşı korunma süresini artırdığı tespit edildi. Tüm epruvasyon günlerinde SA ve PLGA oral aşı gruplarının koruyuculuğu arasında istatiki bir farklılık olmaması, SA oral aşıların PLGA'ya göre çok daha az maliyetle hazırlanabilmesi nedeniyle SA oral aşıların gökkuşığı alabalıklarında laktokokkozise karşı kullanımında tercih edilebileceği görüldü.

Anahtar sözcükler: *Lactococcus garvieae*, Gökkuşığı alabalığı, Oral immunizasyon, Sodyum aljinat, Poli (laktid-ko-glikolit)



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INTRODUCTION

Lactococcus garvieae is a facultatively anaerobic, non-motile, non-spore forming, Gram positive ovoid coccus occurring in pairs and short chains and producing α -haemolysis on blood agar (BA). It is the etiological agent of lactococcosis, an emergent disease which affects many fish species and causes important economical losses both in marine and freshwater aquaculture when water temperature increases over 16°C in summer months¹. The first outbreak of lactococcosis in rainbow trout from Spanish fish farms has occurred in 1988. The spread of *L. garvieae* throughout Mediterranean Europe has been rapid; *L. garvieae* infections of trout were recorded in Spain in 1991, the same pathogen was detected in Italy. The pathogen and accordingly the disease have stated to spread rapidly throughout the southern part of the European continent, including countries such as Portugal as well as the Balkans¹. In Turkey, *L. garvieae* has been firstly isolated in 2001 at an outbreak occurred in rainbow trout farms^{2,3}. Since then, such infections have been reoccurred, especially during the warm summer months. Therefore, *L. garvieae* is now considered one of the most important pathogens at in the rainbow trout industry in Turkey^{2,3}. The existence of two serological groups associated with the presence of capsular material has been demonstrated for *L. garvieae*. In addition, capsulated strains are more virulent to rainbow trout than non-capsulated strains⁴.

Ravelo et al.⁵, have reported that *L. garvieae* were separated into three genetic groups, composed of the Spanish, Portugues, English and Turkish strains (Group A), the Italian and French strains (Group B) and the Japanese strains (Group C). Altun et al.³ have determined that Turkish strains are 99-100% identical to Spanish and English strains in the point of view sequence analysis of 16s rDNA of *L. garvieae*.

Mortality of lactococcosis has stated to be more than 50% in rainbow trout in summer season and the treatment of the disease is not successful with chemotherapeutics because of development of the resistance and recurrent infection. Therefore, development of effective vaccines is needed to prevent lactococcosis outbreaks⁶. Attempts to develop effective vaccines against lactococcosis have been carried out⁷⁻⁹. Fish vaccines can be administered by immersion, injection or oral route. Injection is evaluated as an effective immunization method in inducing immunity, but is stated to be not suitable for extensive aquaculture because of handling stress and high labor costs. For Gram-positive fish pathogens^{10,11}, good protection levels are could have been achieved when vaccines are had

been administered intraperitoneally (IP)⁷. However, the short duration of the immunity (2 to 3 months) constitutes the main inconvenience for the success of these vaccines, since this period is not enough to protect during warm seasons in which water temperature is higher than 16°C when and the majority of lactococcosis outbreaks occur⁸. To overcome such problems, several approaches including use of adjuvants in the vaccine formulation, the combination of selection of genetically resistant fish and vaccine strains, and use of booster immunization have been evaluated. Oral vaccines have been evaluated as good alternatives since there is no need to handling of the fish; it is not a stressful method and does not require extensive labor¹². Activation of mucosal immunity has stated to be appeared an important factor since many pathogens can enter into the body of the fish through mucosal surface³. However, oral vaccination has stated to have some drawbacks; i) the applied antigen is often destroyed due to protease activity present in the intestinal tract, (ii) oral tolerance can be evoked and (iii) the antigen does not necessarily enter the gut mucosa and consequently an immune response is not initiated¹³⁻¹⁵.

An oral vaccine should be in such form that the formulation may protect the antigen from inactivation and digestion during passage through the stomach and the anterior gut¹⁶. One option is acid-stable coating. Nevertheless, this method has stated to be relatively expensive. Piganelli et al.¹⁷ have described the coating of antigen microspheres (ECAMs) with Eudragit LD-30 copolymer for oral vaccine delivery. Vervarcke et al.¹⁸ have examined the effect of lag time of the coated pellets on uptake and immune response to inactivated *V. anguillarum*. Joosten et al.¹⁹ and Romalde et al.⁸ have successfully used the encapsulation of inactivated bacteria in alginate microparticles for vaccination. The aim of this study is to test determine the efficacy and protection level of different oral vaccine formulations against lactococcosis as well as their usefulness as primary or secondary immunization method materials. Also, duration of protection was investigated in order to propose an appropriate vaccination program to prevent *L. garvieae*.

MATERIAL and METHODS

Selection of Bacterial Strains for Vaccine Formulation

The vaccine was prepared from *L. garvieae* M1 strain. This strain has been previously isolated at a natural outbreak that occurred in a rainbow-trout farm which located in Fethiye - Mugla, Turkey⁴. The strain was selected among several lab strains on the basis of

previous studies in which we have determined its antigenic characteristics determined in a previous study ^{9,20}. The reference strain NCDO 2155 (ATCC-43921) was also used in that study to compare its property with the lab strain. The strains were inoculated on tripticase soy agar (TSA, Difco Laboratories, Detroit, MI) and incubated at 25°C for 24-48 h. Pure bacteria were transferred to triptic soy broth (Difco Laboratories, Detroit, MI). Then, pure culture stocks were stored at -80°C in triptic soy broth (TSB, Difco) including 15% glycerol.

Preparation of the Bacterin

The formalin killed bacterin was prepared as stated in the previous studies ^{9,20}. Bacterial cells were inactivated by adding formalin until final concentration of 0.7%. The solution suspension was incubated at 25°C for 3 h, and then at 4°C overnight. Thereafter Then, inactivated bacterial cells were washed three times with phosphate buffered saline (PBS pH 7.2) by centrifugation at 6000 rpm for 30 min at 4°C. The formalin killed vaccine was then resuspended in phosphate buffered saline (PBS) and optical density of final suspension was adjusted to OD₆₀₀ of 1.2 to make its cell concentration about 1x10¹⁰ cfu/ml.

Preparation of SA (Sodium alginate) Microbeads and PLGA poly (lactide-co-glycolide) Microspheres

The previously prepared bacterial suspension was mixed separately with SA (Sigma aldrich A2033, Darmstadt, Germany) and PLGA (Sigma-Aldrich P2191, Darmstadt, Germany) solutions at the concentration of (10¹⁰ cells/ml).

Sodium alginate microbeads were prepared by using orifice-ionic gelation method which was previously described by Gonzales-rodriguez et al. ²³. Sodium alginate solution in distilled water (4%-w/v) was prepared and mixed with the bacterial homogenate containing 10¹⁰ cells/ml. The resultant mixture was homogenized with Ultra Turrax T25 at 11.000 rpm. This suspension was added dropwise (30 drops/min) through a 18 G needle into the aqueous solution of CaCl₂ (0.3 %-w/v) stirred on a magnetic stirrer for 24 h for the crosslinking. The microbeads were then filtered (Whatman cellulose acetate filter) and washed with distilled water. Finally, the microbeads were left to dry at room temperature until they reach to a constant weight. The microbeads were kept at 4°C until use ²¹⁻²³.

PLGA microspheres containing bacterial strains were prepared by using spray drying method. Briefly, PLGA (50:50) was dissolved in dichloromethane resulting in the final solution of the polymer at 2% (w/v). This solution was added on the bacterial homogenate to prepare 10¹⁰

cells/ml, and the resulting suspension was homogenized by using Ultra Turrax T25 at 11000 rpm for 2 min. This homogeneous suspension was then sprayed through a 0.5 mm nozzle with the apparatus Büchi B-190 spray dryer (Büchi Labortechnik, Postfach, Switzerland). The flow rate was set as 1 ml/min at 4 atm pressure. Inlet/outlet temperatures of the method were set as 65°C and 35°C, respectively. The microcapsules were stored at +4°C until use ²⁴⁻²⁷.

Preparation of Fish Feed Including Vaccine Materials

A commercially available fish feed (Ecobio, feed No: 3, containing 45% protein, 20% lipids, Ekobio Feed, İzmir-Turkey) was wetted by adding distilled water and the feed was ground in a blade mill to obtain a paste. This paste was subsequently mixed with microparticles or cell suspension with pestle and mortar in order to obtain a homogeneous mixture. The final feed paste was given to fish at a rate of 1% of body weight per day. So, daily feed portion of each fish were included 1x10¹⁰ formalin-killed cells. Since the paste has proper plastic properties, it is extruded through a 20-ml syringe, dried during 24 h at room temperature and cut into pellets. In the case of bacterial suspension, the doses are the same for microparticles formulation.

Immunization Procedure

Juvenile rainbow trout obtained from a farm with no history of lactococcosis were maintained at concrete raceway cage of 2x2x1m for 4 weeks for acclimation. Water temperature was 18±1°C and dissolved oxygen amount was 7.5 mgL⁻¹. The experimental design included eight fish groups (50 fish per group having with 20 g initial average weight were used on each challenge application). Intraperitoneal immunization was performed by injection of 0.1 ml of the formalin-killed bacterin (1x10¹⁰ cfu/ml). For oral immunization, oral vaccine was included in the feeding material and fish were fed with the mixture over 7 day-period with a daily feeding rate of 1% of the body weight. Immunization groups were as [Table 1](#). The tests were done in duplicate.

Challenges were carried out on the 30, 60, 90 and 120 days after vaccination, fish from all groups were IP (injection of 0.1 ml) challenged with the homologous strain ([Table 1](#)). Mortalities were recorded daily over a 3-weeks period and all the dead fish were examined to confirm by the isolation of the inoculated strain from the internal organs. Protection was evaluated by determining the relative percent of survival (RPS) according to Amend ²⁸ in each group using the formula:

$$RPS = 1 - (\% \text{ mortality in vaccinated} / \% \text{ mortality in control}) \times 100$$

Table 1. Experimental groups used for the evaluation of vaccines against *Lactococcus garvieae* in rainbow trout

Tablo 1. Gökkuşluğu alabalıklarında *Lactococcus garvieae*'ye karşı hazırlanan aşılardan etkinliklerinin değerlendirilmesinde kullanılan deneme grupları

Fish Group	Number of Fish*	Challenge Dose (cfu/ml)
I. Challenge (30 Days)		
Group 1	50	1.2x10 ⁵
Group 2	50	1.2x10 ⁵
Group 3	50	1.2x10 ⁵
Group 4	50	1.2x10 ⁵
II. Challenge (60 Days)		
Group 1	50	1.5x10 ⁵
Group 2	50	1.5x10 ⁵
Group 3	50	1.5x10 ⁵
Group 4	50	1.5x10 ⁵
III. Challenge (90 Days)		
Group 1	50	2.3x10 ⁵
Group 4	50	2.3x10 ⁵
Group 5	50	2.3x10 ⁵
Group 6	50	2.3x10 ⁵
Group 7	50	2.3x10 ⁵
Group 8	50	2.3x10 ⁵
IV. Challenge (120 Days)		
Group 1	50	1.8x10 ⁵
Group 4	50	1.8x10 ⁵
Group 5	50	1.8x10 ⁵
Group 6	50	1.8x10 ⁵
Group 7	50	1.8x10 ⁵
Group 8	50	1.8x10 ⁵

Group 1: Intraperitoneally vaccinated with formalin inactivated bacteria on day 0 (positive control)

Group 2: Orally vaccinated with PLGA microspheres on day 0

Group 3: Orally vaccinated with SA microbeads on day 0

Group 4: Non-vaccinated group (negative control)

Group 5: Fish were vaccinated on the 0 day as Group 1 and then subjected to oral booster vaccination with PLGA microspheres on 61st day

Group 6: Fish were vaccinated on the 0 day as Group 1 and then subjected to oral booster vaccination with SA microbeads on 61st day

Group 7: Fish were vaccinated on the 0 day as Group 2 and then subjected to oral booster vaccination with PLGA microspheres on 61st day

Group 8: Fish were vaccinated on the 0 day as Group 3 and then subjected to oral booster vaccination with SA microbeads on 61st day

* Test were done duplicate and totally 2000 fish were used in the study. Arithmetic means of dead fish were used for RPS calculation.

Statistical Analysis

Results were analyzed by ANOVA and significances were determined by Duncan's test (SPSS 9.0 package for Windows).

RESULTS

The levels of protection were given in Table 2. The vaccine groups resulted in a greater level of protection, determined by a lower level of mortality compared with

that of the control group (Group 4). The protection remained high over the time as significant differences in mortality were observed between the control and the treatment groups on the 30th, 60th, 90th and 120th post-vaccination days. As seen in Table 2, "statistically" significant level of protection was achieved in rainbow trout within 30 days when the vaccine (bacterin) was administered via IP injection compared with non-immunized trout (P<0.05).

A significantly high level of protection with a RPS value of 95.34% was achieved in 20 g rainbow trout within 30 days when the bacterin was administered by IP injection (Table 2). Protections obtained with oral PLGA microspheres and SA microbeads were 62.79% and 53.48%, respectively on the 30th day of vaccination (Table 2).

On the basis of these results, a second experiment including booster vaccination by using PLGA microspheres was designed. The results obtained are shown in Table 2.

Table 2. Protective efficacy of the different vaccine formulations administered intraperitoneally (IP) or by the oral route against *L. garvieae* in rainbow trout

Tablo 2. Gökkuşluğu alabalıklarında *Lactococcus garvieae*'ye oral ve İP yolla uygulanan farklı aşı formülasyonlarının koruma etkinliği

Fish Group	Challenge Dose (cfu/ml)	Number of Fish	Mortality (%)	RPS
I. Challenge (30 Days)				
Group 1	1.2x10 ⁵	50	4 ^c	95.34
Group 2	1.2x10 ⁵	50	32 ^b	62.79
Group 3	1.2x10 ⁵	50	40 ^b	53.48
Group 4	1.2x10 ⁵	50	86 ^a	-
II. Challenge (60 Days)				
Group 1	1.5x10 ⁵	50	16 ^c	82.22
Group 2	1.5x10 ⁵	50	50 ^b	44.44
Group 3	1.5x10 ⁵	50	56 ^b	37.77
Group 4	1.5x10 ⁵	50	90 ^a	-
III. Challenge (90 Days)				
Group 1	2.3x10 ⁵	50	40 ^b	52.38
Group 4	2.3x10 ⁵	50	84 ^a	-
Group 5	2.3x10 ⁵	50	8 ^e	90.47
Group 6	2.3x10 ⁵	50	12 ^{de}	85.71
Group 7	2.3x10 ⁵	50	24 ^{cd}	71.42
Group 8	2.3x10 ⁵	50	28 ^{bc}	66.66
IV. Challenge (120 Days)				
Group 1	1.8x10 ⁵	50	50 ^c	42.85
Group 4	1.8x10 ⁵	50	84 ^a	-
Group 5	1.8x10 ⁵	50	14 ^d	83.33
Group 6	1.8x10 ⁵	50	16 ^d	80.95
Group 7	1.8x10 ⁵	50	30 ^{bc}	64.28
Group 8	1.8x10 ⁵	50	32 ^b	61.90

Significant difference (P<0.05) letters shows differences between the groups

RPS value obtained with PLGA microspheres in the first challenge was 62.79% and then decreased to 44.44% in the second challenge. Protection in IP vaccinated animals was noted as 95.34% on the 30th day while it decreased to 82.22% on the 60th day. Thereafter, this value declined until 42.85% in the fourth challenge that was performed on the 120th day post-vaccination. However, increase in RPS was observed in a fourth challenge group that was vaccinated firstly by IP administration of the bacterin and revaccinated on the 61st day with the oral encapsulated vaccine, reaching values of 83.33% (Table 2).

Significant level of protection was achieved on 90th and 120th days with fish immunized intraperitoneally followed by booster vaccination on 61st day with oral PLGA microspheres (90.47% and 83.33% respectively) and SA microbeads (85.71% and 80.95% respectively) as shown in Table 2.

DISCUSSION

It has been observed that good protection levels are only achieved when vaccines are intraperitoneally (IP) administered ⁷. Immersion procedures produce a lesser degree of protection against Gram-positive bacterial infections in salmonids ^{7,8}. Romalde et al. ⁸ have reported the efficacy of vaccination in rainbow trout against lactococcosis ⁸. They applied a combined strategy consisting of a primary immunization with an aqueous bacterin followed by a booster immunization (3 months later) with an oral alginate-encapsulated vaccine ⁸.

In fact, the way of delivery may reflect the efficiency of transferring the immunogenic constituents of the vaccine to the important recognition and effector components of the fish immune system. As in the case of other Gram-positive fish pathogens such as *S. iniae* or *S. parauberis* ^{7,8,29} good protection results were also achieved against *L. garvieae* infections by the IP administration of vaccines but not by immersion procedures ⁷. However, the formulations assayed until now to prevent fish streptococcosis caused by *S. iniae* or *L. garvieae* rendered protection for short periods of time (approximately 3-4 months) ⁸. Similar results were obtained in the present work for the *L. garvieae* bacterin, since 120 days after vaccination, the protection level dropped to RPS values of 42.85%.

Oral vaccination is less stressing for animals and requires less time for application. Another advantage of this technique is that regardless the size of the fish mass vaccination of a pool is possible ^{8,30}. Since the first contact between animal and pathogens in oral immunization occurs through mucosal immune system oral vaccination

becomes more promising ³¹. It has been reported that oral vaccination may induce the appearance of antigen-specific antibodies in skin mucus, bile or intestine in fish species ³². On the other hand, oral vaccination has some disadvantages. High acidity of fish stomach inactivates antigens and prevents absorption in the lower gut ³³. To overcome this problem efficient delivery methods should be improved.

In our study, 95.34% RPS has been obtained 30 days after IP administration of the prepared bacterin. Eldar et al. ¹⁰ have obtained 90.0% RPS against *Streptococcus iniae*; Romalde et al. ¹¹ obtained 83.3% RPS against *L. garvieae*, and Ravelo et al. ³³ obtained 82.6% RPS against *L. garvieae* after IP immunization of fish. This complies with other research results.

There are only one data available regarding the usefulness of oral vaccines in the prevention of fish streptococcosis, regardless of its causative agent. Oral vaccination with encapsulated and non-encapsulated antigens was preliminary evaluated as alternative immunization procedures against trout lactococcosis ⁸. Authors reported that several microparticle systems for protection of the antigen and efficient oral vaccine delivery were tested in trout in comparison to a vaccine produced by adding directly inactivated bacterial cells to the fish food. Only the formulation including bacteria encapsulated in alginate-acetone microspheres rendered significant levels of protection (RPS of 50%), which indicates that these microparticles seem to avoid the antigen degradation, due to low pH and proteases, in the anterior part of the digestive tract. Similar results were obtained in oral vaccination experiments against *Vibrio anguillarum* ¹⁹.

Romalde et al. ⁸ reported the efficacy of oral immunization using alginate-microparticles for booster vaccination in rainbow trout to prevent lactococcosis. The fish were initially IP vaccinated with the aqueous bacterin and they received an oral booster vaccine 90 days later. On the 30th day of revaccination protection reached to 87% RPS.

Although protection obtained in the present study with the alginate microparticles provides good booster vaccination, this vaccination can not be used as primary immunization method According to the European Pharmacopoeia ³⁴.

Similar results were obtained in the present work for the *L. garvieae* bacterin, revaccination of fish 61 days after a first IP immunization resulted in an increase in the protection, RPS levels rising from 42.85 to 83.33%. Although RPS value was under 63% with single oral

administration of either encapsulated SA or PLGA vaccine, application of a booster vaccination increased the protection level until 60% in both systems. Fish vaccinated IP exhibited an RPS value of 82.22% on the 60th day. Thereafter, this value declined gradually over the time and attained to 52.38% and 41.46% on the 90th and 120th days respectively. Application of initial IP vaccination together with SA and PLGA oral vaccines booster resulted with increased protection level. In that case, while protection with SA booster vaccination were 85.71% and 80.95% on the 90th and 120th days respectively, the RPS obtained with PLGA booster for the same measuring days were 90.47% and 83.33%. Statistically significant difference ($P < 0.05$) were obtained when oral application of SA microbead and PLGA microsphere vaccines were used either in combination (oral SA + oral SA; oral PLGA + oral PLGA) or after IP application. These results are in good agreement with those of reported by Romalde et al.⁸ and Yazıcı³⁵.

It can be concluded from the present research that booster application of oral SA and PLGA vaccines provided efficient and sufficient level of immunization against *L. garvieae* and that the protection obtained by rainbow trout lasted up to 120 days.

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The Effect of Vitamin E on Black Sea Trout (*Salmo labrax*) Broodstock Performance

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Summary

This study was conducted to examine the effects of feeding diets, different dose of vitamin E contains, on female and male Black Sea trout's (*Salmo labrax*) reproductive performance. Broodfish were fed with dry feed contained 100 (control), 250, 500, 1000 mg kg⁻¹ vitamin E. Milt volume, spermatozoid number, fecundity and egg size were determined. Milt volume and sperm number were positively affected at a dose of 250 and 500 mg kg⁻¹ vitamin E in the diet. A similar positive trend was also observed at the dose of 1000 mg kg⁻¹. Egg size is not affected by dietary vitamin E dose, but there are positive relationship between total fecundity and dietary vitamin E dose especially at the first stripping (P<0.05).

Keywords: Milt, α -tocopherol, Broodstock, Fecundity, Vitamin

E Vitamininin Karadeniz Alabalığı (*Salmo labrax*) Damızlık Stoklarında Üreme Performansına Etkisi

Özet

Bu çalışma Karadeniz Alabalığı'nda (*Salmo labrax*) 6 hafta süreyle, yemlerine farklı dozlarda ilave edilen E vitamini ile beslemenin üremeye etkisini ortaya koymak amacıyla yapılmıştır. Anaç stoktaki balıklar 1 kg yemde 100 mg (kontrol grubu), 250 mg, 500 mg ve 1000 mg olacak şekilde E vitamini ilave edilen yemle beslenmişlerdir. Sperm hacmi, spermatozoa sayısı, fekondite ve yumurta çapı verileri incelenmiştir. Sperm hacmi ve spermatozoa sayısı 250 ve 500 mg E vitamini içeren yemlerle beslenen balıklarda pozitif yönde etkilenmiştir. Benzer ilişki 1000 mg kg⁻¹ dozda da görülmüştür. Yumurta büyüklüğü yemdeki E vitamininden etkilenmezken, özellikle ilk sağırmda total fekondite ve vitamin E miktarı arasında pozitif ilişki vardır (P<0.05).

Anahtar sözcükler: Sperm, α -tokoferol, Anaç stok, Fekondite, Vitamin

INTRODUCTION

Vitamin E is lipid soluble antioxidant, and it is also important for fish reproduction. The requirement for vitamin E as an essential dietary component in fish has long been recognized and minimum requirements for any fish species have already been established. The recommended dietary allowance for vitamin E is 15 mg day⁻¹ with an adult upper limit of 1000 mg/day¹. On the other hand, the Committee on Animal Nutrition of American National Research Council² suggested a general vitamin E supplement in the diet around 50 mg kg⁻¹ fish. High concentrations of vitamin E content can result in yolk sac hypertrophy and decreased survival caused by

oxidative stress. Unlike vitamin A and D, vitamin E is essentially nontoxic³ and it was first shown to be involved in prevention of sterility and fetal resorption in rats. It may likewise be involved in embryo membrane permeability and hatchability of fish eggs⁴. There are no significant apparent benefits by using higher levels of vitamin E on growth and diet utilization⁵.

Vitamins are organic substances that are necessary for health, growth, maintenance and also spermatogenesis and oogenesis in animals. Especially vitamin E is essential for fertility and reproduction in fish and fish

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cannot synthesize vitamin E, so the maternal dietary content of each prior to oogenesis is an important determinant of reproductive fitness².

Broodstock diet has been considered as one of the factors affecting fecundity, egg, larval and sperm quality in fish^{6,7}. The concentration of sperm in the seminal fluid has been traditionally used for the assessment of sperm quality in fish⁸. Low concentration of anti-oxidants such as vitamin E and vitamin C reduce sperm quality, the rate of egg fertility and affects gamete and fry quality⁹. During the last phase of gonad development, vitamin E is transferred from the muscular tissue to the reproductive apparatus. This process happens during physiological fasting before the final sexual maturation and for this reason and the amount of vitamin E accumulated in the previous months is very important. In some experiments on Salmonid diets supplemented with 60 or 270 mg of vitamin E demonstrated that high levels of fatty acids (almost 60 g kg⁻¹) coupled with high concentration of vitamin E had the best reproductive performances in terms of fry survival rate. Furthermore, the trials showed that the requirement of this vitamin is also related to the level of fatty acids in the diet¹⁰. According to Wen¹, the recommended dietary allowance for vitamin E is 15 mg day⁻¹ with an adult upper limit of 1000 mg/day.

There is no data available regarding the effects of Vitamin E on semen and egg quality in Black Sea trout (*Salmo labrax*). The aim of the study affects of six weeks (42 days) treatment of broodfish with vitamin E added feed on sperm and egg quality in Black Sea trout.

MATERIAL and METHODS

Fish

Two hundred and fifty, four years old, Black Sea Trout (*Salmo labrax*), born and farmed in the experimental commercial farm (in Findikli, Rize, Turkey) were used in the experiment. The fish were randomly divided into four groups. Each group consisted of twenty female (687.5±43.6 g 35.6±0.59 cm) and ten male (555±21.5 g 34.3±0.35 cm) broodfish, and stocked in 8 x 2000 L ponds. Held under a constant natural water flow (8±2°C; 0.5 l sec⁻¹; total hardness 20 mg l⁻¹) and photoperiod (8 h light and 16 h dark) were maintained.

Commercial trout feed was prepared as mentioned from Canyurt and Akhan¹¹. Feed was weighed and vitamin E (purity: 50% D-α-tocopherol acetate; BASF Fine Chemicals, Ludwigshafen, Germany) was added and thoroughly mixed at ratios of 150 mg, 400 mg and 900 mg kg feed⁻¹ mixed. Sunflower oil was then sprayed on

those feeds in order to facilitate absorption of fat soluble vitamin E. Fish were fed *ad libitum* with 250 mg (E250), 500 mg (E500), 1000 mg (E1000) vitamin-E supplemented feeds and no vitamin-added groups (Named as E100 as a control) commercial pellets (6 mm) two times a day for six weeks (from 26 October 2008 - 4 December 2008; 42 days).

Milt and Egg Sampling

After 6 weeks vitamin treatment, a first batch of fish ripened (first spawn) and the others ten days later (second spawn). Milt was taken from all males first and second spawning without anesthetic manipulation, but females were anesthesia with 40 mg l⁻¹ benzocaine. The stripped milt was kept in dry glass vials. Freshly stripped milt was stored on ice in 15 ml glass containers until used. Any samples that were obviously contaminated (e.g. urine, faces) were discarded. Eggs taken from females were fertilized with milt from males of the same group. Eggs data were examined after water hardening.

Sperm concentration was determined using a Thoma hemocytometer under the microscope (40x=400 magnification). Immediately after collection of semen, the volume was determined using a graded level collection tube. The mean of the three counts was calculated for each of the two dilutions, and then the mean of these two values was used to calculate the actual sperm density. The mean number of eggs per spawning was evaluated.

Statistic Analysis

All data were subjected to one-way analysis of variance¹². The significance of differences between groups were determined using Duncan's multiple range test (P<0.05) using SPSS for WINDOWS (Version 13.0). Values are expressed as means ±SE.

RESULTS

Mean body weights (initial and final) of the broodfish showed no significant differences (P>0.05) among groups (E100, E250, E500, E1000); initial (pre-spawning) weights of female: 683±40.1 g and male: 548±15.6 g were determined. Post stripped weight of female was determined as 587±36.7 g. The dietary treatments did not influence the final body weight among the broodfish groups statistically.

Milt Volume and Sperm Number

It is observed that high vitamin E level increased milt volume. In the second milt stripping, milt volumes were diminishing. As milt volume of control group (E100) was

not different from first and second stripping, treatment groups' milt volumes were higher than control. Sperm concentration was high at the first stripping but very diminish was observed second stripping (Fig. 1, Fig. 2).

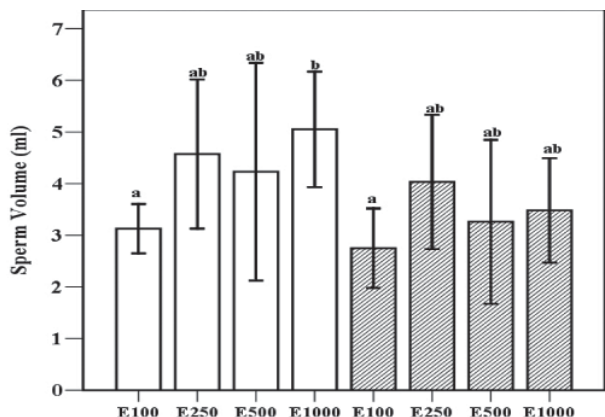


Fig 1. Sperm volume first and second stripping (mean ml per group, n=10 per group). Means with different superscript differs (P<0.05). Error bars: $\pm$ SE. Grey bars represent second spawning

Şekil 1. Birinci ve ikinci sağımdaki sperm hacmi (ml, her grup için n=10). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: $\pm$ SE. Gri sütun ikinci yumurtlamayı göstermektedir

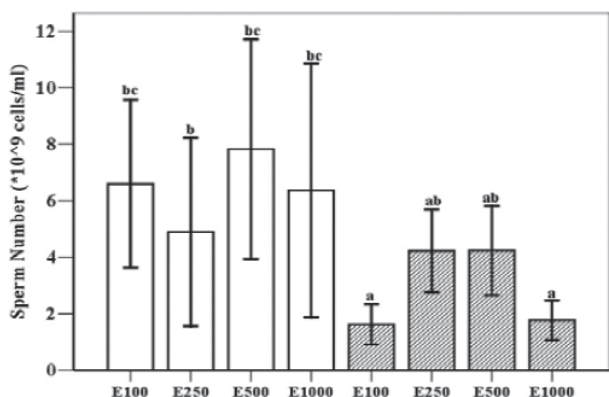


Fig 2. Sperm concentration (number $\times 10^9$ ml⁻¹) in groups (n=10 per group). Means with different superscript differs (P<0.05). Error bars: $\pm$ SE. Grey bars represent second spawning

Şekil 2. Sperm yoğunluğu ortalaması (adet*10⁹ ml⁻¹, n=10). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: $\pm$ SE. Gri sütun ikinci yumurtlamayı göstermektedir

Fecundity

No abnormalities were observed between the groups in egg development. The total and relative fecundity per kg body weight was determined. And egg number was determined gravimetrically (Fig. 3 and Fig. 4).

Each group (E100, E250, E500, E1000) first and second stripping total fecundities were observed as 943 $\pm$ 69, 1165 $\pm$ 140, 1227 $\pm$ 154, 929 $\pm$ 104; 1036 $\pm$ 138, 1002 $\pm$ 104, 951 $\pm$ 68, 1115 $\pm$ 193 respectively. Relative fecundities were determined as 1909 $\pm$ 212, 2110 $\pm$ 232, 1657 $\pm$ 198,

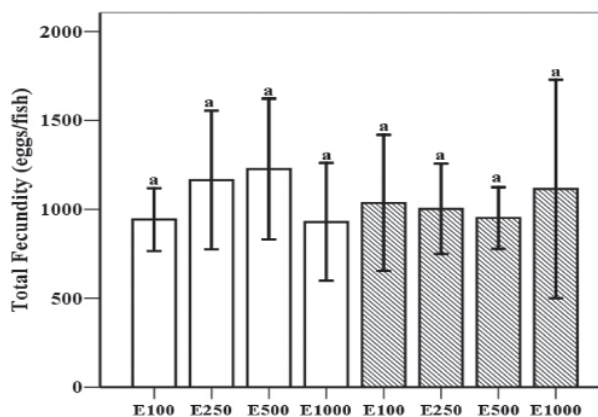


Fig 3. Total Fecundity first (Week 1) and second (Week 2) stripping (eggs per female) in groups. Means with different superscript differs (P<0.05). Error bars: $\pm$ SE. Grey bars represent second spawning

Şekil 3. İlk (birinci hafta) ve ikinci sağımda toplam fekundite (dişi başına den yumurta sayısı). Hata çubukları: $\pm$ SE. Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: $\pm$ SE. Gri sütun ikinci yumurtlamayı göstermektedir

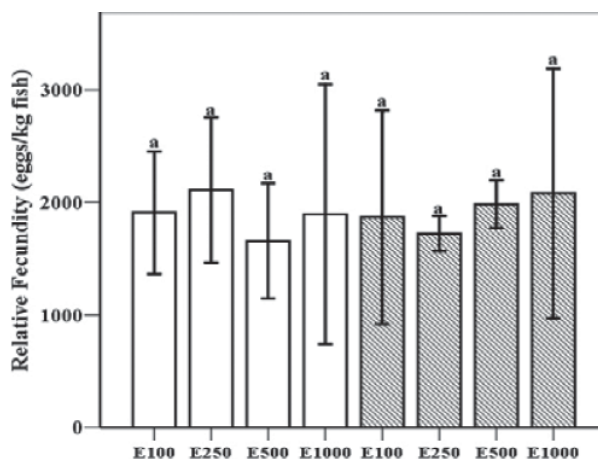


Fig 4. Relative Fecundity (eggs per kg post stripped female weight) between groups. Means with different superscript differs (P<0.05). Error bars: $\pm$ SE. Grey bars represent second spawning

Şekil 4. Relatif fekundite (adet yumurta/sağımlı balık ağırlığı). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: $\pm$ SE. Gri sütun ikinci yumurtlamayı göstermektedir

1893 $\pm$ 362 and 1867 $\pm$ 341, 1723 $\pm$ 63, 1983 $\pm$ 82, 2081 $\pm$ 349 egg kg⁻¹ broodfish respectively

Egg Size

Egg size was determined as egg diameter which was determined 30 eggs length in a Von Bayer trough. There were no differences of the egg size among the groups and egg sizes in the first spawning are presented in Fig. 5. First and second stripping time, egg size was observed as 5.4 $\pm$ 0.1, 5.3 $\pm$ 0.16, 5.3 $\pm$ 0.17, 5.1 $\pm$ 0.05; 5.2 $\pm$ 0.07, 5.3 $\pm$ 0.03, 5.2 $\pm$ 0.15, 5.2 $\pm$ 0.09 respectively.

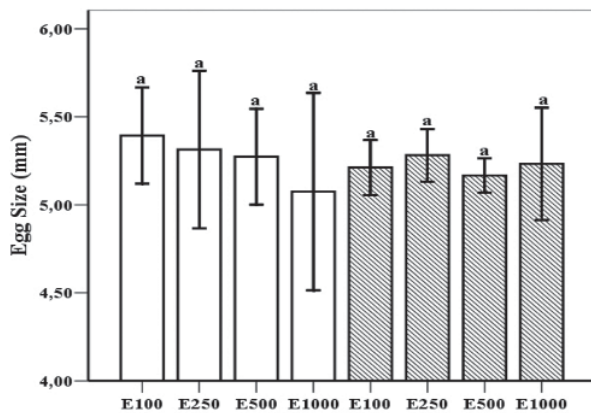


Fig 5. Egg size (mm as diameter) in groups (n=10 per group). Means with different superscript differs (P<0.05). Error bars: $\pm$ SE. Grey bars represent second spawning

Şekil 5. Grupların yumurta büyüklüğü (mm olarak çap). Farklı gruplar farklı harflendirilmiştir (P<0.05). Hata çubukları: $\pm$ SE. Gri sütun ikinci yumurtlamayı göstermektedir

DISCUSSION

The requirement for vitamin E as an essential dietary component in fish has long been recognized, and minimum requirements for many fish species have already been established. For instance, salmonid fish require for normal growth 30-60 mg of vitamin E per kg of diet ¹². But this dose adequate or not for broodstock nutrition is dispute. Commercial feed producers maintained that there is 100-200 mg kg⁻¹ vitamin E in their feed in Turkey. But vitamins must be added in the diet freshly. So, the vitamin dosage in the diet diminished slowly dependent on stock conditions.

There was no significant benefit of using the vitamin E in a diet about weight gain. This result confirmed the findings of Boggio et al.¹³ and Kiron et al.⁵, that no difference occurred in the weight gain of fish fed diets containing either lower (100 mg kg⁻¹) or higher level of vitamin E (1000 mg kg⁻¹ diet). Naziroglu et al.¹⁴ mentioned that vitamin E especially alpha tocopherol form, have very effective role on immune system response, and it is one the few nutrients for which supplementation with higher than recommended levels enhance certain aspects of immune function in fish.

Relative and total fecundity and egg size were not affected vitamin E content either positively nor negatively. There were no differences between groups (P<0.05). Our finding was similar to Tabak et al.¹⁵, which found that relative fecundity as 1747 $\pm$ 70 (1162-2494) and egg size 5.48 $\pm$ 1.1 in Black Sea trout.

Groups E250, E500 and E1000 sperm volumes and sperm numbers differ from E100 statistically (P<0.05),

especially 250 mg kg⁻¹ (E250) and 500 mg kg⁻¹ (E500) vitamin E affected positively. Total amount of spermatozoa and the morphology of the sperm were significantly lowered in cocks with increasing amounts of supplemented vitamin E. As mentioned Canyurt and Akhan ¹¹, our research results did not show any negative effect of vitamin E on male rainbow trout sperm volume. These results showed that dietary vitamin E is useful for improving the sperm quality of male rainbow trout.

In conclusion, our research results did not show any negative effect of additional vitamin E in the diet on neither male nor female sea trout. Supplemented dietary vitamin E up to 250 mg kg⁻¹ can be useful for improving the sperm and egg quality of Black Sea trout.

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***Yersinia ruckeri* Suşlarının Fenotipik ve Serolojik Özelliklerinin İncelenmesi**

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Özet

Araştırmada, ülkemizin farklı coğrafik bölgelerinde bulunan gökkuşağı alabalığı işletmelerinden izole edilmiş olan 19 adet *Yersinia ruckeri* suşunun 2 adet referans suşla (serotip 1 ve serotip 2) karşılaştırmalı olarak fenotipik ve serotipik özellikleri incelenmiştir. *Yersinia ruckeri* suşlarının fenotipik özelliklerinin belirlenmesinde geleneksel mikrobiyolojik ve API 20 E testleri kullanılmıştır. Geleneksel mikrobiyolojik ve API 20 E testlerinde bakterinin oldukça homojen (API 20 E testinde suşlar arasında jelatin hidrolizi, voges proskauer reaksiyonu, sitrat kullanımı testleri yönünden farklılıklar olmakla birlikte) bir yapı gösterdiği belirlenmiştir. Shotts-Waltman medium (SWM) ve Riboz Ornitin Dezoksikolat (ROD) selektif besiyerlerinde *Y. ruckeri* suşlarının spesifik koloniler oluşturduğu saptanmıştır. Serotip 1 ve serotip 2 immünserumlar kullanılarak yapılan mikroaglutinasyon testinde ise ülkemizden izole edilmiş olan 19 suştan 18'inin serotip 1, geri kalan 1 suşın ise serotip 2 özellikte olduğu görülmüştür. Bu sonuçlar, ülkemizde Serotip 1 özellikteki *Yersinia ruckeri* suşlarının yaygın olduğunu göstermiştir.

Anahtar sözcükler: *Oncorhynchus mykiss*, *Yersinia ruckeri*, API 20 E, Mikroaglutinasyon

Investigation of Phenotypical and Serological Properties of *Yersinia ruckeri* Strains

Summary

In the present study, phenotypical and serological properties of 19 *Yersinia ruckeri* isolates that were isolated from different fish cultivating plants located at different geographical area of Turkey as well as two references serotypes (serotype 1 and serotype 2) have been compared. Basic microbiological techniques and API 20 E tests that have been employed for phenotypical characterization provided evidence that isolates have relatively similar structure (although gelatine hydrolysis, Voges-Proskauer and citrate utilisation tests as performed by API 20 E exhibited some differences). Specific colonies of *Y. ruckeri* were observed on the plates of Shotts-Waltman medium (SWM) and Ribose Ornithine Deoxycholate (ROD) medium. Microagglutination tests that were performed by using Serotype 1 and Serotype 2 revealed that 18 out of 19 isolates were grouped as Serotype 1 while only one isolate was serotype 2. Results of the present study have shown that *Yersinia ruckeri* Serotype 1 are the most commonly found serotype in our country.

Keywords: *Oncorhynchus mykiss*, *Yersinia ruckeri*, API 20 E, Microagglutination

GİRİŞ

Gram negatif enterik bir bakteri olan *Yersinia ruckeri*, özellikle *Salmonidae* familyasına ait balıklarda akut veya kronik seyir gösteren yersiniozise (enterik kızıl ağız hastalığına) neden olur. Bu mikroorganizma bugüne kadar salmonid balıkların entansif yetiştiriciliğinin yapıldığı 5 farklı kıtadaki birçok ülkeden (Avustralya, Bulgaristan,

Kanada, Şili, Danimarka, Finlandiya Fransa, Almanya, Yunanistan, İran, İtalya, Yeni Zelanda, Norveç, Güney Afrika, Portekiz, İspanya, İsveç, İsviçre, Türkiye, Hırvatistan İngiltere, Amerika Birleşik Devletleri ve Venezuela) bildirilmiş olup, hastalığın yayılım gösterdiği ülke sayısı günden güne artış göstermektedir ¹⁻⁴. Yersiniozis, alabalık ve



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diğer salmonidlerin septisemik karakterli bir hastalığıdır. Fakat gökkuşağı alabalığı (*Oncorhynchus mykiss*) hastalıktan en fazla etkilenen türdür. *Y. ruckeri*'nin Ülkemizde ilk defa, 1991 yılında Denizli ve İzmir'deki işletmelerden izole edildiği bildirilmiştir ^{5,6}.

Y. ruckeri suşları arasında serolojik farklılıklar bulunmasına rağmen, biyokimyasal özellikleri açısından serotiplerin çok benzer olduğu belirtilmektedir ^{1,7}. Ancak *Y. ruckeri* ile yapılan biyokimyasal çalışmalarda inkübasyon sıcaklığının 22-25°C arasında olması gerektiği, optimum sıcaklığın dışında yapılan testlerde hatalı negatif veya pozitif sonuçların elde edilebileceği bildirilmiştir ^{1,8,9}.

Y. ruckeri serotipleri arasında sorbitolü fermente etme yeteneğinin değişebileceği, serotip 2 suşlarının sorbitolü fermente etme yeteneğiyle diğer serotiplerden ayrılabilceği belirtilmiştir ¹⁰⁻¹².

Y. ruckeri suşları farklı serotip özellikler göstermektedir. *Y. ruckeri*'nin en yaygın ve en virulent tipi serotip 1 (Hagerman suşu) olarak bildirilmiştir ^{7,13}. Daha sonra salmonlardan, virulensi serotip 1'den daha düşük olan serotip 2 izole edilmiştir ^{14,15}. Avustralya'da 1973 yılında izole edilen *Y. ruckeri* suşunun ise serotip 3 (Avustralya suşu) olduğu belirtilmiştir ¹⁶. Bu üç serotip dışında daha sonraki yıllarda IV, V, VI adı verilen serotiplerde de bildirilmiştir ^{11,17}. Daha sonraki araştırmalarda suşlar arasındaki serolojik ilişki ve antijenik determinantlarına (LPS ve membran proteinleri) göre *Y. ruckeri*'nin 4 serotipik (O₁, O₂, O₃, ve O₄) gruba ayrıldığı saptanmıştır ¹⁸.

Y. ruckeri suşlarının fenotipik özelliklerinin belirlenmesinde, geleneksel testler, ve API 20 E test kitleri kullanılmaktadır ^{1,8}. *Y. ruckeri* izolatları üzerine API 20 E hızlı teşhis kitleriyle yapılan çalışmalarda elde edilen verilere göre araştırmacılar; *Y. ruckeri*'nin bazı suşlarının identifikasyon sırasında *Hafnia alvei* ile karıştırılabileceğini bildirmişlerdir ^{4,9,19}.

Bu çalışmada, ülkemizin farklı bölgelerinden izole edilmiş olan *Y. ruckeri* suşlarının identifikasyonlarında geleneksel mikrobiyolojik yöntemler, selektif besiyerleri, API 20 E hızlı teşhis kitleri kullanılarak; API 20 E hızlı teşhis kitlerinin kullanımı sırasında karşılaşılabilecek identifikasyon hatalarının belirlenmesi ve mikroaglutinasyon testiyle belirtilen bakteri suşlarının serolojik özelliklerinin ortaya konulması amaçlanmıştır.

MATERYAL ve METOT

Y. ruckeri Suşları

Araştırmada 21 adet *Y. ruckeri* suşu kullanılmış olup, bu suşlardan iki tanesini ATCC 29473 (serotip 1) ve

1850621-1116B Danimarka (serotip 2) referans suşlar oluştururken, diğerlerini ülkemizin farklı coğrafik bölgelerinden izole edilmiş izolatlar oluşturmaktadır. Bu suşların kökenleri ve kaynağı **Tablo 1**'de verilmiştir.

Tablo 1. Çalışmada kullanılan *Yersinia ruckeri* suşları

Table 1. *Yersinia ruckeri* strains

Suş No	Köken	Kaynak
1	Isparta	Gökkuşağı Alabalığı
2	Bilecik	Gökkuşağı Alabalığı
3	ATCC 29473	Gökkuşağı Alabalığı
4	1850621-1116B Danimarka	Gökkuşağı Alabalığı
5	Denizli	Gökkuşağı Alabalığı
6	Kırklareli	Gökkuşağı Alabalığı
7	Bolu	Gökkuşağı Alabalığı
8	Tekirdağ	Gökkuşağı Alabalığı
9	Çanakkale	Gökkuşağı Alabalığı
10	Elazığ	Gökkuşağı Alabalığı
11	Aydın	Gökkuşağı Alabalığı
12	Bursa	Gökkuşağı Alabalığı
13	Yalova	Gökkuşağı Alabalığı
14	Trabzon	Gökkuşağı Alabalığı
15	Rize	Gökkuşağı Alabalığı
16	Kütahya	Gökkuşağı Alabalığı
17	Kayseri	Gökkuşağı Alabalığı
18	Urfa	Gökkuşağı Alabalığı
19	Ordu	Gökkuşağı Alabalığı
20	Manisa	Gökkuşağı Alabalığı
21	Denizli	Gökkuşağı Alabalığı

Hiperimmün (Serotip 1 ve Serotip 2) ve Negatif Serumlar

Araştırmada kullanılan *Yersinia ruckeri* suşlarının serotiplendirilmesinde kullanılan hiperimmün serumlar (pozitif kontrol serum) Ege Üniversitesi Su Ürünleri Fakültesi Balık Hastalıkları Anabilim Dalından temin edilmiştir. Negatif kontrol serumlar ise sağlıklı gökkuşağı alabalıklarından elde edilmiştir.

Y. ruckeri Suşlarının Fenotipik Özellikleri

Y. ruckeri suşlarının fenotipik özelliklerinin belirlenmesinde geleneksel mikrobiyolojik testler, API 20 E hızlı teşhis kitleri ve selektif besiyerleri (Shotts - Waltman medium, Riboz Ornitin Dezoksikolat medium) kullanılmıştır ^{1,8}.

Etkenin İzolasyonu ve Saflaştırılması

Y. ruckeri suşları stok kültürden TSA besi yerine alınarak 22°C'de 48 saat inkübe edilmiştir. Inkübasyon sonunda incelenen bakteri suşlarının 1-2 mm çapında, yuvarlak, kenarları düz görünümde, beyazdan krem renge kadar değişen renklerde, yarı şeffaf koloniler verdiği görülmüştür. Bu kolonilerin saflıkları, Shotts - Waltman medium (SWM, %1 tripton, %1 yeast ekstrakt, %0.125 fenilalanin, %0.12 ferrik amonyum sitrat, %0.0003 brom timol mavisi, % 0.1 bile salts, %1.5 agar pH 7.0 121°C'de otoklavlandıktan sonra membran filtre ile steril edilmiş

%0.35 mannitol ve 10 µg/ml kolitsin sülfat ilave edilmiştir) ve Riboz Ornitin Dezoksikolat (ROD, %0.3 yeast ekstrakt, %0.1 sodium deoksikolat,%0.5 sodyum klorid, %0.68 sodyum tiyosülfat, %0.08 ferrik amonyum sitrat, %0.75 maltoz, %0.375 riboz, %0.375 ornithin hidroklorid %0,1 sodyum dodeksil sülfat, %0.008 fenol red, %1.25 agar, pH 7.4 ortam 121°C'de otoklavlandıktan sonra membran filtre ile steril edilmiş 0.5 g/ml sukroz ilave edilmiştir) besiyerinde gösterdikleri koloni özellikleri yönüyle incelemeler yapılarak kontrol edilmiştir ^{1,20,21}.

Klasik Mikrobiyolojik Testlerin Kullanımı

Araştırmamızda; Gram boyama, oksidasyon-fermentasyon (O/F), Simmons sitrat, jelatin hidrolizi, sitokram oksidaz, katalaz, %1, %3, %7 NaCl'de büyüme, 37°C'de büyüme, 22°C, 37°C'de hareket, indol, metil kırmızısı, voges-proskauer reaksiyonu (VP), sorbitol, ksiloz, maltoz, inositol, glukoz'dan asit/gaz üretimi SWM ve ROD besiyerlerindeki koloni morfolojileri yönünden mikrobiyolojik çalışmalar yapılmıştır ^{1,8,20,21}.

API 20 E Testi

API 20 E hızlı teşhis testinin kullanımı üretici firmanın (Biomérieux, 20 100) yönergesi doğrultusunda yapılmış ve değerlendirilmiştir ^{1,8,22,23}.

Antijen Hazırlanması

Kültür edilen *Y. ruckeri* suşları PBS (bu solüsyon 8 g NaCl, 1.21 g K₂HPO₄, 0.34 g KH₂PO₄, 1.000 ml deiyonize su içinde eritilip, otoklavda sterilize edilerek hazırlanmıştır) içinde 100°C'de 30 dak. süreyle otoklavda bekletilmiştir. Kültürler 5.000 devir/dak. 15 dak. santrifüje edilerek, çökelti kısmı ayrılmış ve PBS ile sulandırılmıştır. Bu süspansiyonun (antijenin) yoğunluğu spektrofotometrede (Shimadzu UV 160 A) 525 nm dalga boyunda 0.65 Optik Dansite'ye ayarlanarak belirlenmiştir. Antijen, somatik O antijeni olarak mikroplyet aglutinasyon testinde kullanılmıştır ^{18,24}.

Mikroaglutinasyon

Y. ruckeri suşlarının serotiplendirilmesi sırasında mikroaglutinasyon testi kullanılmıştır ²⁵⁻²⁸.

BULGULAR

Y. ruckeri Suşlarının Fenotipik Özellikleri

Y. ruckeri suşlarının fenotipik özelliklerinin belirlenmesinde, klasik testler ve API 20 E test kitleri kullanılmış olup, elde edilen sonuçlar **Tablo 2** ve **3**'te verilmiştir.

Tablo 2. *Yersinia ruckeri* Suşlarında API 20 E Testi Kullanılarak Belirlenen Fenotipik Özellikler

Table 2. Phenotypic characteristics in *Yersinia ruckeri* strains with API 20 E Test

Fenotipik Özellikler	Yersinia ruckeri Suşları																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sitrat Üretimi	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
H ₂ S Üretimi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ureaz Üretimi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
İndol Üretimi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-
Jelatin Hid.	+	+	+	-	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+
Glukoz*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
İnositol*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol*	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ramnoz*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sakkaroz*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melibioz*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amygdalin*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinoz*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Oksidaz	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO ₂ Üretimi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
McC'de Ür.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1. ONPG: * - Galaktosidaz, **2. ADH:** Arginindihidrolaz, **3. LDC:** Lizindekarboksilaz, **4. ODC:** Ornitindekarboksilaz, **5. TDA:** Triptofandeaminaz, **6. VP:** Vogesproskauer, **7. McC'de Ür.:** MacConkey Agarda Üreme, * : Karbonhidratlardan Asit Üretimi

Tablo 3. *Yersinia ruckeri* suşlarında klasik mikrobiyolojik testler kullanılarak belirlenen fenotipik özellikler**Table 3.** Phenotypic characteristics in *Yersinia ruckeri* strains with using conventional microbiological tests

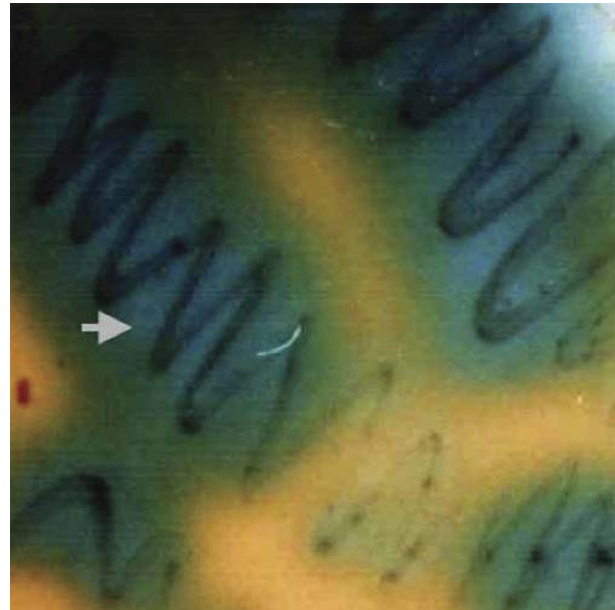
Fenotipik Özellikler	Yersinia ruckeri Suşları																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Gr Boyama	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. Oksidaz	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Katalaz	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Jelatin Hid.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
%3 NaCl*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
%7 NaCl*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30°C Büyüme	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C Büyüme	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22°C Hareket	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C Hareket	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SWM**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ROD***	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H ₂ S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
İndol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metil Red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol *	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ramnoz *	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
İnositol*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glukoz* (A/G)	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Ksiloz*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*, % 1, 3, 7 NaCl'de Büyüme, ** ; Shotts-Waltman Ortamında Tween 80'nin Hidrolizi sonucunda buzlu cam görünümünde yeşil renkli koloni oluşturma, *** ; ROD besiyerinde kırmızı zemin üzerinde ribozun fermentasyonuna bağlı olarak sarı renkli koloni oluşturma

API 20 E ve klasik testlerle ilgili sonuçlar incelendiğinde, *Y. ruckeri* suşlarının fenotipik özelliklerinin büyük ölçüde benzer olduğu ancak yalnızca sorbitol, sitrat, jelatin ve voges proskauer testleri açısından bazı farklılıklar görüldüğü tespit edilmiştir. Sitrat, jelatin ve voges proskauer testlerinde suşlar arasındaki farklılıkların API 20 E hızlı teşhis kitlerinde görülüp, geleneksel mikrobiyolojik testlerde görülmemesi, API 20 E hızlı teşhis kitiyle *Y. ruckeri*'nin teşhisi sırasında *H. alvei* ile karışabilme riskine karşı dikkatli olunması gerektiğini göstermektedir.

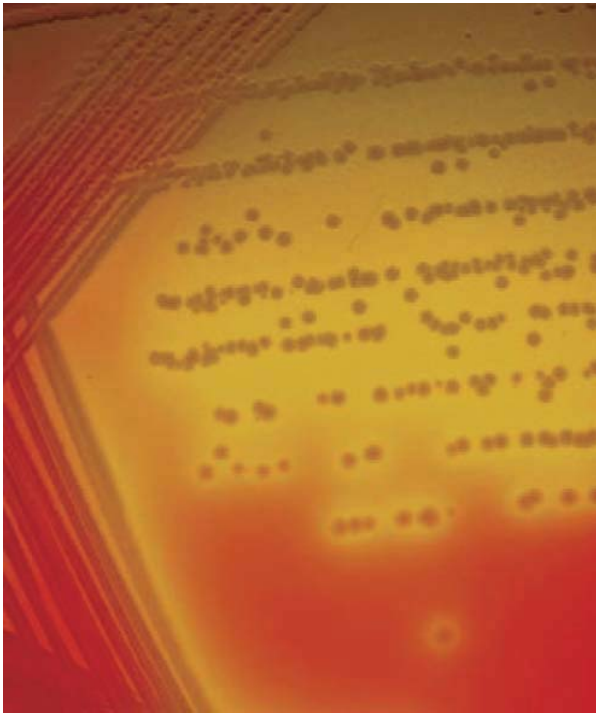
Sorbitol testi yönünden 4 ve 5 nolu izolatin pozitif sonuç vermesi (hem API 20 E hem de geleneksel testlerde) bu suşun serotip 2 özellikte olmalarından kaynaklanmaktadır.

SWM besiyerinde *Y. ruckeri*'nin, yeşil renkli koloniler oluşturarak bu kolonilerin etrafında kalsiyum tuzlarının presipitasyonu ve Tween 80'nin hidrolizine bağlı olarak buzlu cam görünümünde bir zon meydana getirdiği tespit edilmiştir (Şekil 1). *Y. ruckeri*'nin, ROD besiyerinde ise ribozun fermentasyonu ve sodyum dezoksikolat tuzlarının presipitasyonu sonucunda sarı renkli koloniler verdiği görülmüştür (Şekil 2).



Şekil 1. SWM besiyerinde *Y. ruckeri*'nin yeşil renkteki kolonilerinin çevresinde buzlu cam görünümündeki hidroliz zonu

Fig 1. Green colonies and surrounded by a zone of hydrolysis of *Y. ruckeri* in SWM medium



Şekil 2. ROD besiyerinde *Y. ruckeri*'nin sarı renkli kolonileri
Fig 2. Yellow colonies of *Y. ruckeri* in ROD medium

Aglutinasyon Testleri

Y. ruckeri suşlarıyla (21 adet) yapılan mikropleyt aglutinasyon testleri sonucunda; 19 suşun serotip 1, 4 ve 5 numaralı izolatların ise serotip 2 özellik gösterdiği tespit edilmiştir. Serotip 1 ve serotip 2 immunsorumlarına karşı, ülkemizden izole edilmiş olan serotip 1 ve serotip 2 karakter gösteren suşlarda olduğu gibi iki referans suşa ait antijenik yapıların birbirleriyle kros reaksiyon vermedikleri görülmüştür. Ayrıca gerek serotip 1 suşlarının gerekse serotip 2 suşlarının hepsinde, immün serumlarla (serotip 1 ve 2) pozitif sonuç veren antijen dilusyonu 1:256 olarak belirlenmiştir. Negatif kontrol serumlarının kullanıldığı pleyt bölmelerinde ise aglütinasyon görülmemiştir.

TARTIŞMA ve SONUÇ

Gram negatif, hareketli, çubuk şeklinde enterik bir bakteri olan *Y. ruckeri*'nin teşhisi; genellikle fenotipik (morfolojik, fizyolojik, biyokimyasal) özellikleri incelemek yapılmaktadır ^{1,7,8,16}.

Beş kıtaya yayılmış olan *Y. ruckeri* suşlarıyla yapılan çalışmalarda; suşlar arasında serolojik farklılıklar olmasına rağmen, fenotipik özelliklerinin çok benzer olduğu belirtilmiştir. *Y. ruckeri*'nin fenotipik özelliklerinin belir-

lenmesinde, klasik mikrobiyolojik testler ve API 20 E hızlı teşhis kiti kullanılmaktadır ^{1,8,12,29}. Bu çalışmada geleneksel mikrobiyolojik testleri kullanarak elde ettiğimiz sonuçlar açısından suşlar arasında bir farklılık görülmemiştir. Bu yönüyle ülkemiz *Y. ruckeri* suşları oldukça homojen bir yapı göstermiştir. Elde ettiğimiz bu sonuçlar, ülkemizden izole edilen *Y. ruckeri* suşlarıyla çalışmış araştırmacıların sonuçlarıyla benzeşmektedir ^{5,19,30}.

Yapılan araştırmalarda, bazı *Y. ruckeri* suşlarının tween 80'i hidrolize edemediği bildirilmiştir ^{1,5,7,16,29}. Davies ve Frerichs ²⁹; hareketsiz *Y. ruckeri* suşlarının tween 80'i hidrolize edemediği, Bush ⁷ ise, hareketlilik ile tween 80 hidrolizi arasında bir ilişkinin olmadığını bildirmiştir. Çağırğan ⁵, çalıştığı 20 adet *Y. ruckeri* suşunun hepsinin hareketli olduğunu, ancak bu suşlardan bir kısmının tween 80'i hidrolize etmediğini saptamıştır. Bu çalışmada ise, 21 adet *Y. ruckeri* suşunun hepsinin hareketli olduğu ve 21 numaralı suş dışındaki diğer suşların tween 80'i hidrolize edebildiği tespit edilmiştir. Tween 80'i hidrolize etmeyen 21 nolu suşun hareketli olması Davies ve Frerichs'dan ayrılırken ²⁹, Bush ⁷ ve Çağırğan ⁵ ile benzerlik göstermiştir.

Bakteriyel hastalık etkenlerinin fenotipik özelliklerinin belirlenmesinde ve teşhisinin yapılmasında en çok kullanılan yöntemlerden birisi de API 20 E test kitleridir. *Y. ruckeri* suşlarının fenotipik özelliklerinin API 20 E test kitiyle belirlendiği araştırmalarda; *Y. ruckeri* suşlarının 20, 22 ve 25°C'lerde inkübe edilme sürelerine (24, 48, 72 saat) bağlı olarak elde edilen sonuçların, arjinin dihidrolaz üretimi, jelatin hidrolizi, voges proskauer reaksiyonu ve sorbitol fermentasyonu açısından farklılık gösterebileceği bildirilmiştir ^{19,29,31,32}. Yine bir çok araştırmacı, API 20 E ile teşhiste özellikle *Hafnia alvei* ile karıştırılma riskinin olduğunu belirtmişlerdir ^{1,4,8,9,19,29,31,32}. Yaptığımız çalışmada 22°C'de 48 saat inkübasyon sonucunda *Y. ruckeri* suşları arasında arjinin dihidrolaz üretimi yönünden farklılık görülmezken, voges proskauer reaksiyonu, sorbitol fermentasyonu, sitrat kullanımı ve jelatin hidrolizi testlerinde bazı farklılıklar tespit edilmiştir. Sorbitol fermentasyonundaki farklılıklar, geleneksel mikrobiyolojik testlerde belirlenen sonuçlara paralel olarak yalnızca serotip 2 suşlarında görülmüştür. Ancak sitrat kullanımı negatif (1, 10, 11, 16 ve 17 nolu izolatlar), voges proskauer reaksiyonu pozitif (2, 6, 8, 14 ve 19 nolu izolatlar) ve jelatin hidrolizi negatif (4, 5, 9, 13 ve 20 nolu izolatlar) olarak sonuç veren suşlar, geleneksel mikrobiyolojik testlerden farklılık göstermiştir. Candan ve Yazıcı ¹⁹ *Y. ruckeri*'nin API 20 E testiyle teşhis edilmesinde en uygun sıcaklık ve zamanı belirlemek amacıyla 15 *Y. ruckeri* suşuyla çalıştıkları araştırmada 22°C'de 48 saat inkübasyon sonucunda, jelatin hidrolizi negatif (2 izolat), voges proskauer reaksiyonu pozitif (9 izolat), sitrat

kullanımı negatif (2 izolat) sonuçlar veren suşlar tespit etmişlerdir. API 20 E testiyle ilgili araştırma sonuçlarımızda, geleneksel mikrobiyolojik testlerden farklı olarak sitrat kullanımı, voges proskauer reaksiyonu ve jelatin hidrolizi testlerinde farklılıklar görülmesi, bu konuda çalışan diğer araştırmacıların sonuçlarıyla benzerlik göstermektedir ^{4,9,19,33-36}.

Bir çok araştırmacı tarafından API 20E ile yapılan identifikasyonlarda *Y. ruckeri* ile *Hafnia alvei*'nin karışabildiği bildirilmesine karşın, *Y. ruckeri* suşlarının 37°C'de ürettiğinde hareketsiz, β - galaktosidaz, Jelatin hidrolizi, pozitif, Ramnoz, Arabinoz negatif sonuç verirken *H. alvei* bakterisinin ise 37°C'de ürettiğinde hareketli, β - galaktosidaz, Jelatin hidrolizi negatif, Ramnoz, Arabinoz pozitif sonuç verdiği görülmektedir ^{1,31,37}. Ayrıca *Y. ruckeri* SWM ve ROD selektif besiyerlerinde oluşturdıkları özel koloni morfolojileri ile diğer bakterilerden kolayca ayırt edilebilmektedir.

Y. ruckeri'nin serotipik şeması, yersiniozisin ilk ortaya çıkışından bu yana yeni antiserumların çıkarılması ^{11,16,17,38} veya yeni antijenik şemaların oluşturulmasıyla ^{18,22,39-41} sürekli değişime uğramıştır. Bütün bu çalışmalar *Yersinia ruckeri*'nin serolojisi ve antijenik özelliklerinin karışık bir yapı gösterdiğini ortaya koymaktadır.

Sonuç olarak ülkemizin 7 farklı coğrafik bölgesindeki gökkuşağı alabalık işletmelerinden izole edilmiş olan *Y. ruckeri* suşlarının fenotipik ve serolojik özelliklerinin incelenmiş olduğu bu çalışmada; *Y. ruckeri* suşlarının fenotipik ve serolojik özelliklerinin çok benzer bir karakter gösterdiği ortaya konulmuştur. *Y. ruckeri* suşlarının API 20 E hızlı teşhis kitiyle teşhisi sırasında Jelatin hidrolizi, VP ve Sitrat kullanımı testlerinde oluşabilecek hatalı sonuçların önüne geçmek için klasik metotlarla mukayeseli çalışmanın yararlı olacağı görülmüştür. SWM ve ROD besiyerleri *Y. ruckeri* suşlarının identifikasyonu ve kolonilerin saflıklarının kontrolünde başarılı sonuçlar verdiği tespit edilmiştir. Referans serotipler kullanılarak (serotip 1 ve serotip 2) *Y. ruckeri* suşlarının mikroaglutinasyon testiyle serotiplendirilmesi yapılmış ve serotipler arasında antijenik yapıların birbirleriyle kros reaksiyon vermedikleri saptanmıştır. Ülkemizde de Avrupa ülkelerinde olduğu gibi serotip 1 suşunun yaygın olduğu belirlenmiştir. Bu araştırmanın, ülkemizin 7 farklı coğrafik bölgesini kapsayan işletmelerden izole edilmiş olan *Y. ruckeri* suşlarıyla çalışılmış olması nedeniyle, elde edilen araştırma sonuçları bundan sonraki zamanlarda *Y. ruckeri* bakterisiyle yapılacak çalışmalara katkı sağlayacaktır.

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Evaluation of the VecTest™ Malaria Antigen Panel Assay using *Anopheles sacharovi* Specimens in an Endemic Area, Sanliurfa Province, Turkey

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Summary

In recent years, malaria is located in southeastern Anatolia of Turkey. Because of no information is available about sporozoite rates in the mosquitoes in Turkey, *Anopheles (An.) sacharovi* were collected in Sanliurfa province and examined by the VecTest™ Malaria Antigen Panel Assay, a rapid immunochromatographic assay intended for the qualitative determination of three *Plasmodium* circumsporozoite antigens (*P. falciparum*, *P. vivax* 210, *P. vivax* 247) in infected Anopheline mosquitoes. *Anopheles sacharovi* specimens were collected using CDC light traps in 5 villages belonging to Sanliurfa province in July 2004. The pools containing 10 mosquitoes were prepared and totally 390 *Anopheles sacharovi* females were used for the VecTest™. Test was performed in the field conditions just after collection. The positivity was observed only in one pool and *P. vivax* 247 antigen line. Infection rate was detected as 0.25% in mosquitoes. The detection of *P. vivax* 247 antigen in *An. sacharovi* is an important natural evidence of vector capacity of this species for *P. vivax* variant 247 in Turkey. Results were suggestive of most likely involvement of *An. sacharovi* in malaria transmission in Sanliurfa province.

Keywords: *Anopheles*, *Plasmodium vivax*, Variant 247, Turkey

VecTest™ Sıtma Antijen Panel Yönteminin Sıtmanın Endemik Olduğu Şanlıurfa'dan Toplanan *Anopheles sacharovi* Örnekleri Kullanılarak Değerlendirilmesi

Özet

Son yıllarda sıtma genelde Güneydoğu Anadolu'da lokalize olmaya başlamıştır. Türkiye'deki sivrisineklerde sporozoit oranı hakkında veri bulunmadığı için Şanlıurfa'da *Anopheles (An.) sacharovi* örnekleri toplanmış ve enfekte Anopheline sivrisineklerde üç *Plasmodium* sirkumsporozoit antijenini (*P. falciparum*, *P. vivax* 210, *P. vivax* 247) kalitatif olarak saptayan bir test olan VecTest™ Sıtma Antijen Panel Yöntemi ile incelenmiştir. *Anopheles sacharovi* örnekleri CDC ışıklı tuzaklarla Şanlıurfa iline bağlı 5 köyden Temmuz 2004'de toplanmıştır. Yakalanan toplam 390 *An. sacharovi* dişi her biri 10 örnekten oluşan havuz yapılarak teste tabi tutulmuştur. Test, toplanmanın hemen sonrasında saha koşullarında yapılmıştır. Pozitiflik sadece bir havuzda ve *P. vivax* 247 antijen çizgisinde gözlenmiş ve buna göre de sivrisineklerdeki enfeksiyon oranı %0,25 olarak belirlenmiştir. *Plasmodium vivax* 247 antijeninin saptanması, Türkiye'de *P. vivax* varyant 247 antijen için bu türün vektöryal kapasitesinin doğal kanıtı olmasından dolayı önemlidir. Sonuçlar, *An. sacharovi*'nin Şanlıurfa ilinde sıtma bulaşımındaki rolünü göstermektedir.

Anahtar sözcükler: *Anopheles*, *Plasmodium vivax*, Varyant 247, Türkiye

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INTRODUCTION

More than 2 billion people are at risk of malaria, which primarily affects poor populations in tropical and subtropical areas, where the temperature and rainfall are most suitable for the development of the malaria-causing *Plasmodium* parasites in *Anopheles* mosquitoes. Four *Plasmodium* species, *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* cause malaria in humans. Recently, a fifth species, *P. knowlesi* which was originally described as a malaria parasite of long-tailed macaque monkeys, was also reported as a causative agent of natural human malaria infection ¹.

Malaria parasites are transmitted by more than 70 species of *Anopheles* mosquitoes world-wide but, in each malarious area, only a few species serve as vectors while the majority of *Anopheles* females are not infected ². The climatic conditions in Turkey are suitable for malaria vectors to proliferate. Agricultural infrastructural changes, Southeastern Anatolian Dams and Irrigation Project, insufficient environmental conditions, urbanization, national and international population moves are thought to be key factors that can contribute to malaria control. In recent years, malaria is located in south-eastern Anatolia. Sanliurfa, Batman, Diyarbakir, Siirt, and Mardin provinces are the most affected areas. In western provinces of Anatolia, like Aydin and Manisa, an increase in the number of autochthonous cases might be observed from time to time. This is due to workers moving from malaria districts to western parts in search of job opportunities.

The number of malaria cases is lowest in winter and reaches its peak in summer and autumn. In the past years, the comprehensive malaria prevention program has started bear fruits. The program was very successful and malaria cases decreased from 84.345 in 1994 to 796 cases in 2006.

Almost all malaria cases are caused by *P. vivax* in Turkey. There is also imported *P. vivax* and *P. falciparum* cases. The districts where malaria cases occur are the places where there is high population movement, agriculture is the main occupation, the increase in the population is high and the education/cultural level is low. The conventional method for the diagnosis of malaria has relied on the microscopical examination of Giemsa stained blood smears in local malaria centers.

Environmental factors, behavioral patterns of vectors and movement of human populations combine to provide favorable conditions for malaria transmission. The most important vector species is *Anopheles sacharovi* Favre, 1903 in Turkey. This species has wide

distribution throughout Turkey. Nevertheless, Kasap et al.³ in 1987 and De Sousa et al.⁴ proved under laboratory conditions that *Anopheles superpictus* Grassi, 1899 is also a vector of the human malarias *P. vivax* and *P. falciparum*.

Detection of advance-stage sporozoites in mosquitoes provides evidence incriminating particular vector species. Traditional methods of determining sporozoite rates by direct dissection of fresh mosquito salivary glands ⁵ or by using a parasite antigen capture enzyme linked immunosorbent assay (ELISA) procedure ⁶ is relatively time consuming, requires specialized equipment and trained personnel.

Bangs et al.⁷ evaluated a dipstick assay for the determination of specific circumsporozoite protein (CSP) of *P. falciparum*, *P. vivax* 210, or *P. vivax* 247.

The VecTest™ Malaria Panel Assay is a rapid immunochromatographic assay intended for the qualitative determination of three *Plasmodium* circumsporozoite antigens (*P. falciparum*, *P. vivax* 210, *P. vivax* 247) in infected Anopheline mosquitoes. In this study, we used the VecTest™ Malaria Panel Assay in a preliminary study for the detection of the risk of malaria transmission in Sanliurfa province, most endemic malaria site in Turkey.

MATERIAL and METHODS

Mosquitoes were collected using CDC light traps in 5 localities belonging to Sanliurfa province (villages of Sekerli, Mezra, Sandi and Pamuklu; Birecik town) in July 2004. In total, 390 *An. sacharovi* females were used for the VecTest™ (Medical analysis Systems, Inc., CA, USA, MAS™). Test was performed in the field conditions just after collection.

The mosquitoes were identified to species and pooled into groups of 10 mosquitoes and placed in eppendorf tubes. In total, 20 pools from Sekerli village, 9 pools from Birecik village, 5 pools from Birecik town, 4 pools from Pamuklu village and 1 pool from Sandi village were prepared. Following the kit's instructions, 13 drops (250 µL) of the grinding solution provided in the dropper bottle were added to the sample. The mosquitoes were ground by hand using the grinding pestle provided in the kit. The pestles were washed twice with phosphate buffered saline - Tween 20 (PBST) and wiped clean with a tissue between samples. Strips were placed into the mosquito suspensions in the grinding tube and results were read after 15 min by two people and the photographs were taken in 15 min. Results were not accepted as true after 30 min.

RESULTS

In this study, we found a *P. vivax* 247 antigen positivity (Fig. 1) in one pool of *An. sacharovi* mosquitoes collected from Birecik town (37° 1' 46 N; 37° 59' 25E; alt. 411 m) located in east part of Sanliurfa province. The minimum sporozoite rate among *An. sacharovi* was found to be 0.25% (1/390).

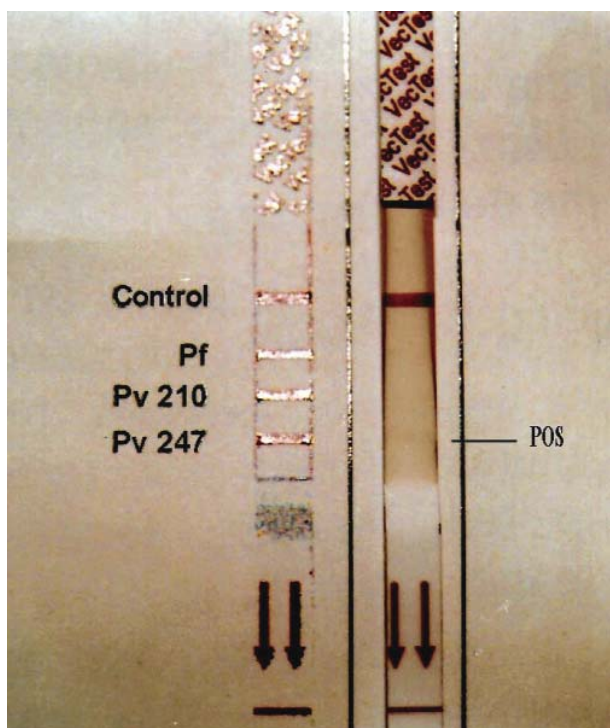


Fig 1. The VecTest showing positivity in the *An. sacharovi* mosquitoes collected from Birecik village, Sanliurfa

Şekil 1. Şanlıurfa Birecik köyünden toplanan *An. sacharovi* sivrisineklerinde VecTest ile saptanan pozitifliğin gösterilmesi

DISCUSSION

Plasmodium vivax is the most geographically widespread and prevalent malaria parasite in some regions, which accounts annually for 70-80 million clinical cases across much of the tropics and subtropics of the world. The *Csp* sequences of *P. vivax* are of two types, VK210 and VK247, which differ in the amino acid composition of the central repetitive region of the gene, but also by three diagnostic amino acid replacements, one in each of the 5- and 3- terminal regions of the gene and the third in an insertion region (IR) between the central repeat (CR) and the 3- terminal region. Both *P. vivax* variants are present in the New and Old World⁸. On this subject, there is only one study showing the presence of *P. vivax* variant 210 and *P. vivax* variant 247 variants in

human malaria patients in Turkey. In Sanliurfa province, 88 malaria patients were diagnosed as *P. vivax* by microscopy and PCR. Among these patients, variant 210 and variant 247 genes were determined in 65.5% and 34.5% of the samples by Restriction Fragment Length Polymorphism (RFLP) analysis after PCR, respectively. There was no mixed variant type in any patients⁹.

It is known that the CS ELISA is considered to be reference method of choice or gold standard for malaria parasite detection in mosquitoes. It is specific for *P. falciparum* and *P. vivax* variant 210 and variant 247. In our study, CS ELISA could not be performed because of some logistic reasons. But, previous studies^{2,7,10,11} showing detailed results about comparison between CS ELISA and the VecTestTM were demonstrated that VecTestTM is sensitive, specific, simple to use, rapid, can identify mixed infection (v210 and v247) and can be used in the field. The 100% positive correlation between CS ELISA and VecTestTM was also shown in different studies^{2,7,10}. We agree with Ryan et al.² that the quick and easy dipstick assay offers practical advantages for field workers and performs at an acceptable level for testing the mosquitoes in the field.

In most malaria endemic situations more than one species of *Anopheles* may transmit *P. falciparum* and *P. vivax*, although the relative status and bionomics of each species of vector remains poorly known in many localities². In order to understand the epidemiology of the disease, there is clearly a need for a field assay capable of rapidly detecting *Plasmodium* infected mosquitoes. Malaria rapid panel (MRP) assays were developed to be capable of detecting several different species and variants of human malaria sporozoites in mosquitoes. Sensitivity of the MRP was demonstrated as roughly equivalent to the original CSP ELISA assays that it is based upon. MRP assays can detect Pf, Pv210 and Pv247 from the same *Anopheles* specimen with a sensitivity of at least 1ng/mL (200 pg) of antigen of any of the three CS proteins. A complete correlation was found between VecTestTM and CS protein ELISA for detection of Pf, Pv210 and Pv247 malaria sporozoites in field-caught *Anopheles* mosquitoes of several species¹⁰.

In a previous study, three species of genus *Anopheles*, four species of genus *Culex* and two each of genus *Culiseta* and genus *Ochleratatus* were collected in Sanliurfa Province. Of the total *Anopheles* collected, *An. sacharovi* was found to be the most abundant (72%) species¹². The vectorial importance of *An. sacharovi* was highlighted in the previous papers^{13,14}. In the present preliminary study, the detection of *P. vivax* 247 antigen in *An. sacharovi* specimens collected in Birecik town is an important natural evidence of vector capacity of this

species for *P. vivax* variant 247 in Turkey. If we accept that only one mosquito among 10 mosquitoes in the pool is infected by sporozoites, the mosquito infection rate will be calculated as at least 0.25%. There is a need to perform VecTest™ dipstick analysis, CS-ELISA or molecular studies using more *An. sacharovi* specimens as well as other *Anopheles* species from different endemic areas in Turkey in order to better understand; (i) the infection rate in the mosquitoes, (ii) the role in transmission of the other *Anopheles* species present in this region for both variants, VK210 and VK247 and (iii) epidemiology of the disease.

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Endocrine Cells in the Gastrointestinal Tract of *Garra rufa*

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Summary

The distribution and relative frequency of endocrine cells in the gastrointestinal tract of the *Garra rufa* were studied immunohistochemically using the peroxidase anti-peroxidase complex method. The aim was to determine the distribution of specific immunoreactivities in some regions of digestive tract of the *Garra rufa* against the antisera VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin. The samples were taken from the enlarged areas of after oesophagus, anterior intestine, middle intestine and posterior intestine. The most intensive region of immunoreactive cells were determined as the anterior intestine. Generally, eight types of endocrine cells declined in number from the enlarged areas of after oesophagus to the intestines. These immunoreactive cells were found in the posterior intestine at very low frequencies. As a result of the immunohistochemical studies, all the peptides studied were determined to be localized generally in different distribution within the stomachs of *Garra rufa* (the enlarged areas of after oesophagus) as well as within their intestines (anterior, middle and posterior intestine). Density of the peptides was concluded to be associated closely with functions in *Garra rufa*.

Keywords: *Garra rufa*, Gastrointestinal tract, Endocrine cells, PAP

Garra rufa'nın Gastrointestinal Kanalındaki Endokrin Hücreler

Özet

Peroxidase anti-peroxidase (PAP) yöntemi ile *Garra rufa* gastrointestinal kanalındaki endokrin hücrelerin dağılım ve yoğunlukları çalışıldı. Bu çalışmada VIP, substance-P, histamin, glukagon, gastrin, CCK-8, somatostatin and serotonin antiserumlarına karşı spesifik immunoreaktivitelerinin *Garra rufa* sindirim kanalının bazı bölümlerindeki dağılımlarının belirlenmesi amaçlandı. Materyal özofagustan sonraki genişlemiş bölgeden, ilk, orta ve son bağırsaktan alındı. Immunreaktif hücrelerin en yoğun olduğu bölgenin ilk bağırsak olduğu belirlendi. Genelde bu çalışmada araştırılan 8 tip immunoreaktif hücrelerin özofagustan sonraki genişlemiş bölgeden son bağırsağa doğru azaldığı gözlemlendi. Bu hücrelerin son bağırsakta oldukça az yoğunlukta olduğu saptandı. Yapılan immunohistokimyasal çalışmalar sonucunda *Garra rufa*'nın mide (özofagustan sonraki genişlemiş bölge) ve bağırsak bölümlerinde (ilk, orta ve son bağırsak) genel olarak çalışılan tüm peptidlerin farklı yoğunlukta lokalize oldukları tespit edildi. Peptid lokalizasyon ve yoğunluğunun fonksiyonlarına göre *Garra rufa*'da da bölgesel olarak yerleşim gösterdiği gözlemlendi.

Anahtar sözcükler: *Garra rufa*, Gastrointestinal kanal, Endokrin hücre, PAP

INTRODUCTION

Gastrointestinal endocrine cells dispersed throughout the epithelia and gastric glands of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract ¹. Many reports have dealt with the identification of regulatory peptides of the alimentary

tract in fish species using silver-staining techniques and either radioimmunochemical or immunohistochemical methods ¹⁻⁸.

Secretions of many endocrine cells act in digestion process together in fishes. The secretions, located in the



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gastrointestinal tract, are the chemicals regulating tracts structure and functions. These chemicals in gastrointestinal tract, accepted as the largest endocrine organ, are mainly secreted by endocrine cells ^{3,8,9}. A large number of endocrine-type secretions are produced in the gastrointestinal tract mucosa of the fish. These secretions, which are called peptide and/or amine, are detected in lamina epithelialis, glands, several connective tissue cells, mucosal nerve ganglions ^{10,11}, and inter-muscular nerve plexus ¹². These secretions assure the mobility as well as the proper functioning of the nervous system, regulation of the secretion through the cellular interaction ¹³, cellular proliferation ^{9,14}, regulation of the intestinal epithelium ¹⁵, and the contraction of the smooth muscles ¹⁶.

This paper examines the distribution and localization of gut endocrine cells in the gastrointestinal tract of the *Garra rufa*, using specific immunohistochemical methods. This species is known as a doctor fish. It apparently feeds on skin scales of men and is used in the treatment of neurodermitis. The fish lives in Sivas thermal waters (28°C-34°C) and is used to treat psoriasis. It is also known as an oily fish ¹⁷. Environmental condition of this fish are pH 7.0-7.4 ¹⁸.

MATERIAL and METHODS

Ten adult (length 8-10 cm, weight 10-20 g) *Garra rufa* (Fig. 1, 2) were used in this study without sexual distinction. After being anaesthetized, the intestinal tract of *Garra rufa* was divided into four portions from proximal to distal; the enlarged area after oesophagus and anterior, middle and posterior intestine. All samples were fixed for 12 h in Bouin's solution and embedded in paraffin. Serial, transverse 6-7 µm sections of these portions were cut. Each representative section was deparaffinized, rehydrated and immunostained using the peroxidase anti-peroxidase (PAP) method ¹⁹. Sections were treated with methanol containing 0.3% H₂O₂ for 30 min, to block any endogenous peroxidase.

Subsequently, the sections were incubated for 1 h at room temperature in normal goat serum (1:100), then stained immunohistochemically to identify specific endocrine cells using PAP. The sections were incubated with specific antisera for individual peptides for 12 h at 4°C. Details of primary antisera used in this study are listed in Table 1. After rinsing with phosphate buffered saline (PBS, 0.01 mol/L, pH 7.4), the sections were incubated in secondary antiserum (ant-rabbit IgG serum raised in goats; 1:200) for 1 h at room temperature. Sections were then washed with PBS buffer and incubated with PAP complex (1:400) for 1 h at room

temperature. The peroxidase reaction was carried out in 0.02% 3,3-diaminobenzidine tetrahydrochloride solution containing 0.01% H₂O₂ in Tris-HCl buffer (0.05 mol/L, pH 7.6). After immunostaining, the sections were lightly counterstained with Mayer's haematoxylin and immunoreactive (IR) cells were observed with light microscopy. Light microscope with magnification 10x40 in total 4 different preparations were made VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin immunoreactive cells numbers. Total cell numbers in the 1 mm² area of the cross sections were



Fig 1. *Garra rufa* were used in this study (lateral view) (original)

Şekil 1. Çalışmada kullanılan *Garra rufa* (lateral görünüm) (orijinal)



Fig 2. *Garra rufa* were used in this study (ventral view) (original)

Şekil 2. Çalışmada kullanılan *Garra rufa* (ventral görünüm) (orijinal)

Table 1. List of primary antisera used in the study

Tablo 1. Bu çalışmadaki primer antiserumların listesi

Antiserum	Code	Dilution	Source
CCK-8	C2581	1:200	Sigma USA
Gastrin-1	G0785	1:200	Sigma USA
Substance P	S1542	1:200	Sigma USA
VIP	V3508	1:200	Sigma USA
Somatostatin-14	S0694	1:200	Sigma USA
Glucagon	G2654	1:200	Sigma USA
Histamine	H7403	1:200	Sigma USA
Serotonin	S5545	1:200	Sigma USA

All antisera were raised in rabbit

CCK: Cholecystokinin, VIP: Vasoactive intestinal peptide

determined. Data were subjected to analysis of variance SPSS 10 statistical software programme and analysis of variance results were significant in the region of average density immunoreactive cell type were compared with ANOVA test ²⁰.

RESULTS

Eight types of endocrine cells were detected with the antibodies against VIP, substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin. The distribution patterns and relative frequencies of these endocrine cells in the gastrointestinal tract of the *Garra rufa* are shown in Table 2. Generally, all antisera used showed specific immunoreactivity within endocrine cells in the mucosa, and they were higher in number in the crypt epithelia than in superficial epithelia.

VIP-positive cells were detected the whole gastro-intestinal tract and most predominant frequencies were detected anterior intestine (Fig. 3, 4). The numbers of these cells appeared to be low in the middle and posterior intestine. Substance P-IR cells were demonstrated in both the enlarged area after oesophagus and gastro-intestinal tract but were more numerous in the former (Fig. 5). The number of cells was decreased in the middle intestine. A great number of histamine-immunoreactive cells were detected in the enlarged area after oesophagus (Fig. 6). Histamine immunoreactive cells were the most predominant cell types in the study. In intestines (Fig. 7, 8), density of the cells lowered towards posterior end.

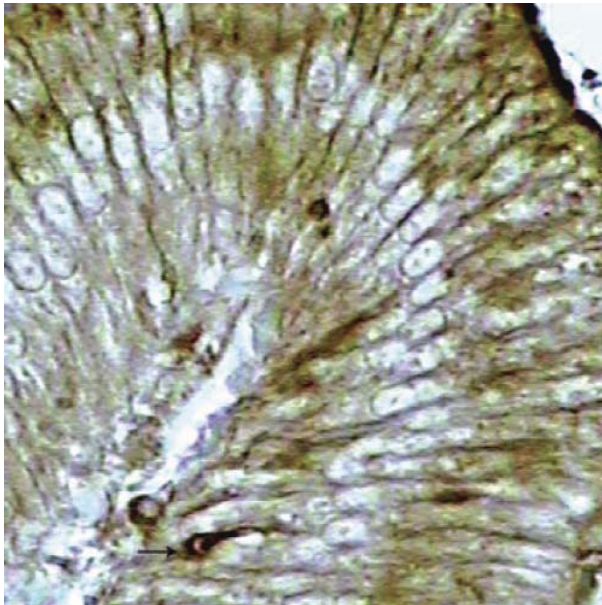


Fig 3. VIP IR cell, anterior intestine, PAP, X400
Şekil 3. VIP IR hücre, ilk bağırsak, PAP, X400



Fig 4. VIP IR cell, anterior intestine, PAP, X400
Şekil 4. VIP IR hücre, ilk bağırsak, PAP, X400



Fig 5. Sub-P IR cell, anterior intestine, PAP, X400
Şekil 5. Sub-P IR hücre, ilk bağırsak, PAP, X400

Cells immunoreactive for glucagon were numerous in the enlarged area after oesophagus. Glucagon IR cells, which were at the highest frequency in the anterior intestine, were found in the epithelia throughout the tract at various frequencies. These cells increased in number from the posterior intestine to the anterior intestine.

Gastrin immunoreactive cells decreased gradually from anterior intestine to posterior intestine. In enlarged region after oesophagus and anterior intestine, Gastrin-IR cells were denser in middle and posterior intestines. Gastrin immunoreactive cells were observed in large

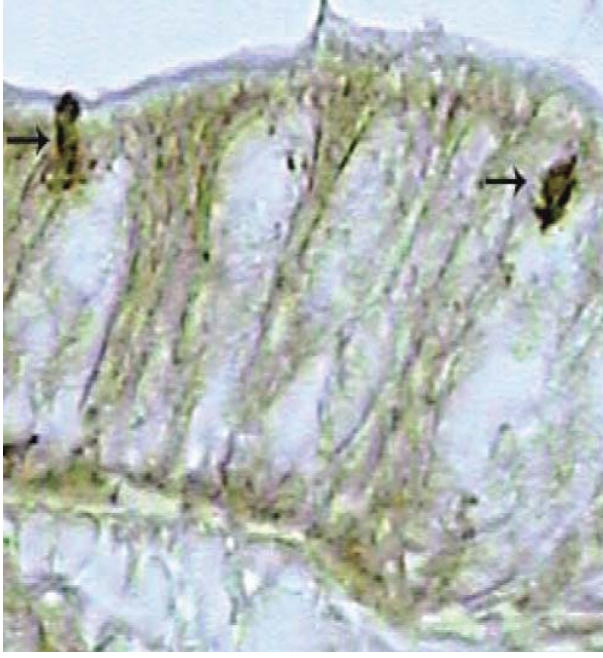


Fig 6. Histamine IR cells, the enlarged area after oesophagus, PAP, X400, *Garra rufa*

Şekil 6. Histamin IR hücreleri, özofagustan sonraki genişlemiş bölge, PAP, X400



Fig 7. Histamine IR cell, anterior intestine, PAP, X400

Şekil 7. Histamin IR hücre, ilk bağırsak, PAP, X400

numbers in the anterior intestine compared to the other regions. Somatostatin immunoreactivity was observed in several cells of the middle and posterior intestines, whereas higher somatostatin immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine (*Fig. 9*). CCK-8 positive cells were observed from area after oesophagus to intestines and

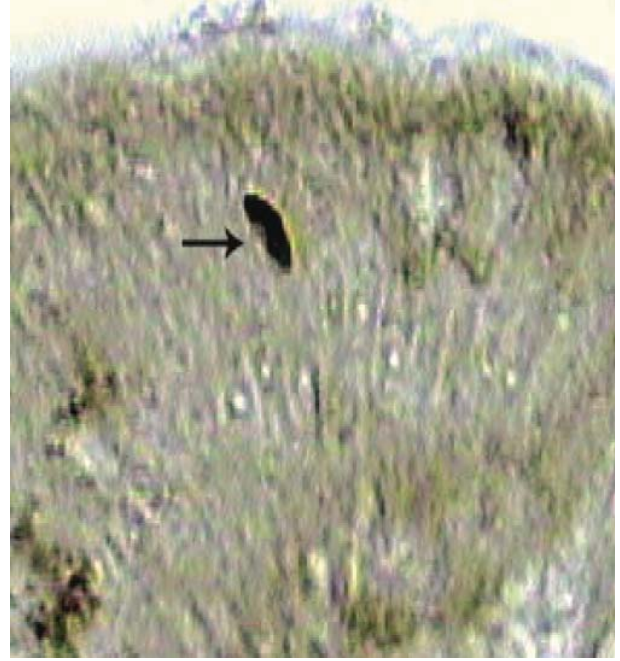


Fig 8. Histamine IR cell, anterior intestine, PAP, X400

Şekil 8. Histamin IR hücre, ilk bağırsak, PAP, X400

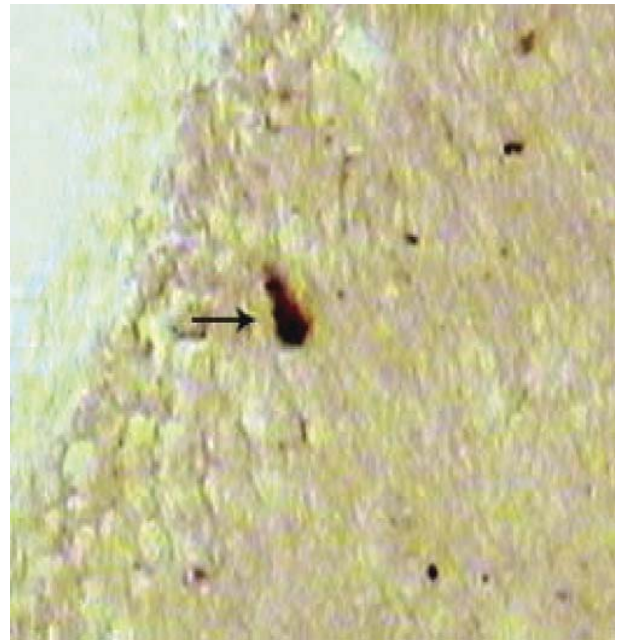


Fig 9. Somatostatin IR cell, anterior intestine, PAP, X400

Şekil 9. Somatostatin IR hücre, İlk bağırsak, PAP, X400

showed the highest frequency in anterior intestine. The numbers of serotonin immunoreactive cells in the enlarged area after oesophagus showed regional distribution to that in the anterior intestine (Fig. 10, 11). These cells were found at a very low frequency in the middle and posterior intestines. Generally, eight types of endocrine cells declined in number from the enlarged area after oesophagus to the intestines. These immunoreactive cells were found in the posterior intestine at very low frequencies.



Fig 10. Serotonin IR cell, anterior intestine, PAP, X400

Şekil 10. Serotonin IR hücre, ilk bağırsak, PAP, X400

ganglion cells. The present study describes 8 types of endocrine cells were observed in the gastrointestinal tract of *Garra rufa*. The endocrine cells were immunoreactive for VIP, Substance-P, histamine, glucagon, gastrin, CCK-8, somatostatin and serotonin.

VIP immunoreactive cells were determined the whole gastrointestinal tract and most predominant frequencies were detected in anterior intestine. Similar results were reported by Çınar et al.²¹, who also found that VIP-IR cells



Fig 11. Serotonin IR cells, the enlarged area after oesophagus, PAP, X400

Şekil 11. Serotonin IR hücre, özofagustan sonraki genişlemiş bölge, PAP, X400

Table 2. Mean number of endocrine cells per intestinal fold (mean $\pm$ SD) in *Garra rufa*

Tablo 2. *Garra rufa* ($\pm$ standart sapma) bağırsağındaki endokrin hücre sayısı

Area/Type of Endocrine Cells	VIP	Sub-P	Histamine	Glucagon	Gastrin	CCK	Somatostatin	Serotonin
The enlarged area after oesophagus	5.75 $\pm$ 4.27	24 $\pm$ 12.83	30 $\pm$ 14.71	11.75 $\pm$ 8.46	10.5 $\pm$ 5.80	13.5 $\pm$ 5.32	12.5 $\pm$ 15.00	7.5 $\pm$ 7.54
Anterior intestine	17 $\pm$ 6.68	13 $\pm$ 5.59	12 $\pm$ 1.41	19.75 $\pm$ 9.17	19 $\pm$ 6.05	19.25 $\pm$ 7.63	11.25 $\pm$ 11.29	6.25 $\pm$ 6.13
Middle intestine	1.75 $\pm$ 2.06	0.5 $\pm$ 0.57	3.75 $\pm$ 2.21	2.25 $\pm$ 0.95	2.5 $\pm$ 0.57	6.25 $\pm$ 2.62	1.5 $\pm$ 1.29	1.25 $\pm$ 0.5
Posterior Intestine	4 $\pm$ 3.77	5.75 $\pm$ 2.06	1 $\pm$ 0.81	1.75 $\pm$ 0.95	2 $\pm$ 1.41	2 $\pm$ 0.81	0 $\pm$ 0.00	1.25 $\pm$ 0.5

DISCUSSION

IR cells were generally observed in crypt, while weak or no IR cells were seen in connective tissues and

were sparsely distributed in the digestive tract of *Pseudophoxinus antalyae* species. While VIP immunoreactive cells were not observed in the stomach of *Oncorhynchus mykiss*¹⁶ and in the whole digestive tract

of *Gadus morhua* L.²², *Oncorhynchus mykiss*¹⁴, *Coreoperca herzi*⁴ and *Zacco platypus*¹, Rajjo et al.²³ found these cells were present in all regions of *Amia calva* L. stomach. In the present study, numerous VIP immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine, except in the middle and posterior intestines. As in the present study, VIP immunoreactive cells have been determined in the intestine of many fish species, such as *Barbus conchoni*²⁴, *Anguilla anguilla*²⁵, *Stizostedion lucioperca*²⁶. These results suggest that VIP immunoreactive cells provide contraction of intestinal wall and blood flow.

Çınar and Diler²⁶ showed that Sub-P IR cells were present throughout the intestine of *Stizostedion lucioperca*. Similar results were reported by Pan et al.¹², Domeneghini et al.²⁵, Rombout and Reinecke^{2,3}. Sub-P immunoreactive cells were not observed in *Zacco platypus*¹, *Coreoperca herzi*⁴. In this study, numerous Sub-P immunoreactive cells were observed in the enlarged area after oesophagus and anterior intestine. These results suggest that Sub-P immunoreactive cells inhibit gastrin secretion.

Histamine is peptide functioning in contraction of smooth muscles and stimulation of acide secretion in stomach^{1,4,27}. Like *Oncorhynchus mykiss*²⁸, *Salmo salar*²⁹, *Dicentrarchus labrax*³⁰ gastrointestinal Histamine IR cells were found densely. Dezfuli et al.²⁶ reported that Glucagon-like immunoreactive ECs were found in proximal intestine of uninfected and parasitised *Salmo trutta*. These findings agree with Bosi et al.³¹ reports. Glucagon IR cells were observed in the digestive tract of *Coreoperca herzi*⁴, *Zacco platypus*¹ and teleost fishes¹². Similar results were determined in this study. These results imply that Histamine and Glucagon immuno-reactive cells are kept a certain level of acid secretion in stomach and intestines.

Gastrin secreted by the intestinal G cell promoted gastric acid secretion. In this study, gastrin immunoreactive cells were detected from the enlarged area after oesophagus to the posterior intestine. These results corresponded with other reports on the fish^{12,26,31,32}. These results suggest that Gastrin immunoreactive cells are most predominant frequencies in the enlarged area after oesophagus in order to increase gastric acid secretion. CCK immunoreactive cells are distributed from the segment II of the gut to the segment V of the gut in the *Zacco platypus*¹. As in the present study CCK immunoreactive cells have been determined in the gastrointestinal tract of many fish species, such as *Coreoperca herzi*⁴, *Salmo trutta* L.²⁷, *Pseudophoxinus antalyae*^{21,32}, *Anguilla anguilla*²⁵, *Stizostedion lucioperca* L.³³. These results imply that CCK and Gastrin peptides

are affecting each other.

Somatostatin, which consist of 14 amino acids, was isolated from the hypothalamus of sheep for the first time and it could be subdivided into straight form and cyclic form¹. As in the present study, somatostatin -14 immunoreactive cells have been determined in the gastrointestinal tract of many fish species, such as *Oncorhynchus mykiss*¹⁶, species of the *Osteoglossomorpha*³⁴, *Zacco platypus*¹, *Coreoperca herzi*⁴. These results suggest that Somatostatin immunoreactive cells inhibit gastrin secretion. Serotonin immunoreactive cells were determined the whole gastrointestinal tract and most predominant frequencies were detected the enlarged area after oesophagus and anterior intestine. Similar results were reported by Ku et al.¹ who also found that serotonin IR cells were sparsely distributed in the *Zacco platypus* segments of the gut. As in the present study, serotonin immunoreactive cells have been determined in the intestine of many fish species, for instance *Coreoperca herzi*⁴, *Stizostedion lucioperca*³³, *Anguilla anguilla*²⁵. These results imply that Serotonin immunoreactive cells regulate gastrointestinal motility.

In conclusion, the regional distribution and relative frequency of immunoreactive cells in the *Garra rufa*, are essentially similar to those of other fish. However, some characteristic differences are observed in this species, which may be due to differences in the antisera tested, the methods used and/or the species investigated in the various studies. Density of the peptides was concluded to be associated closely with functions in *Garra rufa*.

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A Novel Site for Hematopoietic Organ in *Bombyx mori* L. (Lepidoptera: Bombycidae)

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Summary

A novel site for hematopoietic organ closely associated with the dorsal vessel (HPO-DV) in the fifth-final larvae of *Bombyx mori* L. was described. HPO-DV is composed of several lobes located very close to the dorsal vessel in the thorax and in the abdominal segments of the larvae. The anterior lobes contain big secretory cells. Loose hemocyte clusters among the secretory cells were observed particularly in the posterior lobes. When the HPO-DV was cultured in the absence of hemolymph; prohemocytes, plasmatocytes, and granulocytes were released from the hematopoietic organ. Cultured hemocytes from two sources, released from HPO-DV and circulating in the hemolymph, were compared under inverted microscope. They remained the suspended in culture medium. Conversely, the cells in the hemolymph immediately adhered on the flask. This different behavior of the cells obtained from different sources implies that the cells produced from the HPO-DV probably continue to differentiate in the hemocoel.

Keywords: Hematopoietic Organ, Cell and organ cultures, Hemocytes, *Bombyx mori*

Bombyx mori L. (Lepidoptera:Bombycidae)'de Yeni Hematopoietik Organ

Özet

Bombyx mori L.'nin son evresinde dorsal damar ilişkili yeni hematopoietik organ (HPO-DD) belirlendi. HPO-DD larvanın toraks ve abdomen segmentlerinde dorsal damarın iki yanında, onunla yakın ilişkili yer alan ve loplardan oluşan bir organdır. Anterior loblar büyük salgı hücrelerini içermektedir. Özellikle posterior loblarda gruplar halindeki hemositler salgı loplarnın arasında yer aldığı gözlemlendi. Doku kültürü ortamında, organdan prohemosit, plasmatosit ve granulosit hücre tipleri salınmıştır. HPO-DD'dan ve dolaşımdaki hemositlerin hücre kültürleri inverted mikroskop altında karşılaştırıldı. Bu hücreler kültür ortamında zemine tutunmayıp ortamda asılı bir şekilde kaldı. Diğer taraftan hemolenfteki hemositler kültür kaplarına aktarıldıktan sonra hemen zemine tutunduğu gözlemlendi. Bu davranış farklılığı HPO-DD'dan salınan küçük hemositlerin hemosölde farklılaşmaya devam ederek dolaşıma katıldıklarını düşündürmektedir.

Anahtar sözcükler: Hematopoietik organ, Hücre ve organ kültürleri, Hemositler, *Bombyx mori*

INTRODUCTION

The sites of the hematopoietic organs (HPOs) producing the hemocytes and their structural complexity vary with the insect's phylogenetic position ^{1,2}. HPOs in Diptera are generally connected to both sides of the dorsal vessel ². In *Drosophila*, 4-6 pairs of lobes are associated with the dorsal vessel in the thorax ^{2,8}. On the other hand, in *Calliphora*, 5-8 islets of dividing cells are

found in abdominal segments ². In Lepidoptera species; *Bombyx mori* ⁹⁻¹¹, *Manduca sexta* ^{12,13}, *Pseudoplusia includens* and *Spodoptera frugiperda* of HPOs are attached to imaginal wing discs ¹⁴. The most highly organized organ is found in the housecricket, *Gryllus*, a member of the order Orthoptera ². In general, HPOs in insects with the exception of lepidopteran species are

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located near the dorsal vessel in the thorax and the abdominal segments.

The lepidopteran hematopoietic process is poorly understood¹⁰ and questions concerning hemocyte proliferation, differentiation and mobilization still remain unsolved^{9,10,15}. Little is known about the development or the transformation of the different hemocyte types into one another in the silkworm, *B. mori*¹¹. The physiological properties of hematopoiesis in cultured hematopoietic organs have not been studied in detail. Therefore, *in vitro* tissue culture of the hematopoietic organ (HPO) can be a useful tool for the study of insect hematopoiesis¹⁰ and reveal much information about the differentiation of hemocytes¹⁶.

In this paper, a novel site for HPO, in addition to HPOs attached to imaginal wing discs^{9,12,14}, was described that lies adjacent to the dorsal vessel in *B. mori*. The hemocytes are discharged from the lobes of HPO into the medium, and it has been confirmed by tissue culture that this organ is an HPO. Finally, this study is the first demonstration of the presence of an HPO closely associated with dorsal vessel in *B. mori* that had not previously been described in the silkworm larvae.

MATERIAL and METHODS

The Silkworm and Its Hemocytes

The larvae of silkworm, *Bombyx mori* were cultured on the leaves of the mulberry at 24±1°C, 70% relative humidity, and under a photoperiod of 12:12 (light and dark)^{17,18}. In this study, we used the fifth or last instar larvae of silkworm, designated in text as L5. The prohemocytes, plasmatocytes and granulocytes, being effector cells to the immune response and hematopoiesis^{2,19,20}, comprised approximately 85-95% of all hemocytes in the last instar larvae of Lepidoptera^{12,13,16}.

Silkworm hemocytes were classified into three morphotypes using the criteria described by Lackie², Yamashita and Iwabuchi¹⁶ and Nakahara et al.¹⁰: (1) the prohemocyte is a small, round non-adhesive cell and thin cytoplasm without cytoplasmic inclusions; (2) the plasmatocyte is a pleomorphic, usually oval, adhesive cell; (3) the granulocyte is a round adhesive cell with numerous small granules.

Preparation and Culture of Hematopoietic Organ and Hemocytes

Hematopoietic organs of L5 larvae were dissected under binocular microscopy (Carl Zeiss, 9901). HPOs were washed three times with 0.01 M Phosphate buffer

saline (PBS) (pH:7.3) and transferred to a plastic petri dish (Falcon® 35x10 mm) with 2 ml of culture medium. These culture dishes were incubated at 26°C and natural humidity. The release of hemocytes from the HPOs was observed under an inverted microscope (Olympus CK40) and photographs were taken at 24 h intervals.

The cell culture medium was composed of Grace's Insect Medium (Sigma, G8142) with fetal calf serum (Sigma, F3018) and antibiotic-gentamycin (Gibco). In order to carry out the cell culture, 2 ml medium and 1 ml medium with hemolymph were added to each disposable plastic petri dish. The cells were maintained at 26°C and natural humidity.

Light Microscopic Structure of Hematopoietic Organ

Light microscopic investigation was performed on the semi-thin sections of Epon 812 embedded materials. For this purpose, the dissected HPOs were fixed in Karnovsky²¹ fixative, pH 7.4 at 4°C, and post fixed in 1% OsO₄ in phosphate buffer²². After dehydration, the organ samples were embedded in Epon 812. Semi-thin sections (1 µm) stained with Toluidin Blue were used for light microscopic investigation.

RESULTS

In the investigation of HPO, a lobulated compact structure was identified along the dorsal vessel in the thorax and the abdomen segments of larvae of *Bombyx mori*. To determine whether this structure is an HPO, an *in vitro* tissue culture system was established. During the monitoring under the inverted microscope, a number of hemocytes released from the folds of the HPO were observed after 24 h (Fig. 1). According to the description by Lackie², Yamashita and Iwabuchi¹⁶ and Nakahara et al.¹⁰, these cells were prohemocytes, plasmatocytes and a small number of granulocytes. Granulocytes appeared round, while plasmatocytes were spindle or star-shaped. Prohemocytes were also round cell but noticeably smaller than the other types of hemocytes. In addition, the hemocytes that were discharged from the cultured HPO lobes remained suspended, and they did not adhere to the surface of the flask (Fig 2a). Conversely, when hemocytes in circulation were kept in the culture environment, they immediately adhered and spread across the flask bottom to form a layer (Fig 2b). These observations suggest that differentiation of hemocytes released from HPO still continues into morphotypes in the hemolymph.

We have termed the organ as a novel site of HPO associated with the dorsal vessel as Hematopoietic Organ Related Dorsal Vessel (HPO-DV). The organ is

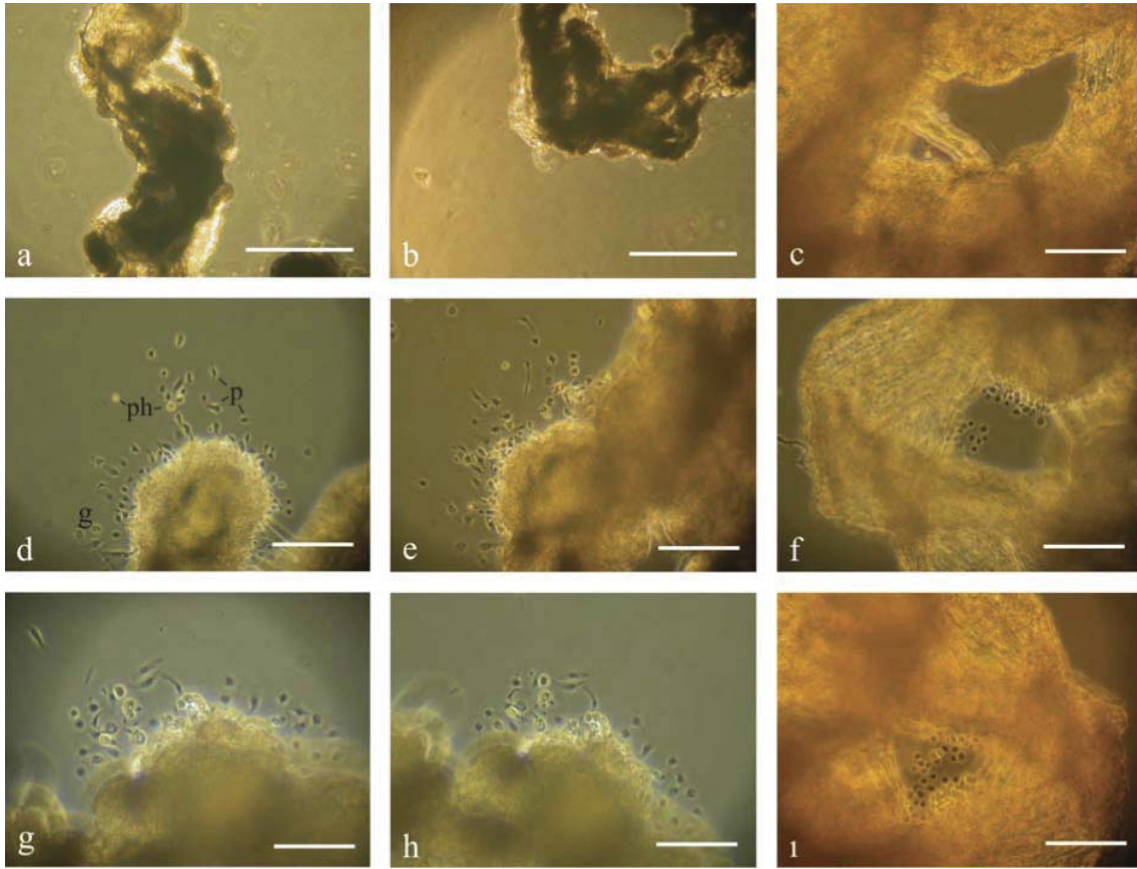


Fig 1. Cultured HPO-DV lobes from L5-larvae. **a, b, c.** No hemocyte release after the first 30 min in culture medium, **d, e, f.** Hemocyte release on the second day, **g, h, i.** Hemocyte release on the third day. **p:** plasmotocytes, **g:** granulocytes, **ph:** prohemocytes (Bar 100 μ m)

Şekil 1. Kültür ortamında HPO loplarından hemositlerin salınması. **a, b, c.** Kültür ortamına koyulan HPO'nun ilk 30 dakika sonraki yapısı ve hemosit salınımı yok, **d, e, f.** İkinci gün HPO'nun loplarından salınan hemositler, **g, h, i.** Üçüncü gün loplardan salınan hemositler. **p,** plazmatositler, **g,** granulositler, **ph,** prohemositler (Bar 100 μ m)

composed of several lobes (Fig 2c). They are located along the surface of the dorsal vessel and maintain very close contact with it. The inner surface of the organ's lobes lies up against the dorsal vessel and outer surface faces the hemocoel. All lobes were surrounded by a continuous layer of basement membrane or stroma (Fig 2d).

HPO-DV exhibits morphological differences between the anterior and the posterior lobes along the extension of the organ in the thorax and the abdominal segments of the larvae. Anterior lobes were primarily composed of big secretory cells. Also, there are some differences among the anterior lobes of HPO-DV according to the included granules. Some of lobes contain cells with small and round granules. In neighboring lobes, similar big cells were filled with secretory granules very different in appearance (Fig 2e). The posterior lobes of HPO-DV include secretory cells and loose-associated different hemocyte clusters (Fig 2f).

DISCUSSION

It has been suggested that the HPO of lepidopteran insects, *Bombyx mori*⁹⁻¹¹, *Manduca sexta*¹², *Pseudoplusia includens* and *Spodoptera frugiperda*¹⁴ are located in imaginal wing discs. However, we have identified a novel site for hematopoietic organ in silkworm larvae. The morphology and the structure of the organ are very similar to the HPO described in *Drosophila* sp. It is suggested that in *Drosophila* larvae, anterior lobes of HPO contain especially secretory cells while the posterior lobes consist of essentially prohemocytes like in *B. mori*. These anterior lobes produce various proteins including collagen, peroxidase and antifungal peptides following an immune challenge³. Secretory cells in the anterior lobes of the HPO-DV of *B. mori* can also be responsible for the synthesis of similar defense molecules for humoral and cellular immune response.

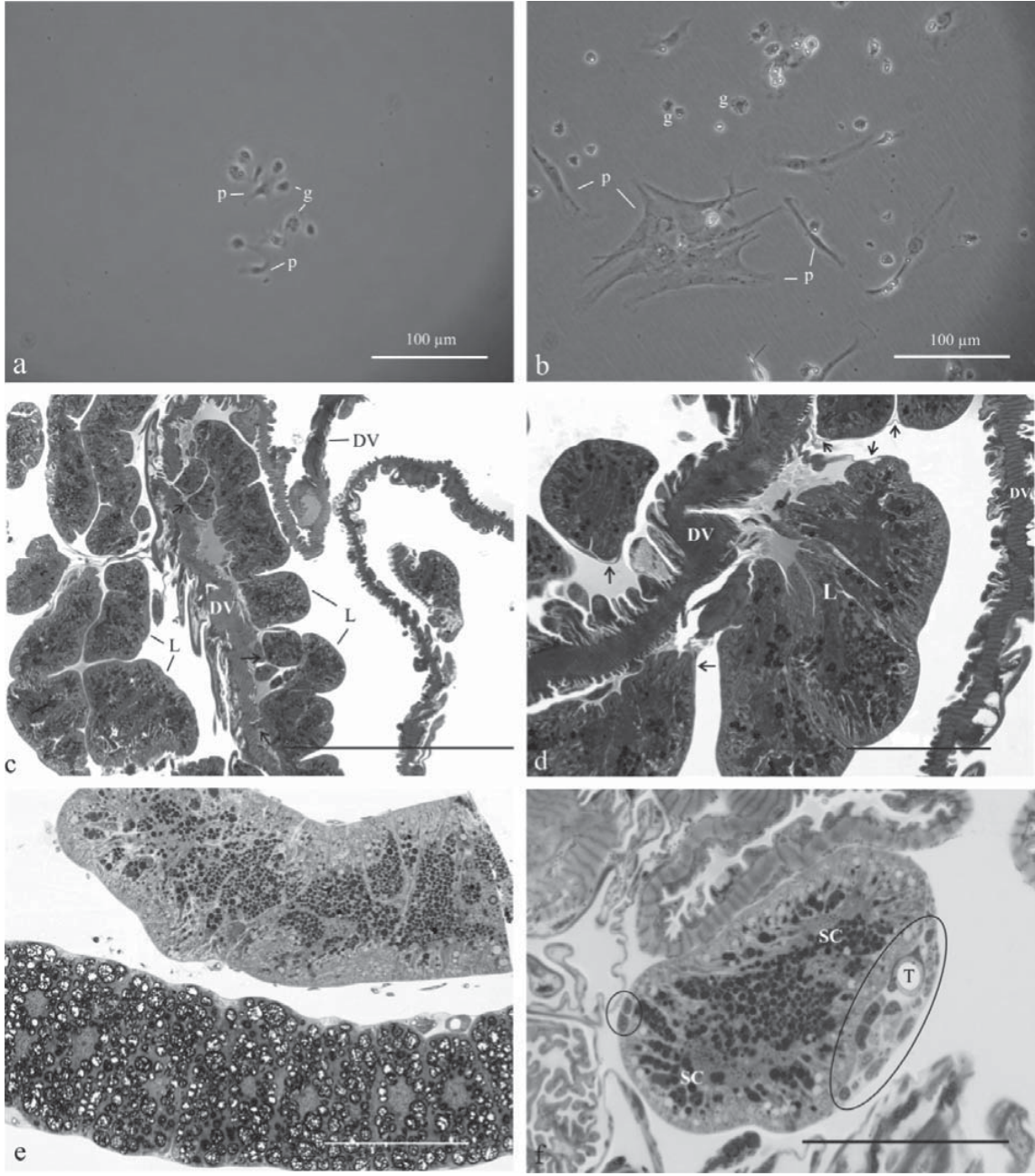


Fig 2. Hemocytes in culture medium (a, b): **a.** released from HPO-DV to culture medium, **b.** obtained from circulating hemolymph. **p.** plasmotocytes, **g.** granulocytes (Bar 100 µm). The general structure of HPO-DV composed of several lobes (c, d): **c.** close contacts (→) between the lobes of HPO-DV and the dorsal vessel (Bar 200 µm), **d.** a stromal coat (→) surrounding the lobes (Bar 50 µm). **L;** lobe, **DV;** dorsal vessel (1 µm epon section stained with Toluidin blue). **e.** anterior lobes of HPO-DV include secretory cells containing different secretory granules (1 µm epon section stained with Toluidin blue) (Bar 50 µm). **f.** posterior lobe of HPO-DV containing both secretory cells (SC) and different loose hemocyte clusters (in circles). **T;** trachea (1 µm epon section stained with Toluidin blue) (Bar 50 µm)

Şekil 2. Kültür ortamındaki hemositler (a, b): **a.** HPO-DV'dan kültür ortamına salınan hemositler, **b.** hemolenfte dolaşan hemositlerin kültürü, **p.** plazmatositler, **g.** granulositler, **ph,** prohemositler (Bar 100 µm). Loplardan oluşan HPO-DV'nin genel yapısı (c, d): **c.** dorsal damar ile HPO-DV arasındaki yakın ilişkisi (→) (Bar 200 µm), **d.** lobları saran bir bazal örtü (→) (Bar 50 µm). **L;** lop, **DV;** dorsal damar (1 µm'lik epon kesit, Toluidin mavisi). **e.** farklı salgı granüllerine sahip salgı hücrelerinden oluşan HPO-DV'nin anterior lopları (1 µm'lik epon kesit, Toluidin mavisi) (Bar 50 µm). **f.** salgı hücreleri (SC) ve gevşek yapıda hemosit topluluklarını (daire içinde) içeren HPO-DV'nin posterior lobu. **T;** trake (1 µm'lik epon kesit, Toluidin mavisi) (Bar 50 µm)

Several types of circulating hemocytes were characterized in larval Lepidoptera, but there is no agreement as to where blood cells are produced and how their populations are maintained ¹⁴. Nakahara et al. ¹⁰ and Ling et al. ¹¹ showed that most of the cells discharged from the larval HPO near to the imaginal wing discs in *B. mori* were plasmatocytes, prohemocytes and granulocytes. They concluded that young hemocytes produced from the organ have a great ability to multiply by mitotic division in circulation. In another study, hemocytes discharged from cultured HPO of *M. sexta* either adhered and spread on the culture surface or remained suspended in the culture medium. The only adherent cells spreading to the glass substrate were plasmatocytes. The prohemocytes were non-adherent and remained in suspension ¹². The behavior of the cells derived from the HPO-DV in *B. mori* was quite similar to the observations made in earlier studies. When circulating hemocytes were added to culture dishes, they did not adhere or spread at first. The cells were later allowed to settle and adhere to the glass coverslip for a time. Consequently, we propose that hemocytes discharged from HPO-DV are likely to continue to differentiate in the hemocoel.

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Effect of Duo-Culture on Growth Performance of Brook Trout (*Salvelinus fontinalis* Mitchell, 1814) and Black Sea Trout (*Salmo trutta labrax* Pallas, 1811) in Tank Reared Condition

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Summary

The objectives of the present study were to compare growth performance and feed conversion ratios of brook trout (*Salvelinus fontinalis*) and Black Sea trout (*Salmo trutta labrax*) in monoculture and duo-culture tank reared conditions. The fish were about 9-month-old hatchery reared brook trout and Black Sea trout with initial weights of 24.18 ± 0.64 (n=60) and 23.87 ± 1.11 (n=60) g, respectively. Fish were kept in 0.2 m³ fiberglass tanks. Nine tanks were used and the fish were equally allotted to 3 groups with three replicates and fed for 240 days. Each tank contained 20 fish from each species for monoculture and 10+10 fish from each species for duo-culture. At the end of the study, mean live weights of brook trout and Black Sea trout increased to 265.47 ± 17.25 g and 206.18 ± 2.95 g in monoculture, 235.53 ± 10.25 g and 222.47 ± 14.29 g in duo-culture, respectively, and significant differences were found among the groups ($P < 0.01$). The highest and lowest specific growth rates were found in brook trout and Black Sea trout in monoculture ($P < 0.01$), respectively. The highest and lowest final condition factors were calculated in duo-culture for brook trout and in monoculture for Black Sea trout ($P < 0.01$), respectively. The feed conversion ratio (FCR) for Black Sea trout was higher than it was for the other groups ($P < 0.01$).

Keywords: Brook trout, Black Sea trout, Duo-culture, Growth performance, Feed conversion ratio

Tank Koşullarında İkili Yetiştiriciliğin Kaynak Alabalığı (*Salvelinus fontinalis* Mitchell, 1814) ve Karadeniz Alabalığı (*Salmo trutta labrax* Pallas, 1811)'nin Büyüme Performansı Üzerine Etkisi

Özet

Bu çalışmanın amacı, tank koşullarında tekli ve ikili yetiştirilen kaynak alabalığı (*Salvelinus fontinalis*) ve Karadeniz alabalığı (*Salmo trutta labrax*)'nın büyüme performansı ve yem değerlendirme oranını karşılaştırmaktır. Kuluçkahane kökenli yaklaşık dokuz aylık kaynak ve Karadeniz alabalıklarının başlangıç ağırlıkları sırasıyla, 24.18 ± 0.64 (n=60) and 23.87 ± 1.11 (n=60) gramdır. Balıklar 0,2 m³ hacimli fiberglas tanklarda stoklanmıştır. Balıklar dokuz tanka üç tekerrürlü, eşit sayı ve ağırlıkta, tekli grupta 20 balık/tank, ikili grupta ise sayıları 10+10 balık/tank olacak şekilde yerleştirilmiştir ve çalışma 240 gün sürmüştür. Çalışmanın sonunda, kaynak ve Karadeniz alabalıklarının ortalama canlı ağırlıkları tekli grupta sırasıyla 265.47 ± 17.25 g ve 206.18 ± 2.95 g, ikili grupta ise 235.53 ± 10.25 g ve 222.47 ± 14.29 g olarak hesaplanmış ve gruplar arası farklılıklar istatistiksel açıdan önemli bulunmuştur ($P < 0.01$). En yüksek ve en düşük spesifik büyüme oranı değerleri sırasıyla, tekli yetiştirilen kaynak ve Karadeniz alabalığında hesaplanmıştır ($P < 0.01$). En yüksek ve en düşük kondisyon faktörü değerleri sırasıyla, ikili yetiştirilen kaynak alabalığı ve tekli yetiştirilen Karadeniz alabalığında hesaplanmıştır ($P < 0.01$). Tekli yetiştirilen Karadeniz alabalığı grubunda yem değerlendirme oranı diğer gruplara göre yüksek bulunmuştur ($P < 0.01$).

Anahtar sözcükler: Kaynak alabalığı, Karadeniz alabalığı, İkili yetiştiricilik, Büyüme performansı, Yem değerlendirme oranı

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INTRODUCTION

Aquaculture is rapidly gaining importance in Turkey as in many countries of the world. It started with the culture of rainbow trout in the 1970s, but it did not considerable gain a commercial status until the 1990s. The development of a sustainable industry requires species diversification and application of appropriate management procedures. Culturing more than one salmonid species in farms may provide one viable alternative. In this respect, species of genus *Salvelinus* and *Salmo* have drawn considerable attention in recent years ^{1,2}. Brook trout (*Salvelinus fontinalis*) introduced from Europe to Turkey has been farmed in small quantities in rainbow trout farms in the last two decade. Culture possibilities of native Black Sea trout (*Salmo trutta labrax*) were evaluated by the Department of Fisheries Technology at the Faculty of Marine Sciences at Karadeniz Technical University ³, the Ministry of Agriculture and Rural Affairs and the Central Fisheries Research Institute ^{4,5}. Studies of these institutions revealed that the growth performance of Black Sea trout was not at a more desired level than rainbow and brook trout.

Polyculture is the way of simultaneously producing more than one fish species in the same rearing space ^{6,7}. The principle of polyculture is based on the fact that cultured fish species feed on different levels of food chain and environment ⁸, but wild species can be stocked with domesticated species to get them used to artificial feed. In this case, increasing food intake due to competition and social hierarchy can affect fish growth positively. At the same time, uneaten feed by wild species consumed by domesticated fish, and feed efficiency is maintained ⁹.

Between Black Sea trout, a new finfish species for aquaculture ⁴, and domesticated brook trout may occur competition for food in same culture medium. Whether the increase in feed consumption, and growth performance depending on feed consumption of Black Sea trout, dueculture of these species may be practiced by fish farmers. The objectives of the present study are to compare growth performance and feed conversion ratios of brook trout and Black Sea trout in monoculture and duo-culture tank reared conditions.

MATERIAL and METHODS

The growth trial, which lasted 224 days, was carried out between November-June at the Prof. Dr. İbrahim OKUMUŞ Aquaculture Research and Production Unit at Karadeniz Technical University. The fish were about 9-

month-old hatchery reared brook trout and Black Sea trout with initial weights of 24.18 ± 0.64 (n=60) and 23.87 ± 1.11 (n=60) g, respectively. Fish were kept in 0.2 m³ fiberglass tanks. Nine tanks were used and the fish were equally allotted to 3 groups with tree replicates and fed for 240 days. Each tank contained 20 fish from each species for monoculture and 10+10 fish from each species for duo-culture. Rearing tanks were supplied with freshwater at flow rates of 2 to 12 l/min depending on the fish size, temperature and oxygen content of water and the fish were exposed to natural photoperiod (latitude = 41°N) and temperature regimes.

Fish were fed to apparent satiation with commercial dry extruded pellets of 3 and 4 mm containing 48% crude protein and 18.0% crude lipid. Feed was manually given three times a day (at 8:30, 12:30 and 16:30 h). Pellet size and daily feeding frequency were changed according to recommendations of the feed manufacturer. Growth was followed by bulk-weighing the fish in each tank (within 0.1 g accuracy limit) at two weekly intervals, and total lengths (± 1 mm) and individual live weights (± 0.1 g) were noted down to determine condition factors.

The following parameters were calculated from the data collected: Specific Growth Rate (SGR, %/day) = $[(\ln W_t - \ln W_0)/t] \times 100$; Condition Factor (CF) = $(W / L^3) \times 100$; Feed Conversion Ratio (FCR) = Feed consumed/Biomass increment; where W_0 and W_t are live weights (g) of the fish at the beginning and a successive weighing, respectively; t is number of days and L is total length of the fish (cm).

The mean and standard deviation ($\pm$ sd) were calculated for all parameters in each group and one-way analysis of variance (ANOVA) ¹⁰ and Tukey were used to test for differences among the groups. All data analysis and statistical testing were carried out using the Minitab (13.0) statistical software.

RESULTS

Daily water temperatures ranged from 6.5 to 16.5°C, and dissolved oxygen content of incoming water was the highest (over 10 mg/l) in winter, but dropped to a minimum value of 8.7 mg/l in June. At the end of study, mean live weight of brook trout and Black Sea trout increased 265.47 ± 17.25 g and 206.18 ± 2.95 g in monoculture, and 235.53 ± 10.25 g and 222.47 ± 14.29 g duo-culture, respectively (*Fig. 1*), and significant differences were found among the groups ($P < 0.01$) (*Table 1*). The highest and lowest specific growth rates (*Fig. 2*) were found in brook trout and Black Sea trout in monoculture ($P < 0.01$), respectively. The highest and

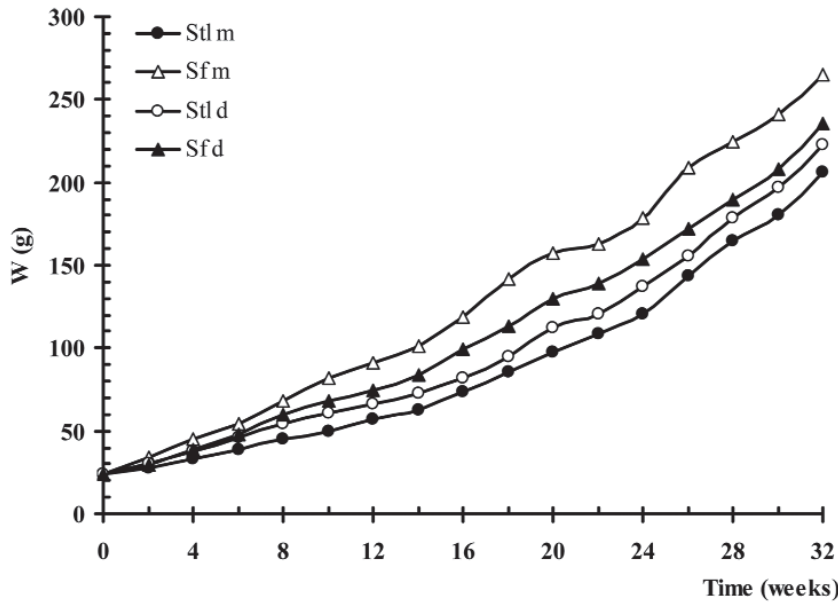


Fig 1. Increments in live weight (W; g) of the experimental fish during the trial period

Şekil 1. Çalışma süresince balıklarda canlı ağırlık (W; g) artışı

Table 1. Mean ($\pm$ SD) growth (Wi: initial and Wf: final weights; SGR: specific growth rate; CF: condition factor; D: stocking density) and, feed conversion ratio (FCR) parameters (m: monoculture; d: duo-culture)

Tablo 1. Ortalama ($\pm$ SD), büyüme (Wi: ilk ve Wf: son ağırlık; SGR: spesifik büyüme oranı; CF: kondisyon faktörü; D: stoklama yoğunluğu) ve yem değerlendirme oranı (FCR) parametreleri (m: tekli kültür; d: ikili kültür)

Parameter	Sf m	Stl m	Sf d	Stl d	F	P
Wi (g)	24.10 $\pm$ 0.34	24.25 $\pm$ 0.77	23.91 $\pm$ 1.11	23.83 $\pm$ 1.12	0.11	>0.05
Wf (g)	265.47 $\pm$ 17.25 ^a	206.18 $\pm$ 2.95 ^d	235.53 $\pm$ 10.25 ^b	222.47 $\pm$ 14.29 ^c	12.66	<0.01
SGR	1.071 $\pm$ 0.026 ^a	0.955 $\pm$ 0.015 ^c	1.021 $\pm$ 0.017 ^b	0.996 $\pm$ 0.018 ^b	18.59	<0.01
CFi	1.079 $\pm$ 0.015	1.068 $\pm$ 0.007	1.092 $\pm$ 0.011	1.088 $\pm$ 0.035	1.32	>0.05
CFf	1.400 $\pm$ 0.051 ^b	1.288 $\pm$ 0.002 ^c	1.578 $\pm$ 0.081 ^a	1.352 $\pm$ 0.04 ^b	17.04	<0.01
FCR	0.909 $\pm$ 0.020 ^a	1.019 $\pm$ 0.014 ^b	0.925 $\pm$ 0.023 ^{a *}		28.35	<0.01
Di (kg/m ³)	2.39 $\pm$ 0.003	2.38 $\pm$ 0.006	2.38 $\pm$ 0.002 ^{a *}		1.39	>0.05
Df (kg/m ³)	26.54 $\pm$ 1.725 ^a	20.62 $\pm$ 0.295 ^b	22.90 $\pm$ 0.853 ^{b *}		22.38	<0.01

*: duo-culture value of Sf and Stl

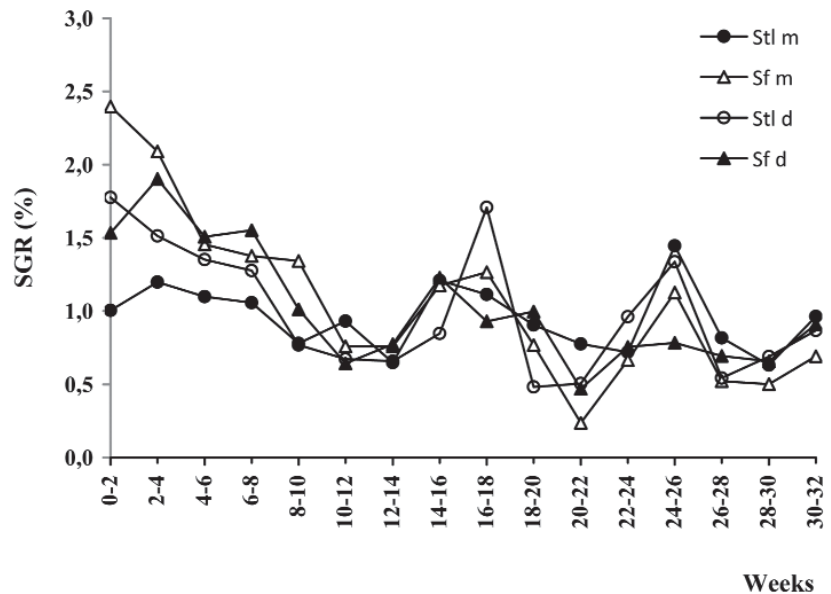


Fig 2. Variations of specific growth rates (SGR; %) of the experimental fish during the trial period

Şekil 2. Çalışma süresince balıklarda spesifik büyüme oranı (SGR; %) değişimleri

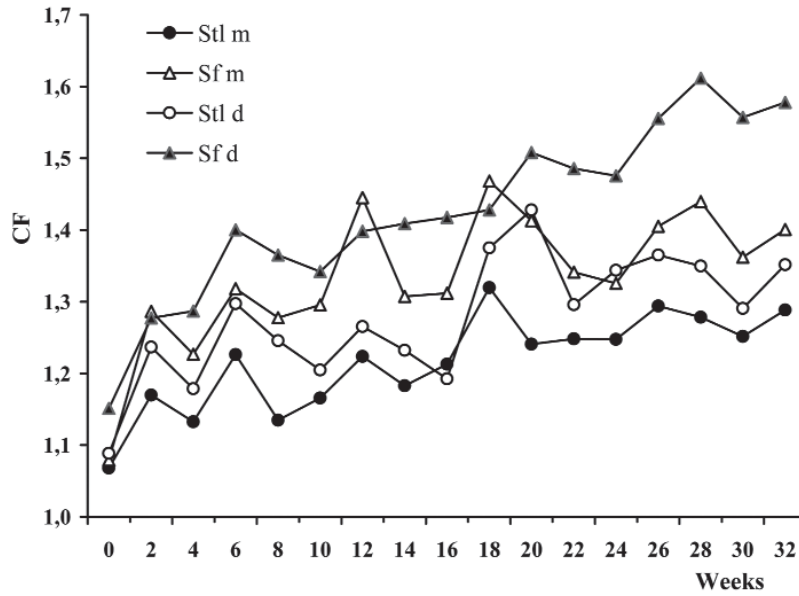
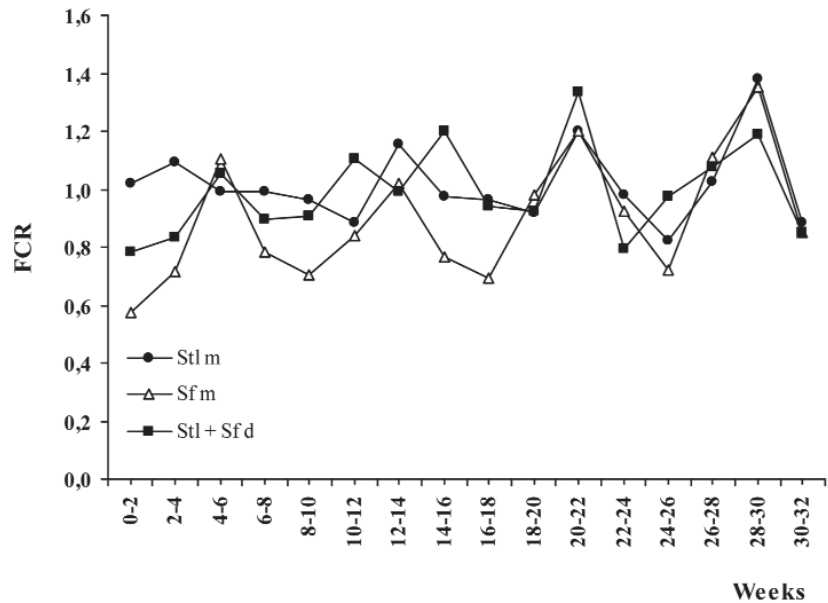


Fig 3. Variations of condition factors (CF) of the experimental fish during the trial period

Şekil 3. Çalışma süresince balıklarda kondisyon faktörü (CF) değişimleri

Fig 4. Variations of feed conversion ratios (FCR) of the experimental fish during the trial period

Şekil 4. Çalışma süresince balıklarda yem değerlendirme oranı (FCR) değişimleri



lowest final condition factors were calculated as 1.578 ± 0.081 in duo-cultured brook trout and as 1.288 ± 0.002 in monocultured Black Sea trout ($P < 0.01$), respectively, and the others were similar (Fig. 3). The feed conversion ratio (FCR) (Fig. 4) for Black Sea trout was higher than it was for the other groups ($P < 0.01$) (Table 1). No fish died during the trial.

DISCUSSION

The fact that mortality is not seen at stocking densities which are not high ($20-26 \text{ kg/m}^3$) shows that feed consumption, food conversion rates (FCR) and

growth ratios and the properties of research environment are acceptable and for this reason, the performance of the fish under the trial conditions is highly satisfying.

Rapid growth, FCR, keeping feed loss at a minimum and the optimal use of stocking container are the most commercially important criteria in fish breeding. The feed which meets qualitative needs of growth in fish must be shared equally among fish in order to secure a rapid and uniform growth. Enequal consumption of feed among individuals may be originating from excessive competitive behaviour because of limited supplies of feed and dominant hierarchy in the group and this results

in a discrepancy in the sizes of individuals¹ and a decrease in the biomass. According to some researchers^{1,11}, zone defence and a dominant hierarchy is inevitable when Salmonidae family individuals are bred in small groups. In such cases, the bigger and more active individuals occupy higher ranks of the hierarchy and they need more feed and this leads to an inequality in feed consumption¹². For this reason, competition among individuals and differences in fish size increase in circumstances where it is not always possible to get feed and where fish are given feed 1 to 3 three times a day for short periods¹ and competition for feed becomes a significant factor that hinders growth in juveniles. So, dominant hierarchy and unequal feed consumption are inevitable, as it is in this study, in an environment where two species with different behavioural characteristics are cultured. It is certain in this study that brook trout which adapt to culture conditions more easily and are more active is in a more advantageous position than Black Sea trout in terms of feed consumption and growth. Therefore, the growth differences between brook trout and Black Sea trout in the polyculture group (Table 1, Fig. 1) can be attributed to the fact that brook trout formed a dominant hierarchy and got a bigger share of the feed.

It is known that the most suitable temperature for brook trout and Black Sea trout growth is 13 to 15°C^{3,9}. In our study, water temperature was at optimum levels only from April to June but, it never dropped below 4°C¹³ at which growth virtually came to a halt and rose to sublethal levels (>20°C). Results indicate that both Salmonidae species show noticeable seasonal variations in feed consumption, growth (Fig. 1, 2) and condition factors (Fig. 3). Similar tendencies in the individuals of the Salmonidae family were observed by other researchers, too¹⁴. Generally, growth is rapid in Spring months and slow in Winter months. Although this variation is due to changes in water temperature, it has been proved that some species (e.g. *Salvelinus alpinus*) living at high latitudes show seasonal variations of growth even when water temperature is kept constant^{12,15}. It is claimed that this reflects the annual rhythms in endogeny, which is controlled by seasonal photoperiods, and longer daytimes in Spring months stimulate growth and shorter daytimes in Autumn and Winter months slow down growth¹⁵. Besides, feed consumption and growth are affected by factors other than water temperature. Fish size is one of these factors^{16,17}. The fact that feed consumption and growth ratios were considerably high at the beginning of the trial and decreased as the fish grew towards the end of the trial confirms this. Actually, it is proved in this study that the relation between water temperature and feed

consumption and growth characteristics is not a significant one. Feed consumption and growth ratios decreased in the second month of the trial and this was because water temperature dropped rapidly. Although, water temperature was relatively lower in the next month, an increase in growth ratio was observed because water temperature did not vary much.

Feed quality, feeding activities of a species, stock density, oxygen content of water and genetic line of fish can be cited among other factors that influence growth¹⁸. It is reported that, generally, daily specific growth rate varied from 0.2% to 1.0% and sometimes reached 4.0% of live weight¹⁹ in brook trout and it varied from 0.17% to 2.04% in Black Sea trout⁵. In the present study, the average daily growth rates were found to be between 0.23% and 2.39% in brook trout and 0.63% and 1.44% in Black Sea trout (Fig. 2). That polyculture Black Sea trout grew more rapidly than monoculture Black Sea trout (Table 1, Fig. 3) is because they wanted to get the upper hand in their competition with brook trout for feed. So, it can be suggested that differences between groups are largely because of feed consumption.

Condition factor increased progressively although it varied in all the groups (Fig. 3). This increase is a direct indicator of feed consumption, rapid weight increase and energy accumulation¹⁴. Although the increasing condition factor is partly due to the fact that stomach and entrails of the fish were filled, it is largely due to tissue growth in the fish since they were deprived of feed before they were weighted¹⁷. Generally, average condition factor values in brook trout were found to be high. This is because brook trout has a relatively deeper body and it consumes more feed and grows rapidly.

In this study, it was found out that both species had good FCR if the conditions are suitable. Feed conversion ratio, which fell below 1.1, in all three groups proves this (Table 1). Since feed conversion ratio is affected by various such factors as biological value of feed, the ratio of main constituents of feed, stock density, genetic line of fish, fish size, basal metabolic rate, water temperature, feeding method and frequency¹⁶⁻¹⁹, comparing results of different trials may not have a practical value. The estimated feed use values during the study shows that there is no need to overfeed. Similar findings in rainbow trout with weights varying between 0.5 and 1.0 kg were obtained by Storebakken and Austreng²⁰. Jobling et al.¹⁶ claims that this is because the maximum feed consumption of the rainbow trout, optimum growth and FCR temperatures showed differences of as much as a few degrees. So, optimum FCR temperature <optimal growth temperature <maximum feed consumption and metabolism rate.

As a result, this study has shown that duo-culture of brook trout and Black Sea trout is disadvantageous for brook trout, which is the more rapidly growing and dominant species, in terms of growth and feed use and it has also shown that polyculture provides a more rapid growth for Black Sea trout compared to monoculture. In the culture mediums, brook trout may be used for increasing growth performance of Black Sea trout, therefore food conversion ratio can be effected positively.

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Hatay Yöresinde Bulunan *Culicoides* (Latreille, 1809) (Diptera: Ceratopogonidae) Türleri

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Özet

Bu araştırma, Hatay yöresindeki *Culicoides* (Latreille, 1809) türlerinin belirlenmesi amacıyla Nisan 2005 - Ekim 2006 tarihleri arasında Antakya Merkez, Reyhanlı, Hassa, Samandağ ve Kırıkhan ilçelerinde yürütülmüştür. *Culicoides* örneklerinin toplanmasında ışık tuzakları kullanılmıştır. Işık tuzakları güneşin batışına yakın saatlerde koyun ve sığır barınaklarının içlerine veya yakınlarına kurulmuş ve iki-üç saat sonra alınmışlardır. Araştırmada 15 türe ait 520 adet *Culicoides* yakalanmış olup, bunlar; *C. badooshensis* Khalaf, *C. cataneii* Clastrier, *C. circumscriptus* Kieffer, *C. dzhafarovi* Remm, *C. festivipennis* Kieffer, *C. gejgelensis* Dzhaferov, *C. imicola* Kieffer, *C. nubeculosus* (Meigen), *C. odiatus* Austen, *C. picturatus* Kremer ve Deduit, *C. puncticolis* (Becker), *C. riethi* Kieffer, *C. sahariensis* Kieffer, *C. schultzei* kompleks (Enderlein) ve *C. shaklawensis* Khalaf'dır. *Culicoides circumscriptus* Kieffer ve *C. schultzei* kompleks baskın türler olurken, *C. festivipennis*, *C. odiatus*, *C. picturatus*, *C. riethi* ve *C. shaklawensis*'in ikiye, *C. dzhafarovi*'nin ise sadece bir örneğine rastlanmıştır.

Anahtar sözcükler: *Culicoides*, *Ceratopogonidae*, Hatay

Culicoides species (Latreille, 1809) (Diptera: Ceratopogonidae) in Hatay Province

Summary

This study was carried out to determine of *Culicoides* species in Hatay province (Antakya, Reyhanlı, Hassa, Samandağ and Kırıkhan districts) between April 2005 and October 2006. The light traps were used for the collection of *Culicoides* samples. They were placed in or nearby sheep or cattle pens at the sunset and was kept for about two-three hours. A total of 520 *Culicoides* specimens were collected and 15 species were identified follow as: *C. badooshensis* Khalaf, *C. cataneii* Clastrier, *C. circumscriptus* Kieffer, *C. dzhafarovi* Remm, *C. festivipennis* Kieffer, *C. gejgelensis* Dzhaferov, *C. imicola* Kieffer, *C. nubeculosus* (Meigen), *C. odiatus* Austen, *C. picturatus* Kremer and Deduit, *C. puncticolis* (Becker), *C. riethi* Kieffer, *C. sahariensis* Kieffer, *C. schultzei* kompleks (Enderlein), *C. shaklawensis* Khalaf. While *Culicoides* circumscriptus and *C. schultzei* kompleks were dominant species, two specimens of *C. festivipennis*, *C. odiatus*, *C. picturatus*, *C. riethi* and *C. shaklawensis* only one specimen of *C. dzhafarovi* were obtained.

Keywords: *Culicoides*, *Ceratopogonidae*, Hatay

GİRİŞ

Culicoides cinsinde yer alan sinekler özellikle güneşin batışına yakın saatlerde uçmaya başlarlar. Uçuş aktiviteleri türlere göre değişmekle ve giderek azalmakla birlikte genel olarak sabaha kadar devam eder ¹. Dişileri kan emerek beslenirler ve bu nedenle alerjik tepkimelerin ortaya çıkmasında, bazı viral ve paraziter hastalıkların

bulaştırılmasında rol oynarlar ². Bazı türler Mavi Dil (MD), Akabane (A), Ephemeral Fever (EF) ve Epizootic Haemorrhagic Disease (EHD) gibi Türkiye'de de görülen viral hastalıkların bulaştırılmasında vektör ödevi görürler ^{2,5}. MD ve EF'a zaman zaman Güney ve Güney Doğu Anadolu Bölgelerinde de rastlanmaktadır ⁶. Üç gün



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hastalığı olarak da bilinen EF 1980'li yılların başlarında Güney Doğu Anadolu, Orta Anadolu ve Güney Anadolu bölgelerimizde görülmüştür ⁷. Bu hastalık en son 2008 yılı sonbaharında Güney ve Güneydoğu bölgelerinde, Gaziantep, Şanlıurfa, Adana, Osmaniye ve Hatay'ın da içinde bulunduğu birçok ilde salgın olarak ortaya çıkmış ve birçok hayvanın ölümüne neden olmuştur ⁸.

Türkiye'de *Culicoides* türleri üzerine sistematik araştırmalar yaklaşık 30 yıldır devam etmektedir. Bununla birlikte, yapılan çalışmalar genel olarak il, ilçe veya yöresel çalışmalarla sınırlıdır ^{4,9-12}. Birkaç araştırma ^{5,6,13-16} ise birkaç bölgeyi içine almakla birlikte, Türkiye genelini içine alan geniş kapsamlı bir çalışma bulunmamaktadır. Dik ve ark.⁴ Türkiye'de *Culicoides* türleri üzerine yapılan araştırmaları değerlendirerek, Türkiye'de görülen türleri ve bölgelere göre dağılımlarını liste halinde vermişlerdir. Her ne kadar İskenderun'da yapılan bir çalışma bulunmakta ise de ¹⁷ bugüne kadar Hatay yöresindeki *Culicoides* türlerinin belirlenmesi üzerine yapılmış bir çalışma bulunmamaktadır.

Bu araştırma, Hatay yöresinde *Culicoides* türlerinin belirlenmesi amacıyla yapılmıştır.

MATERYAL ve METOT

Bu araştırma Nisan-Eylül 2005 ve Haziran-Ekim 2006 tarihleri arasında Antakya Merkez, Reyhanlı, Hassa, Samandağ ve Kırıkhan ilçelerinde yürütülmüştür. Antakya ili 36 10' Kuzey enlemi ve 36 06' Doğu boyları ile yurdumuzun en güneyinde yer alıp, yıllık yağış ortalaması 1173.4 mm, yıllık ortalama nem oranı ise %69'dur. Antakya ve civarında Akdeniz iklimi görülmekte olup, yazlar sıcak ve kurak, kışlar ılık ve yağışlı geçmektedir. Samandağ'da makilik alanlar dikkat çekerken, diğer araştırma merkezleri genellikle düz ova şeklindedir. Antakya il merkezinden Asi nehri geçmektedir.

Culicoides örneklerinin yakalanmalarında ışık tuzakları kullanılmıştır. Reyhanlı'da 8, Samandağ ve Antakya Merkez'de 4, Hassa'da 3 ve Kırıkhan'da sadece bir kez olmak üzere toplam 20 kez ışık tuzağı kurulmuştur. Tuzaklar koyun ve sığır barınaklarının genel olarak içlerine, bazen de yakınlarına kurulmuş ve iki-üç saat sonra alınmışlardır. Toplanan tüm artropodlar küçük kavanozlara veya şişelere alındıktan sonra üzerleri etiketlenerek Selçuk Üniversitesi Veteriner Fakültesi Parazitoloji Laboratuvarına getirilmiş ve stereo mikroskop altında incelenmiştir. *Culicoides* soyuna bağlı olanlar ayrılarak, içlerinde %70'lik alkol bulunan eppendorf tüplere konulmuşlardır. Alkol fenol çözeltisi içinde saydamlaştırılan *Culicoides* örnekleri stereo mikroskop altında baş, gövde, kanat ve abdomenleri ayrılarak Faure-forte ile

lam üzerine yapıştırılmışlar ve kurutulduktan sonra binoküler ışık mikroskopunda tür seviyesinde teşhis edilmişlerdir.

Araştırma merkezleri arasında *Culicoides*'lerin toplanma merkezlerine göre dağılımı ki-kare testine göre değerlendirilmiş, $P < 0.05$ değeri istatistiksel açıdan önemli kabul edilmiştir.

BULGULAR

Araştırma sonunda, 361 adedi (%69.4) dişi, 159 adedi (%30.6) erkek olmak üzere toplam 520 adet *Culicoides* örneği yakalanmış ve 15 tür tespit edilmiştir. Bu araştırma ile tespit edilen türler ve bunların araştırma merkezlerine göre dağılımları **Tablo 1**'de gösterilmiştir. Bu tablodan da anlaşılabileceği gibi *C. circumscriptus* Kieffer ve *C. schultzei* kompleks (Enderlein) diğer türlere oranla daha çok sayılarda yakalanmışlardır. Diğer taraftan *C. festivipennis* Kieffer, *C. odiatus* Austen, *C. picturatus* Kremer ve Deduit, *C. riethi* Kieffer ve *C. shaklawensis* Khalaf'ın, ikişer, *C. dzhafarovi*'nin ise sadece bir örneğine rastlanmıştır. *Culicoides badooshensis* Khalaf, *C. cataneii* Clastrier, *C. dzhafarovi* Remm, *C. imicola* Kieffer, *C. odiatus* Austen, *C. picturatus* Kremer ve Deduit ve *C. riethi* Kieffer'nin sadece dışlarına rastlanmış, erkek bireylere tesadüf edilmemiştir. Reyhanlı'da 11, Hassa'da 8, Antakya merkezde 5, Kırıkhan'da 2, Samandağ'da ise sadece bir *Culicoides* türü tespit edilmiştir.

Çalışma merkezleri arasında *Culicoides* örneklerine en çok Reyhanlı (%51.3) ve Hassa'da (%42.5) rastlanmış olup, onları sırası ile Antakya Merkez (%5.2), Samandağ (%0.6) ve Kırıkhan (%0.4) izlemiştir. Antakya merkez, Reyhanlı ve Hassa'dan yakalanan *Culicoides* örnek sayıları grubun tamamından farklı ($P < 0.05$) bulunurken, Samandağ ve Kırıkhan'dan yakalanan örnek sayıları ise istatistiksel olarak önemsiz ($P > 0.05$) bulunmuştur.

TARTIŞMA ve SONUÇ

MD ve EF gibi viral hastalıklara Türkiye'nin değişik bölgelerinde rastlanmaktadır. Her iki hastalık Hatay'ın da içinde bulunduğu Güney ve Güneydoğu illerinde zaman zaman salgınlar halinde seyrederek koyun, keçi ve sığırlarda ölümlere veya hastalığı atlatan hayvanlarda verim düşüklüklerine neden olmaktadır. Bu hastalıklardan MD Türkiye'nin Batı bölgelerinde salgınlar halinde ortaya çıkmış ve daha sonra ülke geneline yayılmıştır ^{5,6,13,17,18}. EF ilk kez 1980'li yılların ortalarında Güney bölgelerimizde görülmüş ⁷, daha sonraki yıllarda ise birkaç yıl aralıklarla görülmeye devam etmiştir. MD virüsünün Avrupa ve Orta Doğu'daki başlıca vektörünün

Table 1. Hatay yöresinde tespit edilen *Culicoides* türleri**Tablo 1.** *Culicoides* species found in Hatay province

Toplam	Araştırma Merkezleri											
	Antakya		Reyhanlı		Hassa		Samandağ		Kırıkhan		Toplam	
	Dişi	Erkek	Dişi	Erkek	Dişi	Erkek	Dişi	Erkek	Dişi	Erkek	Dişi	Erkek
<i>C. badooshensis</i>	-	-	-	-	2	-	3	-	-	-	5	-
<i>C. cataneii</i>	1	-	4	-	-	-	-	-	-	-	5	-
<i>C. circumscriptus</i>	3	-	62	55	92	68	-	-	-	-	157	123
<i>C. dzhafarovi</i>	-	-	1	-	-	-	-	-	-	-	1	-
<i>C. festivipennis</i>	-	-	1	1	-	-	-	-	-	-	1	1
<i>C. gejjelensis</i>	-	-	3	3	-	-	-	-	-	-	3	3
<i>C. imicola</i>	-	-	3	-	2	-	-	-	1	-	6	-
<i>C. nubeculosus</i>	7	1	-	-	2	-	-	-	-	-	9	1
<i>C. odiatus</i>	-	-	-	-	2	-	-	-	-	-	2	-
<i>C. picturatus</i>	-	-	-	-	1	-	-	-	1	-	2	-
<i>C. puncticollis</i>	6	-	14	1	1	-	-	-	-	-	21	1
<i>C. riethi</i>	-	-	2	-	-	-	-	-	-	-	2	-
<i>C. sahariensis</i>	-	-	8	3	-	-	-	-	-	-	8	3
<i>C. schultzei komp.</i>	8	-	87	17	43	8	-	-	-	-	138	26
<i>C. shaklawensis</i>	-	-	1	1	-	-	-	-	-	-	1	1
Toplam	25	2	186	81	145	76	3	-	2	-	361	159
Genel Toplam	27 ^C		267 ^A		221 ^B		3 ^D		2 ^D		520	

A,B,C,D: Aynı sütundaki farklı harfler istatistiksel açıdan önemlidir ($P < 0.05$)

C. imicola kompleks olduğu bilinmekle birlikte, 1990'lı yılların sonlarında Avrupa'nın değişik bölgelerinde, Balkan ülkelerinde çıkan MD salgınlarında *C. obsoletus* kompleks'in vektörlük yaptığı tespit edilmiştir²⁰⁻²². Sicilya'da, Mavi Dil virüsü salgını çıkan bölgelerde Nested RT-PCR ile *C. pulicaris*'den Mavi Dil viral RNA'sı saptanmıştır²³. Türkiye'de MD virüsünün vektörlerinin belirlenmesi üzerine yapılan bir çalışmada *C. circumscriptus*, *C. kibunensis*, *C. pulicaris* kompleks (*C. punctatus*) ve *C. nubeculosus*'un vektör ödevi gördükleri belirlenmiştir⁵. EF Kenya'da *C. kingi*, *C. nivosus*, *C. bedfordi*, *C. imicola* ve *C. cornutus*'tan oluşturulmuş bir havuzdan, Avustralya'da ise *C. brevitarsis* ve sivrisineklerden izole edilmiştir²⁴. Türkiye'de bu türlerden sadece *C. imicola* bulunmakla birlikte, Hatay yöresinde *C. imicola*'ya çok az sayıda rastlanmıştır. Tilki ve Dik¹⁷ tarafından İskenderun'da yapılan çalışmada da bu türe rastlanmaması, *C. imicola*'nın bu bölgede fazla yaygın olmadığını ve EF'in muhtemelen başka *Culicoides* türleri ve/veya sivrisinek türleri tarafından taşındığını akla getirmektedir. Bu durumun belirlenmesi için daha kapsamlı araştırmaların yapılması gerekmektedir.

Türkiye'de bugüne kadar *Culicoides* türleri üzerine yapılan araştırmalarda toplam 57 tür tespit edilmiştir⁹. Gerçi, Dik ve ark.¹⁶ tarafından Karadeniz Bölgesi'nde yapılan bir çalışmada, bu türlerin dışında bir türe ait olan iki dişi bireye rastlandığı belirtilmiş, fakat erkek örneklerinin olmamasından dolayı kesin tür teşhisi yapılamamıştır. Hatay yöresindeki *Culicoides* türleri üzerine

yapılan tek bir çalışma bulunmaktadır¹⁷. Hatay'ın İskenderun ilçesinde yapılan bu çalışmada *C. circumscriptus* Kieffer, *C. imicola* Kieffer, *C. longipennis* Khalaf, *C. obsoletus* kompleks (Meigen) ve *C. schultzei* kompleks (Enderlein)'e rastlandığı, *C. longipennis*'in en yaygın tür olduğu, MD'in vektörleri olan *C. imicola* ve *C. schultzei* kompleks'in daha az sayılarda yakalandığı, *C. obsoletus* kompleks'ten ise sadece üç örnek yakalandığı ifade edilmiştir. Burgu ve ark.⁶ Türkiye'nin güney illerinde yaptıkları bir çalışmada, Hatay'daki koyun ve sığırlarda MD'in yaygınlığını serolojik olarak araştırmışlar, fakat diğer bazı merkezlerden örnek yakalamalarına rağmen Hatay'dan *Culicoides* örneği toplamamışlardır. Bu çalışmada Hatay yöresinden az sayıda *Culicoides* örneği yakalanmış ve 15 *Culicoides* türü saptanmıştır. Tilki ve Dik¹⁷ tarafından bildirilen türlerden *C. obsoletus* kompleks dışındaki türlere bu araştırmada da rastlanmış, ayrıca onlar tarafından bildirilmeyen 10 tür daha tespit etmişlerdir. Her ne kadar yakalanan örnek sayısı az olmakla birlikte, tespit edilen tür sayısının 15 olması bir yöre için azımsanamayacak bir sayıdır ve daha kapsamlı yapılacak bir araştırmada bu sayının artması da kuvvetle muhtemeldir. Bu araştırmada en çok görülen tür *C. circumscriptus* olmuş, onu *C. schultzei* kompleks takip etmiştir. MD'in Avrupa'daki başlıca vektörlerinden olan *C. pulicaris* s.s. ve *C. obsoletus* kompleks ile, İç ve İç Batı Anadolu'da⁵ MD virüsü saptanan *C. punctatus* ve *C. kibunensis*'e hiç rastlanmaması, *C. imicola* ve *C. nubeculosus* kompleks'e ise çok az sayıda tesadüf edilmesi oldukça ilginçtir. Diğer taraftan MD'in muhtemel

vektörü olan *C. schultzei kompleks* ile, İç ve İç Batı Anadolu'da bu hastalığa vektörlük yaptıkları tespit edilen *C. circumscriptus*'a bu araştırmada daha sık rastlanması MD ve EF'ın naklinde bu türlerin rol oynamış olabilme ihtimallerini de artırmaktadır.

Sonuç olarak; belirli bir il veya yöredeki *Culicoides* faunasının belirlenmesi üzerine çalışmalar yapmak yerine, bu araştırmaları bölge veya ülke geneline yaymak ve özellikle vektör *Culicoides* türlerinin ekolojik ve epidemiyolojik özellikleri ve yukarıda belirtilen viral hastalıkların bulaştırılmalarındaki rolleri ile ilgili kapsamlı araştırmalara ihtiyaç bulunmaktadır.

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Toxic Effects of Cobalt II Chloride on Tissue Histopathology and Serum Proteins in *Capoeta capoeta capoeta* (Guldenstaedt 1772) ^[1]

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Summary

In this study, the effects of Cobalt (II) chloride (CoCl₂) on *Capoeta capoeta capoeta* (Guldenstaedt 1772) were investigated by electrophoretic and histopathological methods. The fish from Kars Creek were placed in 500 liters tanks and they made to adapt into the medium for 15 days. Later, they were divided into 3 groups. The fish in the 1st group were held in normal water, 2nd and 3rd groups were held in the water containing 1 mg/L and 2 mg/L CoCl₂, respectively for 10 days. At the end of this period, blood and tissue samples were taken from the fish for electrophoresis and histopathological examinations. Serum samples obtained were run in Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Tissue samples were fixed in %10 formaldehyde solution. Paraffin blocks were prepared by routine histological methods and slices 4-5 µ thickness were performed. The slices obtained were dyed with hematoxylen and eosin dying method and examined under light microscope. Thinnings in various protein types were observed in experimental groups in comparison to the control group in the electrophoregram. These thinnings were more in the group that 1 mg/L CoCl₂ applied. In addition, formation of a 32.4 kD new protein band was observed in the group that 1 mg/L was applied; and 33.3 kD, 30.6 kD, and 28.2 kD new protein bands were observed in the group that 2 mg/L CoCl₂ applied. In histopathological evaluations, an increase in the level of degeneration was observed in the livers and intestines tissues of the experimental fish groups in parallel to the increase of the dose. Due to the changes both in the serum protein expressions and in the tissues, we conclude that Cobalt can be dangerous at higher concentrations.

Keywords: Cobalt chloride, *Capoeta capoeta capoeta*, Serum protein, SDS-PAGE, Histopathology

Capoeta capoeta capoeta (Guldenstaedt 1772)'nın Serum Proteinleri ve Doku Histopatolojisi Üzerine Kobalt (II) Klorür'ün Toksik Etkileri

Özet

Bu çalışmada, *Capoeta capoeta capoeta* (Guldenstaedt 1772) üzerine Kobalt (II) klorür'ün etkileri elektroforetik ve histopatolojik yöntemlerle araştırıldı. Kars Çayı'ndan yakalanan balıklar 500 litrelik tanklara konularak 15 gün süreyle ortama adaptasyonları sağlandı. Daha sonra 3 gruba ayrılarak I. gruptaki balıklar normal su ortamında, II. ve III. gruptaki balıklar ise sırasıyla 1 ve 2 mg/L CoCl₂ içeren su ortamlarında 10 gün süreyle bekletildi. Bu süre sonunda elektroforetik ve histopatolojik çalışmalar için balıklardan kan ve doku örnekleri alındı. Elde edilen serum örnekleri Sodyum dodesil sülfat poliakrilamid jel elektroforezi (SDS-PAGE)'nde yürütüldü. Doku örnekleri ise %10'luk formaldehit solüsyonunda tespit edilerek rutin histolojik yöntemlerle parafin bloklar hazırlandı ve 4-5 µ kalınlığında kesitler alındı ve elde edilen kesitlerin tamamı hematoksilin ve eosin boyama metoduna göre boyanarak ışık mikroskopunda incelendi. SDS-PAGE'den elde edilen elektroforegramda kontrol grubuna göre deney gruplarındaki birçok protein bandında incelmeler olduğu belirlendi. Bu incelmeler 1 mg/L CoCl₂ uygulanan grupta daha fazla olduğu, bununla birlikte 1 mg/L'lik grupta 32.4 kD, 2 mg/L'lik grupta ise 33.3 kD, 30.6 kD ve 28.2 kD'luk yeni proteinlerin sentezlendiği saptandı. Histopatolojik incelemelerde ise; deney gruplarındaki balıkların karaciğer ve bağırsak dokularında doz artışıyla orantılı olarak artan derecelerde dejenerasyonlar gözlemlendi. Gerek serum protein ekspresyonlarındaki gerekse de karaciğer ve bağırsak dokusundaki değişikliklere göre, Kobalt'ın yüksek konsantrasyonlarının tehlikeli olabileceği sonucuna varıldı.

Anahtar sözcükler: Kobalt klorür, *Capoeta capoeta capoeta*, Serum protein, SDS-PAGE, Histopatoloji



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INTRODUCTION

Cobalt is an essential trace element and common all over the world. It is obligated for proper formation of vitamin B₁₂. Deficiency of cobalt in fish and other animals results in anaemia. However, the excessive intake of cobalt by organisms results in toxic effects. Cobalt concentrations in drinking water range from less than 0.1 µg to approximately 5.0 µg¹. Cobalt level in the edible muscle tissue of *Capoeta capoeta capoeta* in Kars creek was found to be 0.0038-0.373 µg⁻¹². Sediments collected in Kars Creek were tested for mutagenicity by means of peripheral erythrocyte in *Orthrias angorae*. Micronuclei frequencies (MN) of all the groups exposed to the sediments were higher than those of the control group. Researchers claimed that the presence of mutagenic agents in Kars Creek sediments³.

Cobalt and its alloys are widely used in many industrial fields such as automobile, fuel, battery, paint, metallurgy, and cosmetic. Due to the common use of cobalt and its derivatives on countless fields, contamination of environment, especially contamination of the natural waters, is a big environmental concern⁴. Due to high levels of cobalt contamination, majority of organisms are in the risk. Despite the fact that cobalt possesses a serious environmental risk, the studies that evaluated the effects of cobalt compounds on serum proteins and other tissues are rare⁴⁻⁶.

The aim of the present study was to investigate the effects of CoCl₂ on *Capoeta capoeta capoeta*. We particularly studied the histopathological changes in liver, intestine and the serum protein levels after the fish was exposed to Cobalt (II) chloride.

MATERIAL and METHODS

Experimental Design

Eighteen *Capoeta capoeta capoeta*, weighing 200-250 g, were caught in Kars Creek, Turkey. Water quality of the creek during the collection of fish was as follow; pH 7.8-8.0, dissolved oxygen 5.1-8.8 mg/L and temperature 17-18.5°C. Following the collection of the fish, they were divided into three equal groups and placed in 500-L aquariums. Tap water temperature was adjusted to 18±1°C with a thermostatic thermometer and dissolved oxygen was supplied at 5±0.3 mg/L concentration in the aquariums.

The fish in group I (control) were kept in a tank that contains tap water, and the fish in groups II and III were kept in tanks that contain 1 mg/L and 2 mg/L cobalt (II)

chloride, respectively, for 10 days. The fish were fed daily with standard fish-feed.

Electrophoresis

Blood samples were taken from the dorsal aorta of fish by using an injection syringe, they were centrifuged for 10 min at +4°C and 805X g, the serums were separated and total protein contents were measured by the Biuret method⁷. SDS-PAGE was performed according to the methods of Laemmli⁸ and O'Farrell⁹. The gels were photographed and molecular weights of proteins were calculated according to the method of Weber et al.¹⁰. Calf albumin (66 kD), egg albumin (45 kD), carbonic anhydrase (29 kD) and β-lactoglobulin (18 kD) were used as standard protein. The percentages of the changes in serum protein bands were calculated according to,

$$\frac{\text{The number of protein bands changed}}{\text{The number of total protein bands}} \times 100$$

formula.

Histopathology

The tissue samples collected for histopathological analyses were fixed in 10% phosphate buffered formaldehyde solution for 48 h. Gill samples were decalcified with Osteodec (Bio-Optica, Italy). Paraffin blocks were prepared from the collected tissues by routine methods, and slices of 4-5 µm thickness were taken. All the obtained slices were stained according to hematoxylen and eosin staining method, and examined under the light microscope.

RESULTS

It was determined that in the SDS-PAGE obtained through electrophoregram there were thinning in various protein types of experimental groups in comparison with control group and these thinning were more in the group that 1 mg/L CoCl₂ applied. Furthermore, it was found that the fish kept in 1 mg/L CoCl₂ synthesized a new 32.4 kD protein band, and those kept in 2 mg/L synthesized three new protein bands of 33.3 kD, 30.6 kD, and 28.2 kD (Fig. 1). As a result of exposure to cobalt (II) chloride, the percentages of change occurring in the serum protein bands were shown in Table 1.

Table 1. As a result of exposure to cobalt (II) chloride, the percentages of change occurring in the serum protein bands

Tablo 1. Kobalt klorür maruziyetine bağlı olarak serum protein bandlarında meydana gelen değişim yüzdeleri

Concentrations	Thinnings	New Protein Bands
1 mg/L	%80	%10
2 mg/L	%30	%30

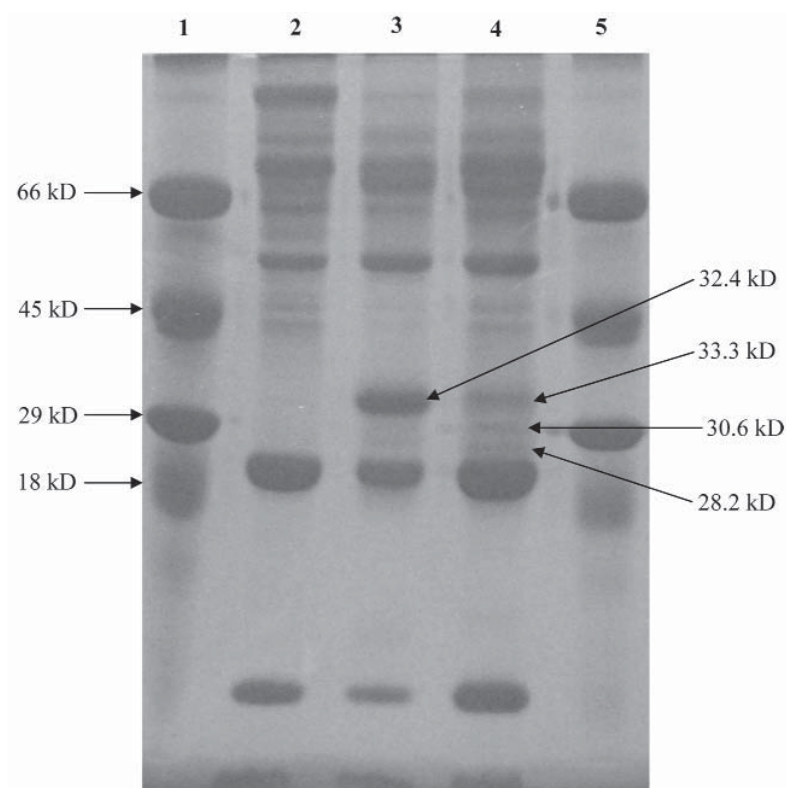
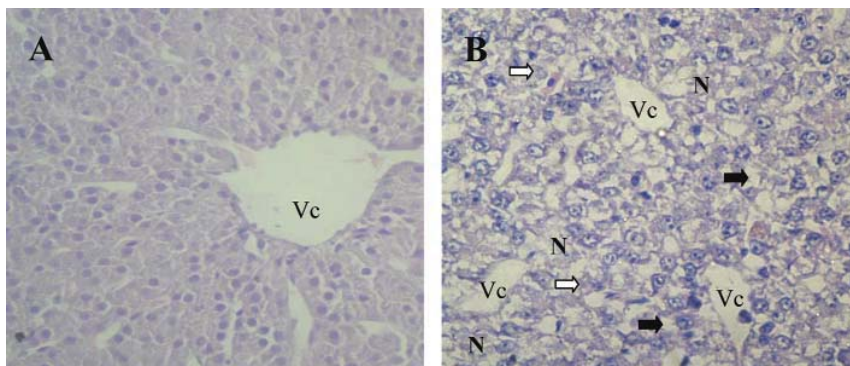


Fig 1. Electrophoregram obtained from the SDS-PAGE of the serum proteins of the fish exposed to cobalt (II) chloride. **1-5.** Standard proteins; **2.** Control group; **3.** 1 mg/L dose group; **4.** 2 mg/L dose group

Şekil 1. Kobalt (II) klorür'e maruz kalan balıkların serum proteinlerinin SDS-PAGE'den elde edilen elektroforegramı. **1-5.** Standart proteinler; **2.** Kontrol grubu; **3.** 1 mg/L'lik grup; **4.** 2 mg/L'lik grup

Fig 2. A- Liver tissue in the control group, **B-** Widespread areas of necrosis (N) of hepatocytes in the liver tissue of the fish exposed to CoCl_2 , hidropic (white arrows) and areas of dissociation with vacuolar degenerations (black arrows) (H&E X 40)

Şekil 2. A- Karaciğer dokusu kontrol grubu. **B-** CoCl_2 uygulanan balıklardan elde edilen karaciğer dokusunda yaygın nekroz alanları (N), hidropik (beyaz oklar) ve vakuolar (siyah oklar) ile birlikte dissosiasyon alanları (H&E X 40)



When slices obtained from liver and intestine examined under the light microscope, the most remarkable histopathological changes were found in liver. Liver (*Fig. 2A*) and intestine (*Fig. 3A*) slices of the control group did not deviated from the normal architecture. In histopathological evaluations, common growing areas of necrosis, hidropic degenerations and dissociation with vacuolar degenerations were observed (*Fig. 2B*) in parallel to increase in the dose. Hemorrhage, desquamation and necrosis in villous epithelium were detected in the intestinal tissue (*Fig. 3B, C*).

DISCUSSION

For humans, food and beverages represent the main

source of cobalt intake. Traces of cobalt are also present in cement and various household products. In industry, the potential for exposure to cobalt is particularly important during the production of cobalt powder, the production, processing and use of hard metals, the polishing of diamonds with cobalt containing disks, and the processing of cobalt alloys ¹¹.

Effects of cobalt toxicities on various organisms have been documented ^{4,12,13}. Effects of cobalt on hepatotoxicity in adult rats and their suckling pups have been investigated and histological studies revealed an infiltration of mononuclear cells and vascular congestion in liver pups and their mothers ¹². In another study, genotoxic and histopathological effects of heavy metal accumulation (Cd, Cu, Co, Pb and Zn) on *Chondrostoma*

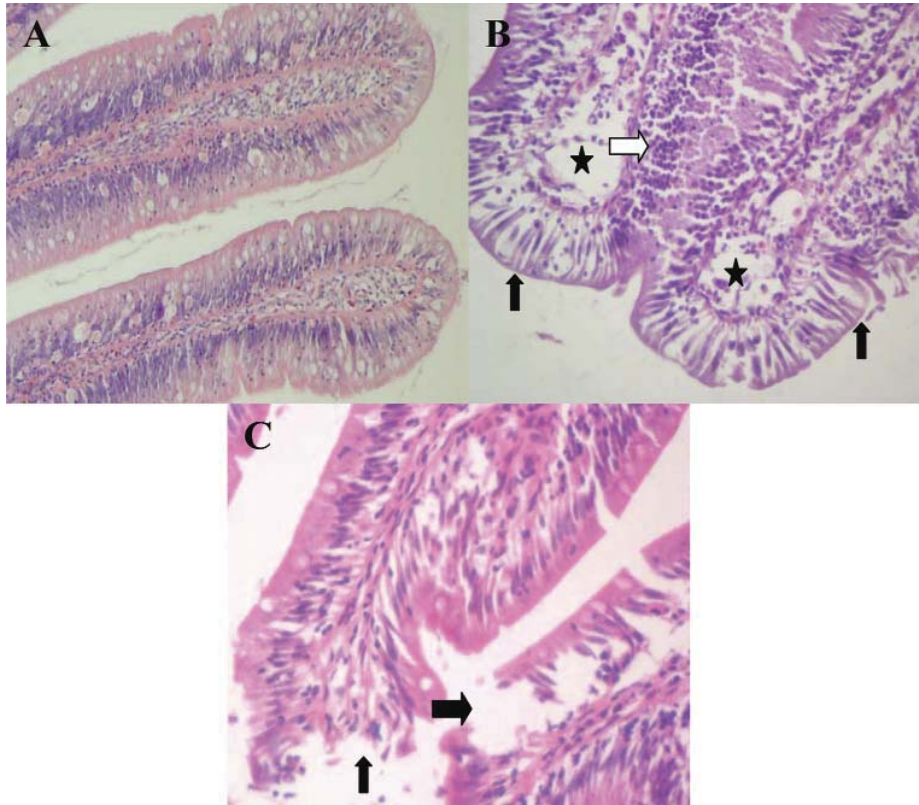


Fig 3. **A-** Intestine tissue in the control group, **B, C-** Hemorrhage, desquamation in intestine tissue of the fish exposed to CoCl_2 (white arrows), necrosis in villous epithelium (black arrows) and degeneration in lamina propria (stars) (H&E X 40)

Şekil 3. **A-** Bağırsak dokusu kontrol grubu. **B, C-** CoCl_2 uygulanan balıklardan elde edilen bağırsak dokusunda deskuomasyon, hemoraji (beyaz ok), villus epitelinde nekroz (siyah oklar) ve lamina propriada dejenerasyon (yıldızlar) H&E X 40)

nasus and *Barbus capito pectoralis* in Büyük Menderes River and Cine stream (Turkey) were investigated. The histopathological results indicated significant decreases of mean lengths of primary and secondary lamellae. The changes in gill epithelia included in cellular proliferation with secondary fusion, ballooning degenerations, club deformation of secondary lamellae, and cystic structures in secondary lamellae. The changes in liver included in swollen and ruptured parenchymal cells, some loss of cord structure, vacuoles filled with cellular debris, focal necrosis, and a significant increase in Kupffer cells¹⁴. The concentration of the heavy metals (Fe, Zn, Cu, Pb, Cd and Co) in the water and various fish organs of *Oreochromis niloticus* and *Lates niloticus* obtained from Lake Nasser, Egypt were investigated by atomic absorption spectrophotometer. Likewise, histopathological alterations due to higher concentrations of these metals were observed. Results showed that several histopathological alterations were observed including vacuolar degeneration with focal areas of necrosis in liver, proliferation in the epithelium of gill filaments, fusion of secondary lamellae, severe degenerative and necrotic changes in the intestinal mucosa, seminiferous tubules, degeneration and atrophy in cardiac muscle fibers, and degeneration in muscle bundles⁵.

Serum albumins are synthesized in liver, and changes in serum proteins are inevitable during the metal toxicity.

In our previous study, toxic effects of cobalt parahydroxybenzoate in liver, gills and intestine and serum proteins of *Capoeta capoeta capoeta* were investigated. We observed a decrease in the expressions of proteins with molecular weights of 85.8, 82.6, 73.9, and 68.5 kD while an increase in the expression of the proteins with molecular weights of 23.0 and 15.2 kD. Therefore, we concluded that the change in serum proteins may be a response to cobalt exposure especially in the liver. In histopathologic examination, degeneration in hepatocytes and dissociation in hepatic cords were observed in the treatment groups. The severity of the degeneration and the dissociation increased, and necrosis was noted in the liver of fish exposed to higher concentration of cobalt parahydroxybenzoate. In gill, degeneration and occasional necrosis in the epithelial cells of secondary lamellae were detected in both groups. Degeneration and necrosis of villous epithelium in the intestine were observed only in fish exposed to higher concentration of cobalt parahydroxybenzoate⁴. Histological aspects of liver and intestinal tissue of the current study show similarities to the findings of abovementioned study. Nevertheless, the serum proteins showed differences in direction. In the previous study in which we evaluated the effects of cobalt parahydroxybenzoate application, synthesis of a new serum protein and inhibition in the expression of some other proteins were observed.

However, in the present study new proteins were synthesized when fish exposed to cobalt (II) chloride. Based on finding in this study, we propose that the newly synthesized proteins are a part of response to cobalt exposure.

In conclusion, different cobalt concentrations can have different toxic effect on fish, and the excessive cobalt exposure causes damage in the liver and the intestine of *Capoeta capoeta capoeta*. Results of this study can be used as a guide for biomonitoring studies of cobalt toxicity on fish.

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Molecular Analysis of Three Local Silkworm Breeds (Alaca, Bursa Beyazı and Hatay Sarısı) by RAPD-PCR and SDS-PAGE Methods ^[1]

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Summary

In this study, PCR-based RAPD method was used to determine the genetic variation in three Turkish local silkworm breeds (Alaca, Bursa Beyazı and Hatay Sarısı) and SDS-PAGE method was used to analyse the sericin proteins of their cocoons. Three local breeds were analysed by randomly chosen 40 primers and 68 total RAPD bands observed with 7 of them. Percentages of polymorphic loci were determined higher in Hatay Sarısı (55.88), lower in Bursa Beyazı and Alaca (44.12). Nei's genetic distance was determined 0.0637 between Bursa Beyazı and Alaca, 0.1012 between Bursa Beyazı and Hatay Sarısı, 0.0793 between Alaca and Hatay Sarısı. In SDS-PAGE sericin proteins of three breeds was yielded a single band of 200 kDa.

Keywords: *Bombyx mori*, RAPD-PCR, Genetic polymorphism, SDS-PAGE

Üç Yerli İpekböceği Irkının (Alaca, Bursa Beyazı ve Hatay Sarısı) RAPD-PCR ve SDS-PAGE Yöntemleri ile Moleküler Analizi

Özet

Bu çalışmada, Türkiye'nin üç yerli ipekböceği ırkında (Alaca, Bursa Beyazı ve Hatay Sarısı) genetik varyasyonu belirlemede PCR-temelli RAPD yöntemi, bu üç ırkın kokonlarında bulunan serisin proteinlerinin analizinde ise SDS-PAGE yöntemi kullanıldı. Üç yerli ırk rasgele seçilen 40 primer ile analiz edildi ve bunlardan 7 tanesi ile toplam 68 RAPD bandı gözlemlendi. Polimorfik lokusların yüzdesi, en yüksek Hatay Sarısı'nda (55.88) en düşük ise Bursa Beyazı ve Alaca'da (44.12) belirlendi. Nei'nin genetik mesafesi Bursa Beyazı ile Alaca arasında 0.0637, Bursa Beyazı ile Hatay Sarısı arasında 0.1012, Alaca ile Hatay Sarısı arası 0.0793 belirlendi. Her üç ırkın serisin proteinlerine ait SDS-PAGE analizinde 200 kDa'lık tek bant görüldü.

Anahtar sözcükler: *Bombyx mori*, RAPD-PCR, Genetik polimorfizm, SDS-PAGE

INTRODUCTION

The domesticated silkworm ¹, *Bombyx mori*, an economic silk secreting insect, comprises a large number of varieties in temperate and tropical countries ^{2,3}. The tropical varieties (nondiapausing) produce poor quality of silk while the temperate varieties (diapausing) produce good quality of silk ^{2,3}.

Recently, genetic markers have used in animal and plant improvement programmes for varietal and parentage identification, construction of linkage maps and evaluation

of polymorphic genetic loci affecting quantitative economic traits ⁴. Development of molecular markers is important in the silkworm for construction of linkage map and fingerprinting of strains for breeding ⁴. Recently, PCR-based techniques have been widely used to detect the polymorphic genetic markers in the silkworm ^{4,5}. Random amplified polymorphic DNAs (RAPDs) is one of the PCR-based techniques used as a tool for genetic mapping and strain identification ^{2,4,6-9}. Because of its relative simplicity, RAPD method is being extensively used in genetic analysis ⁵.



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The *Bombyx mori* cocoon consists of fibroin and sericin proteins¹⁰. Sericin is a group of glue proteins produced in the middle silk gland (MSG) of the silkworm. It surrounds fibroin fibers and binds them to each other in the cocoons¹¹. Recently, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method, has frequently used to determine the molecular weight of sericin protein^{10,11}. SDS-PAGE is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight). In this study, SDS-PAGE method was also preferred to determine the molecular weight of sericin proteins.

Turkish local silkworm breeds are inbred in Bursa Provincial Directorate of Agriculture. Alaca and Bursa Beyazı are diapausing genotypes (temperate genotype; high-quality silk) but Hatay Sarısı is non-diapausing genotype (tropical genotype; low-quality silk). Bursa Beyazı have a higher silk quality than others.

The aim of this study was to estimate the genetic polymorphism between three Turkish silkworm breeds by using RAPD-PCR method and to analyze the sericin proteins of three breeds by SDS-PAGE.

MATERIAL and METHODS

Collection of Silkworm Stocks

Individuals of three Turkish silkworm breeds and cocoons were obtained from Bursa Silkworm Breeding Research Institute.

Genomic DNA Isolation

Genomic DNA was isolated from adult's tissue. Firstly, tissue was pulverized in liquid nitrogen with mortar and pestle. Then powder was placed in a 1.5 ml micro-centrifuge tube and resuspended in TE buffer. Fermentas Genomic DNA Purification KIT® was used in DNA isolation. Lysis solution (KHCO₃, NH₄Cl, 0.5 M EDTA, pH: 8.0) was put into sample and incubated at 65°C for 10 min. Chloroform was added and gently emulsified by inversion and centrifuged. The upper phase containing DNA was transferred to a new tube and precipitation solution was added. Supernatant was removed and DNA pellet was dissolved in 100 µl of 1.2 M NaCl solution by vortexing. Cold ethanol was added and DNA was precipitated at -20°C for 10 min. Then ethanol was poured off and pellet was washed once with 70% cold ethanol and DNA was dissolved in sterile deionized water by gentle vortexing. DNA concentration was measured by spectrophotometer and diluted to 5 ng/µl.

Amplification and Separation of DNA

PCR reaction in a 25 µl volume contained 0.2 u/µl Taq DNA polymerase, 1X PCR buffer, 3 mM MgCl₂, 1.25 mM dNTP, 0.6 µM primer. For this study 40 primers were chosen arbitrarily (Table 1). The reactions were initiated at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 34°C for 30 sec and 72°C for 45 sec. Final extension time was 72°C for 10 min. The amplified products were separated on 1.7% agarose gel in 1X TBE buffer. The products were run at 100 V for 2.5 h. Amplified products were detected under UV light by staining 0.5 µg/ml ethidium bromide. DNA band profiles were evaluated with 100 bp DNA marker.

Table 1. Primer base sequences and the ratio of guanine-cytosine bases (G-C)

Tablo 1. Primerlerin baz dizileri ve G-C bazlarının oranı

Primer	Primer Sequence (5'---3')	G- C (%)
1	CTGGGGACTT	60
2	CTGAGACGGA	60
3	AGCGTCCTCC	70
4	TGGGCGTCAA	60
5	GGGCGGTACT	70
6	AGCCTGAGCC	70
7	ACCACCCACC	70
8	ACAACGCCTC	60
9	GGCGGTTGTC	70
10	GGGACGTTGG	70
11	ACTGAACGCC	60
12	ACAACGGGGG	60
13	GTCAGAGTCC	60
14	TCGGCGGTTT	70
15	GAGGTCCACA	60
16	GTCAGTGCGG	70
17	GTCCGGAGTG	70
18	GTGACCGAGT	60
19	ACGCAAGAGG	60
20	AGACGATGGG	60
21	GGGTCGCGGT	80
22	GGTCGATCTG	60
23	AGTCGCCCTT	60
24	GGGCCAATGT	60
25	GTGGAGTCAG	70
26	TGAGGGTCCC	70
27	AGCCGTGGAA	60
28	GTGTCGCGAG	70
29	CTCTGGAGAC	60
30	GGGAATTCGG	60
31	TGTCATCCCC	60
32	AAGCCTCGTC	60
33	GGAAGGAGT	60
34	GGTGACGCAG	70
35	CCTTGACGCA	70
36	TTCCCCCGCT	70
37	AAGCTTGATTGCC	50
38	AAGCTTCGACTGC	50
39	AAGCTTTGGTCAG	50
40	AAGCTTCTCAACG	50

Data Analysis

Data were analysed using POPGENE Software (POPGENE VERSION 1.31 Microsoft Window-based Freeware for Population Genetic Analysis) program. Polymorphism was detected according to presence or absence of bands. Absence of band was recorded as 0 and presence of band was recorded as 1. Percentage of polymorphic loci, number of polymorphic loci, observed number of alleles (na), the gene diversity (h =the mean percentage of heterozygous per locus), the diversity within the populations (H_s), the magnitude of differentiation among the populations (GST), Nei's genetic distance ¹² (accounts for multiple mutations per locus in populations) was determined.

Preparation of the Silk Sericin Protein

The cocoon samples (450 mg) cut into the pieces and washed repeatedly with hot water. Then it was immersed in 3 ml of distilled water overnight. It was put into a high-pressure boiler at 120°C for 1 h. The degumming solution was filtered to remove precipitates. The resulting solution with yellow color due to the higher molecular weight of the sericin peptide ¹⁰.

SDS-PAGE Analysis

Sericin proteins of three local breeds were determined by sodium dodecyl sulfate-polyacrylamide gel electro-

phoresis (SDS-PAGE) according to the method previously by Laemmli ^{13,14} with 12% acrylamide gel and 5% condensing gel. Then gel was stained with 0.25% Coomassie Brilliant Blue R-250 ¹⁰.

RESULTS

RAPD-PCR: In this study 40 arbitrarily primers were used and 61 polymorphic loci were produced with only seven of them. These seven primers and RAPD-DNA bands are given in [Table 2](#).

The primer called OPN-05 produced 900 bp DNA bands in some individuals of Bursa Beyazı and Alaca. But 900 bp DNA bands weren't observed in Hatay Sarısı. DNA band profile obtained with OPN-05 is given in [Fig. 1](#).

Table 2. Approximate size of alleles (bp) in silkworm breeds

Tablo 2. İpekböceği ırklarından elde edilen allellerin yaklaşık büyüklükleri (bç)

Primers	Approximate Base Pairs (bp)
OPL12	200-1500
OPL18	225-900
OPN05	350-1100
OPU19	200-1500
OPY11	200-800
OPY13	250-425
OPY15	275-1000

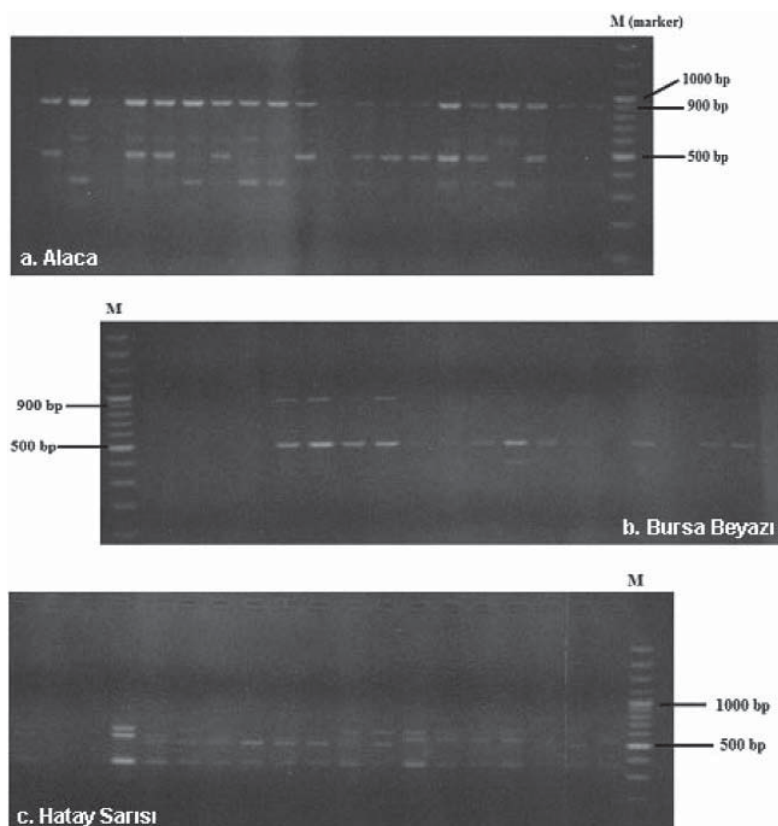


Fig 1. RAPD bands obtained with OPN-05 primer

Şekil 1. OPN-05 primeri ile elde edilen RAPD bantları

OPU-19 produced 650 bp DNA bands in some individuals of Hatay Sarısı. DNA band profile obtained with OPU-19 is given in [Fig. 2](#).

POPGENE program was used to analyze data. According to these data, percentage of polymorphic loci varied between 44.12 (Bursa Beyazı and Alaca) and 55.88 (Hatay Sarısı). Number of polymorphic loci was the highest in Hatay Sarısı but the lowest in Bursa Beyazı and Alaca. Na, ranged between 1.4412 and 1.5588. The highest gene diversity was recorded in Hatay Sarısı (0.1396) and the lowest gene diversity was observed in Bursa Beyazı (0.1176). Hs was 0.1299 and the GST was 0.2586. Nei's genetic distance was 0.1012 between Bursa Beyazı - Hatay Sarısı, 0.0637 between Bursa Beyazı - Alaca and 0.0793 between Alaca - Hatay Sarısı. According to these data, Bursa Beyazı and Alaca are the closest breeds but Hatay Sarısı and Bursa Beyazı are the most divergent breeds as genetic.

SDS-PAGE: In SDS-PAGE sericin proteins of three local breeds yielded a single band of 200 kDa ([Fig. 3](#)).

DISCUSSION

In this study three Turkish silkworm breeds were analysed by RAPD-PCR method to obtain molecular DNA markers. For this analysis, 40 arbitrarily primers were used and 61 polymorphic loci were obtained from only seven of them. Diapausing (Bursa Beyazı and Alaca) and non-diapausing (Hatay Sarısı) local breeds were separated by these primers successfully.

Similarly, Thanananta ¹⁵ used RAPD-PCR and separated five morphological races to diapausing and non-diapausing varieties. Nagaraja and Nagaraju ² used 40 random primers in 13 silkworm genotypes. Diapausing and non-diapausing genotypes were

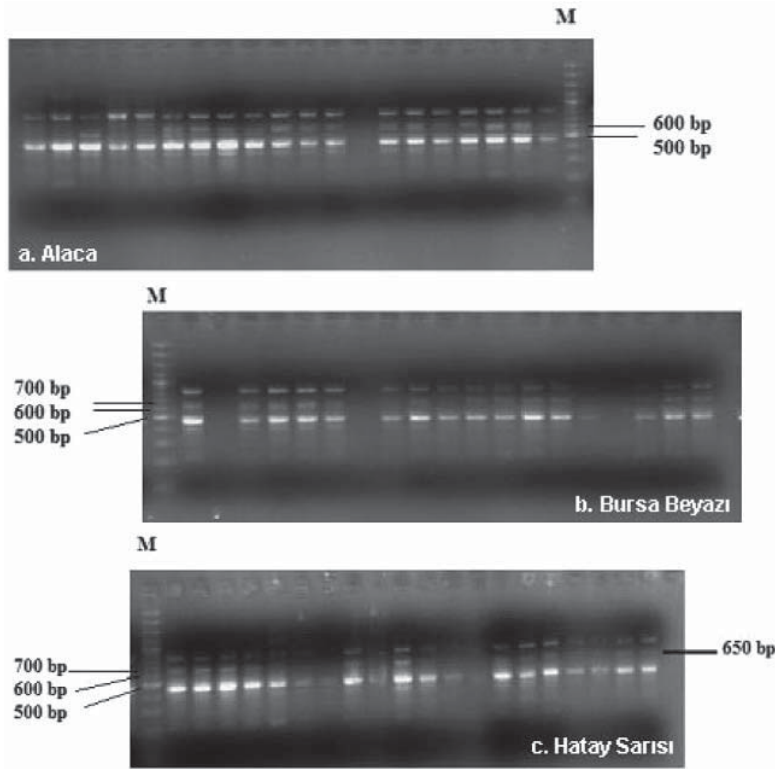
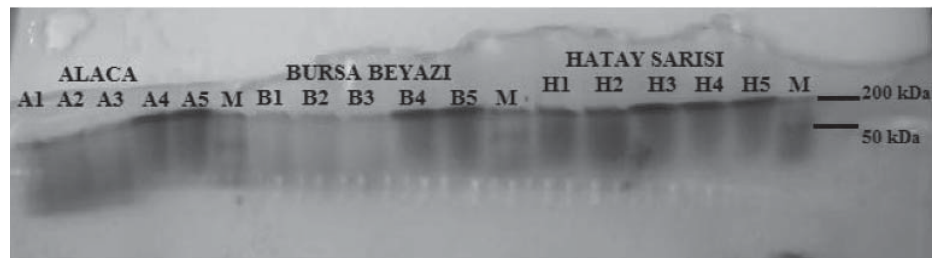


Fig 2. RAPD bands obtained with OPU-19 primer

Şekil 2. OPU-19 primeri ile elde edilen RAPD bantları

Fig 3. Band profiles of sericin proteins in SDS-PAGE

Şekil 3. SDS-PAGE'de serisin proteinlerinin bant profilleri



distinguished by 5 specific markers. The marker OPA-02 (800) was observed in only non-diapausing genotypes. Similarly ¹⁶ the marker OPA-02 (800) was observed in Hatay Sarısı which is a local nondiapausing genotype of Turkey. OPN-05 (900 bp), OPU-19 (650 bp), OPA-02 (800 bp) can be used in characterization of three local breeds.

Sprague ¹⁷ reported the presence of more than 15 sericin polypeptides that have molecular masses ranging from 20 to 220 kDa. Takasu et al.¹¹ found three polypeptides with molecular masses of the 400, 250 and 150 kDa estimated by SDS-PAGE. Zhang et al.¹⁰ characterized the sericin protein with the wide range of molecular weight from about 50 kDa to over 200 kDa. Dash et al.¹⁸, characterized the sericin of the tropical tasar silkworm, *Antheraea mylitta* by the SDS-PAGE. A single band of approximately 200 kDa was detected. Similarly in our study, sericin protein of three breeds yielded a single band of 200 kDa by the SDS-PAGE.

There is no sufficient study about molecular characterization of local silkworm breeds. In this study, three Turkish silkworm breeds were examined by RAPD-PCR and SDS-PAGE. Local silkworm breeds were separated as diapausing (Bursa Beyazı and Alaca) and non-diapausing (Hatay Sarısı) varieties by RAPD-PCR method. OPN-05 (900), OPU-19 (650) RAPD bands and 200 kDa of sericin protein band can be used in characterization of local breeds. This and other molecular studies are important to have a body of knowledge about national gene resources. In conclusion, the results obtained from this study can be used for improvement programmes of Turkish silkworm breeds in future.

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The Effects of Diets Supplemented with CaCl₂ and Rock Salt on Growth, Feed Efficiency and Some Serum Mineral Levels in Rainbow Trout, *Oncorhynchus mykiss* ^[1]

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Summary

In this study, the effects of CaCl₂ (2%) supplemented diet, rock salt (RS, 2%) supplemented diet and control diet (no supplement) on the weight gain, specific growth rate, daily feed intake, feed conversion rate, protein efficiency rate and serum mineral concentration (Na, K, Cl) were investigated in rainbow trout. RS was used for increasing the CAD and Na/K ratio of the control diet and CaCl₂ was used for decreasing the CAD level of the control diet. A total of 300 fish, 2 replications for each treatment consisting of 50 fish with a mean initial body weight of 88.2 g were used in the experiment. The experiment lasted fifteen weeks. The diet supplemented with 2% RS improved significantly the weight gain, feed conversion rate and protein efficiency rate in rainbow trout compared to 2% CaCl₂ supplemented group and control (P<0.05). Dietary RS supplementation also improved the specific growth rate and daily feed intake. However, this improvement was not statistically significant (P>0.05). Dietary 2% CaCl₂ supplementation did not significantly affect the weight gain, specific growth rate, daily feed intake, feed conversion rate and protein efficiency rate in rainbow trout compared to control diet (P>0.05). Na, K and Cl level of the serum and mortality rate were not statistically different among the groups (P>0.05). The dietary 2% RS supplementation produced a better growth and feed efficiency for rainbow trout compared to dietary 2% CaCl₂ supplementation and control diet. Added RS to diet was appropriate for making cationic the diet and increasing the CAD level and Na/K ratio of diet.

Keywords: Rainbow trout, CaCl₂, Rock salt, Cation-anion difference (CAD), Growth, Feed efficiency, Serum mineral level

CaCl₂ ve Kaya Tuzu İlave Edilmiş Rasyonların Gökkuşaağı Alabalığının (*Oncorhynchus mykiss*) Büyüme ve Yemden Yararlanma ve Kimi Serum Mineral Düzeylerine Etkisi

Özet

Bu çalışmada, CaCl₂ (%2) ve kaya tuzu (%2) ilave edilmiş diyetlerin kontrol diyetine (ilave katkı yok) kıyasla Gökkuşaağı alabalıklarında (*Oncorhynchus mykiss*) canlı ağırlık kazancı, spesifik büyüme oranı, günlük yem alımı, yem dönüşüm oranı, protein etkinlik oranı ve serum mineral konsantrasyonu (Na, K, Cl) üzerine etkileri araştırıldı. Kaya tuzu ilavesi kontrol diyetinin Katyon-anyon farkını ve Na/K oranını arttırmak için, CaCl₂ ilavesi ise kontrol diyetinin katyon-anyon farkını düşürmek için kullanıldı. Her birinde 50 balığın kullanıldığı 2 tekerrürlü denemelerde başlangıç ortalama ağırlıkları 88.2 g olan, 300 gökkuşaağı alabalığı kullanıldı. Deneme on beş hafta sürdürüldü. %2 Kaya tuzu ilave edilmiş diyet, %2 CaCl₂ ilave edilen diyet ve kontrol diyetine göre gökkuşaağı alabalığında canlı ağırlık kazancını, yem dönüşüm oranını ve protein etkinlik oranını önemli şekilde iyileştirmiştir (P<0.05). Yeme kaya tuzu ilavesi spesifik büyüme oranını ve günlük yem alımını da ilerletmiştir. Ancak bu ilerleme istatistiksel olarak önemsiz bulunmuştur (P>0.05). %2 CaCl₂ ilave edilen diyet ile kontrol diyeti arasında canlı ağırlık kazancı, spesifik büyüme oranı, günlük yem alımı, yem dönüşüm oranı, protein etkinlik oranı açısından fark bulunmamıştır. Serum Na, K ve Cl seviyeleri ve ölüm oranları açısından gruplar arasında farklılık bulunmamıştır (P>0.05). Diyetle %2 Kaya tuzu ilavesi, kontrol grubuna ve %2 CaCl₂ ilave edilen gruba kıyasla gökkuşaağı alabalığında daha iyi büyüme ve yem değerlendirme sağlamıştır. Gökkuşaağı alabalığı diyetine kaya tuzu ilavesi, diyeti katyonik hale getirmek ve diyetin katyon-anyon farkını ve Na/K oranını arttırmak için uygundur.

Anahtar sözcükler: Gökkuşaağı alabalığı, CaCl₂, Kaya tuzu, Katyon-anyon farkı (KAF), Büyüme, Yem değerlendirme, Serum mineral seviyesi



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INTRODUCTION

Dietary cation anion difference, CAD, is defined as $\text{Na}+\text{K}-\text{Cl}$ and expressed as mEq kg^{-1} . Dietary CAD level has an influence on blood pH, feed intake and growth in dairy cows ¹, dairy calves ², growing steers, lambs ³, chicken ⁴, horses ⁵ and juvenile African catfish, *Clarias gariepinus* ⁶. In juvenile African catfish, Dersjant-Li et al. showed that feed intake and growth increased linearly with dietary cation anion difference from -100 to 700 mEq kg^{-1} , and the level of 700 mEq kg^{-1} was optimal ⁶. In their later study, Dersjant-Li et al.⁷ investigated the effect of dietary Na/K ratios on feed intake, growth and nutrient utilisation of juvenile African catfish at an optimal dietary CAD level of 554-657 mEq kg^{-1} and showed that the dietary Na/K ratio of 1.5 to 2.5 produced the best growth in African catfish.

Some researchers reported that effects of CAD were no clear on growth in rainbow trout, *Oncorhynchus mykiss* ⁸⁻¹¹. We have only limited data on the effects of dietary CAD level and dietary Na/K ratio on growth and feed efficiency in rainbow trout. However Arabacı, Nursoy and Diler ¹² concluded that growth increased linearly, feed conversion rate decreased linearly with increasing dietary Na/K ratio-CAD level and daily feed intake was not significantly influenced in rainbow trout. They observed that the best growth was taken with the dietary Na/K ratio and dietary CAD level between 2.15-560 and 3.53-981 ($\text{mol mol}^{-1}\text{-mEq kg}^{-1}$) for rainbow trout. For regulating the dietary Na/K ratio and dietary CAD level, they supplemented the rainbow trout diet with NaHCO_3 and Na_2CO_3 .

In this study, rainbow trout diet were supplemented with cationic (Rock salt, RS) and anionic salt (CaCl_2). 2% RS was used for increasing the CAD and Na/K ratio of the control diet and 2% CaCl_2 was used for decreasing the CAD level of the control diet.

The objective of the study was therefore to determine the effects of diets, supplemented with CaCl_2 (2%) and rock salt (2%), on growth, feed efficiency and serum mineral concentration in rainbow trout.

MATERIAL and METHODS

Fish and Facilities

The study was conducted at Institute of Mediterranean Fisheries Research, Production and Training, Kepez Unit, Antalya, Türkiye. A total of 300 rainbow trout, 2 replications for each treatment consisting of 50 fish with a mean initial body weight of 88.2 ± 2.4 g (mean $\pm$ SE) were used

in the experiment. Before the commencement of the study, fish were acclimated to rearing conditions for 2 weeks. The study, dietary 2% CaCl_2 and RS supplementation were tested, lasted fifteen weeks. Fish were reared at a temperature of 11.2-15°C, under natural light regime. The O_2 concentration was maintained above 6 ppm. Six concrete ponds were used in the study. Each tank, having 40x40x200 cm of dimensions and with 280 liters capacity, received a constant flow of water (1.0 l.s^{-1}). Hardness and conductivity of water used in the experiment was 370 $\text{mg/l} \text{ CaCO}_3$ and 650 $\mu\text{mho.cm}^{-1}$, respectively.

Diets and Feeding

The basal diet (Control) supplemented with rock salt and CaCl_2 . Supplementation level was selected as 2% for seeing clearly the effects of the salts in the diet. The basal diet was given in [Table 1](#).

Diets were pelleted at the size of 3 mm. During fifteen weeks of feeding period, meals were supplied to fish manually three times daily (at 09:00, 13:00 and 17:00 h) according to feeding rate. Feeding rate was predetermined according to live weight and water temperature (1-20 days, 2% at 15°C; 21-41 days, 1.8% at 13°C; 42-62 days, 1.7% at 12°C; 63-83 days, 1.5% at 11.5°C; 84-105 days, 1.5% at 11.2°C). However, feed was supplied in small quantities, assuring that fish ate to satiation (*ad libitum*) and no feed was wasted. When predetermined feed amount was not sufficient for satiation, more feed was given to fish. The amount of given feed per day was recorded. Live weight was determined 6 times with 21-day intervals from the initiation of experiment to the end of experiment.

Blood (1-2 ml) was sampled from 12 fish in each group, by inserting a syringe into the caudal vessels. The blood samples were centrifuged (1.500 g for 20 min) to obtain serum. Serum samples were stored -20°C until analysis of mineral contents. In each serum sample, Na^+ , K^+ and Cl^- levels were measured in Easy Lyte Plus Na/K/Cl electrolyte analyser by ISE method.

Dietary pH was measured as described by Tarakçı ¹⁶. Dry matter (105°C, overnight), ash (550°C, overnight), crude protein (nitrogen $\times$ 6.25), ether extract, crude fiber of diets were analysed by methods of AOAC ¹⁵. Dietary $\text{Na}^+ + \text{K}^+ - \text{Cl}^-$ or CAD level (mEq kg^{-1}) were determined by Horst et al. and dietary Na/K ratio (mol/mol) was measured from the Na^+ , K^+ and Cl^- in diets which were analysed according to Kaçar ^{1,17}. Calculations used in this study were given in [Table 2](#) ^{18,19}.

Statistical Analysis

Results are presented as mean values $\pm$ standard

Table 1. Ingredients and chemical composition of the diets used in the experiment. RS; Rock salt**Tablo 1.** Deneme diyetlerinin içerikleri ve kimyasal analizler. RS; Kaya tuzu

Ingredients %	Treatment Diets		
	2% CaCl ₂ Suppl.	Control	2% RS Suppl.
Fish meal	45	45	45
Soybean meal	15	15	15
Full fat soybean meal	10	10	10
Blood meal	3	3	3
Wheat middlings	12	14	12
Fish oil	10	10	10
CaCl ₂	2	-	-
Rock salt	-	-	2
Vitamin premix ¹	2	2	2
Mineral premix ²	1	1	1
Vitamin C ³	0.3	0.3	0.3
Choline ⁴	0.15	0.15	0.15
Methionine	0.15	0.15	0.15
Pellet binder	0.4	0.4	0.4
Antioxidant	0.3	0.5	0.3
TOTAL	100.0	100.0	100.0
Analysed Composition			
Dry matter, %	89.25	90.20	89.36
Crude protein, %	46.80	48.01	48.52
Ether extracts, %	13.31	14.81	14.11
Crude fiber, %	2.24	2.01	2.05
Crude ash, %	10.61	10.08	10.66
Na, %	0.23	0.26	0.84
K, %	1.01	1.01	0.93
Cl, %	0.98	0.96	0.94
DE*, kcal kg ⁻¹	3262	3262	3322
Feed pH	5.62	5.96	6.09
Dietary calculated CAD			
** level, mEq kg⁻¹	83	101	338
Dietary Na/K, mol/mol	0.35	0.4	1.39

¹ **Vitamin mixture:** Included of per kg; 18.000 IU Vit A, 2.000 IU Vit D₃, 200 IU Vit E, 12 mg Vit K, 150 mg Vit C, 30 mg Vit B₂, 20 mg Vit B₁, 0.05 mg Vit B₁₂, 20 mg pyridoxine, 10 mg panthotenic acid, 220 mg niacin, 210 mg inositol, 5 mg folic acid, 0.5 mg biotine, 2.000 mg choline. Hoffman La-Roche Inc, Istanbul, Türkiye

² **Mineral mixture:** Included of per kg: 70 mg Zn, 60 mg Mn, 60 mg Mg, 4 mg Fe, 2 mg Cu, 1.5 mg I, 0.5 mg Co, 0.05 mg Se. Hoffman La-Roche Inc, Istanbul, Türkiye

³ **Vitamin C,** Hoffman La-Roche Inc, Istanbul, Türkiye

⁴ **Choline,** Ufuk Kimya İlaç San. ve Tic. Ltd. Sti Istanbul, Türkiye

* **DE:** Digestible Energy which was calculated with NRC (1993) and New (1987) of tables using for diet ingredients ^{13,14}

** Calculated from Na, K, Cl content of diet

error. Results were analyzed by one-way analyses of variance (ANOVA) and the treatment means were compared by Duncan's multiple range tests. Significance was tested at the P<0.05 level. Homogeneity of variance was performed by Levene test and variance was found to be homogenous (SPSS 17.0, released version) ²⁰.

Table 2. Growth performance, feed efficiency and serum mineral levels in experimental groups (Mean±Standard error). RS; rock salt**Tablo 2.** Deneme gruplarında büyüme performansı, yem değerlendirme ve serum mineral konsantrasyonu (Ortalama ±Standart hata). RS; Kaya tuzu

Growth±SE	Treatment		
	2% CaCl ₂ Suppl.	Control	2% RS Suppl.
WG ¹	139.5±1.5 ^b	138.5±3.5 ^b	158±5 ^a
SGR ²	0.92±0.05 ^a	0.88±0.03 ^a	1.01±0.03 ^a
Feed efficiency	-	-	-
DFI ³	1.42±0.03 ^a	1.39±0.01 ^{ab}	1.31±0.01 ^b
FCR ⁴	1.63±0.08 ^a	1.67±0.04 ^a	1.38±0.02 ^b
PER ⁵	1.28±0.06 ^b	1.28±0.03 ^b	1.49±0.02 ^a
Minerals in serum *	-	-	-
Na	152.81±8.31 ^a	144.26±9.7 ^a	150.79±2.79 ^a
K	13.34±1.98 ^a	15.69±3.57 ^a	14.18±0.30 ^a
Cl	125.08±2.56 ^a	121.92±3.26 ^a	133.59±2.61 ^a

Different superscript in a row indicates significant differences

* The initial values for serum mineral levels were 140.04±0.93, 13.24±0.63 and 118.76±0.62 mmol l⁻¹ for Na, K and Cl, respectively (n=12 for each group).

¹ The weight gain (WG, g) = last biomass-first biomass.

² The specific growth rate (SGR, % day⁻¹) = 100 x [(ln w₂-ln w₁)/feeding days]

³ The Daily feed intake (DFI, %) = 100 x (Feed consumed, g/feeding days/fish number)/((initial biomass, g + final biomass, g)/2).

⁴ The feed conversion rate (FCR) = Feed consumed/(Increase in biomass + dead fish biomass).

⁵ Protein Efficiency Rate (PER) = Increase in biomass, g/Protein consumed, g

RESULTS

The diet supplemented with 2% RS improved significantly the weight gain, feed conversion rate and protein efficiency rate in rainbow trout compared to 2% CaCl₂ supplemented group and control (P<0.05, Table 2). Dietary RS supplementation also improved the specific growth rate and daily feed intake. However, this improvement was not statistically significant (P>0.05). Dietary 2% CaCl₂ supplementation did not significantly affect the weight gain, specific growth rate, daily feed intake, feed conversion rate and protein efficiency rate in rainbow trout compared to control diet (P>0.05, Table 2).

Na, K and Cl level of the serum were not statistically different among the groups (Table 2). Mortality was 2% in all groups and insignificantly influenced by supplementation of CaCl₂ and RS.

DISCUSSION

The results of the present study indicated that the diets supplemented with 2% RS improved the weight

gain, feed conversion rate and protein efficiency rate comparing to control and 2% CaCl_2 groups in rainbow trout. Rock salt used in the experiment increased either dietary Na/K ratio or dietary CAD level more than three times (dietary Na/K increased from 0.4 to 1.39 meq kg^{-1} , dietary Na/K ratio increased from 101 to 338 mol/mol, [Table 1](#)). On the other hand, CaCl_2 supplementation decreased the dietary CAD level only 20% ([Table 1](#)). Purity of additive and/or interaction among the ingredients of diet may be cause of this. CaCl_2 supplementation to diet is generally appropriate for making diet anionic and decreasing the dietary CAD level.

The studies about the effects of dietary CAD level and dietary Na/K ratio on growth performance in fish species are few in number. The most studied fish species on this topic are African catfish ^{6,7} and rainbow trout ⁸⁻¹¹.

Different dietary CAD and Na/K regulations caused to different response in African catfish in respect of growth performance and feed efficiency. When only dietary CAD was increased within the range -100 to 700 mEq kg^{-1} , feed intake and growth increased linearly with an increase in dietary CAD and feed conversion rate was not significantly influenced ⁶. When only dietary Na/K ratio was increased (from 0.2 to 2.5 mol mol^{-1}) at optimal dietary CAD level (554-657 mEq kg^{-1}), growth increased quadratically, feed conversion rate decreased quadratically and feed intake was not significantly influenced ⁷. In previous studies, it was reported that effects of CAD were no clear on growth in rainbow trout, *Oncorhynchus mykiss* ⁸⁻¹¹. Recently, Arabacı et al. ¹² reported that growth increased linearly, feed conversion rate decreased linearly with increasing dietary CAD level and Na/K ratio, and daily feed intake was not significantly influenced in rainbow trout.

In the present study, RS supplementation, increasing dietary CAD level and dietary Na/K ratio, improved the weight gain and feed efficiency compared to CaCl_2 supplemented group and control group ($P < 0.05$). However, specific growth rate increased, but this was not statistically significant ($P > 0.05$). The cause of the partial improvement in growth may be an insufficient level of dietary CAD level and dietary Na/K ratio. Because Arabacı et al. ¹² observed that the best growth was taken with the dietary Na/K ratio and dietary CAD level between 2.15-560 and 3.53-981 (mol mol^{-1} -mEq kg^{-1}) for rainbow trout. On the other hand, in this study growth and feed efficiency didn't improve in 2% CaCl_2 supplemented group having low dietary CAD level (83 meq kg^{-1}) and low dietary Na/K ratio (0.35 mol mol^{-1}) like control (CAD level; 101 mEq kg^{-1} , Na/K ratio; 0.4 mol- mol^{-1}).

Improving in daily feed intake in RS supplemented

group was not significantly different from the control group, but was significantly better than CaCl_2 supplemented group ($P < 0.05$). Feed conversion rate decreased by 15-17% in RS supplemented group compared to CaCl_2 supplemented group and control group respectively. Additionally weight gain significantly increased by 12% in RS group compared to CaCl_2 supplemented group and control group ($P < 0.05$). These significant differences in weight gain and feed conversion rate in treatment groups probably associated with differences in energy costs. Because of the significant decreasing at the protein efficiency rate in 2% CaCl_2 supplemented group and control ([Table 2](#)), it can be expected that more energy is needed to maintain mineral balance and homeostasis for the fish feeding with diets having low dietary CAD-Na/K levels. Dersjant-Li et al. also stated that, feed efficiency improved with increasing dietary Na/K in African catfish, so more fat was deposited and increased linearly the body nitrogen, fat and energy levels ⁷. Likewise D'Cruz and Wood, reported that 263 mmol/kg salt (NaCl) supplementation to diet of juveniles of rainbow trout improved the growth and FCR in acidic water conditions ²¹.

The hardness of water used in this study was very high (370 mg lt^{-1} CaCO_3). Water hardness is very important for fish culture. It is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Calcium and magnesium are essential in the biological processes of fish. The presence of free (ionic) calcium in culture water helps reduce the loss of other salts (e.g., sodium and potassium) from fish body fluids. Environmental calcium is also required to re-absorb these lost salts ²². In this study, supplementation of fish diet with 2% RS caused to improve in the weight gain and feed efficiency of rainbow trout in very hard water providing the advantages to fish in respect of ionoregulation and biological processes. We estimated that high hardness of water did not mask the effects of RS supplementation on the weight gain and feed efficiency in rainbow trout. There are need to further studies, for determining the effects of different levels of dietary RS supplementation on growth performance of rainbow trout in water having different hardness values.

Na, K and Cl levels in the serum samples were not significantly influenced with dietary CaCl_2 and RS supplementation. These results are consistent with the research of Arabacı et al. ¹². In previous studies, different regulations in fish diet affected differently the plasma Na, K and Cl levels of fish ^{6-8,11}.

Mortality rate was similar at a range of 2% among groups. Arabacı et al. ¹² were found that there was no clear effect of dietary CAD-Na/K level on mortality.

However Dersjant-Li et al.⁷ observed that mortality and disease rate was high in the fish receiving low dietary Na/K ratio of 0.2. For determining the effect of very low dietary Na/K ratio and/or CAD level on mortality rate in rainbow trout, there is need to further research.

The results of the present study imply that RS supplementation to diet of rainbow trout could be used to increase both dietary CAD level and dietary Na/K ratio and this caused to positive effects on the weight gain and feed efficiency in rainbow trout.

It was concluded that the weight gain and protein efficiency rate increased significantly and the feed conversion rate decreased significantly. Specific growth rate and daily feed intake improved slightly but not a significant level with RS supplementation. Na, K, Cl level in the serum was not significantly influenced with CaCl₂ and RS supplementation. However, there are need to further studies for determining the optimum levels of RS supplementation to rainbow trout diets and for clearing the interactions between dietary salt supplementations and dietary CAD level-Na/K ratios.

According to our present study, 2% RS supplementation produced a better growth and feed efficiency for rainbow trout compared to 2% CaCl₂ supplementation and control diet. Added RS to diet was appropriate for making cationic the diet and increasing the CAD level and Na/K ratio of diet.

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Determining the Relative Abundance of Honey Bee (*Apis mellifera* L.) Races in Kars Plateau and Evaluating Some of Their Characteristics

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Summary

During June-July period 2009, 310 honeybee samples were collected from 32 different colonies in Kars Plateau. The majority of the samples (71.6%, n=222) were hybrid form of *Apis mellifera*. The ratio of *Apis mellifera caucasica* was 26.5% (n= 82) and only 1.9% (n=6) of samples were found to be *A. mellifera remipes*. Tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, metatarsus width of hind leg of *A. mellifera caucasica* and that of hybrid form of *A. mellifera* were measured and the measurements of those morphological characters of different subspecies were compared. In addition of the morphological traits, variation patterns of proteins bades on gel electrophoresis was measured from the two subspecies and their hybrids. Gel electro-phoresis patterns reveal that the total proteins of pure Caucasian race individuals from Ardahan and Kars were similar. On the other hand, the protein bands of pure Caucasian race individuals (66 kD, 45 kD, 29 kD and 18 kD) showed clear differences from that of hybrid individuals.

Keywords: *Apis mellifera caucasica*, *Apis mellifera remipes*, Hybridization, Kars Plateau

Kars Platosu'ndaki Bal Arısı (*Apis mellifera* L.) Irklarının Nispi Yoğunlukları ve Bazı Özellikleri

Özet

Haziran-Temmuz 2009 periyodunda Kars Platosu'ndaki 32 farklı koloniden 310 bal arısı toplandı. Toplanan örneklerin çoğunluğunun (%71.6, n=222) *Apis mellifera*'nın hibrit formu olduğu belirlendi. *Apis mellifera caucasica*'nın oranı %26.5 ti (n= 82). *A. mellifera remipes*'e ait yalnızca 6 birey (1.9%) tespit edildi. *A. mellifera caucasica* ve *A. mellifera* melez formlarının vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği morfolojik ölçümü yapıldı. Bu özelliklerden 3 tanesi (tarsi uzunluğu, çengel sayısı ve femur uzunluğu) saf kaffas ve melez formlar arasında istatistiksel olarak anlamlı farklılık gösterdi (P<0.05). Elektroferez çalışmalarıyla, Ardahan ve Kars'taki saf Kaffas arı ırklarının protein bantlaşmasının oldukça benzer olduğu belirlendi. Diğer taraftan melez formlar ile saf Kaffas ırkına ait protein bantlarında önemli farklılıklar (66 kD, 45 kD, 29 kD and 18 kD) tespit edildi.

Anahtar sözcükler: *Apis mellifera caucasica*, *Apis mellifera remipes*, Melezleşme, Kars Platosu

INTRODUCTION

There has been limited investigation Apoidea in Nort East Anatolia ¹. Honeybees (*Apis mellifera* L.) from Anatolia express very high degrees of genetic diversity at subspecific level ^{2,3} and conventionally being cited as "the place which has no match in the world." Existence of important bee species in different geographical areas of Anatolia is well documented ². Presence of Caucasus race (*Apis mellifera caucasica*) which produces more

honey than other races in North East Anatolia, trans-Caucasian race (*Apis mellifera remipes*) and Armenian race (*Apis mellifera armeniaca*) in Eastern Anatolia ³⁻⁷, the Anatolian race (*Apis mellifera anatoliaca*) in central Anatolia ^{6,8-11}, Karniyol race (*Apis mellifera carnica*) in Thrace region ¹², and Syrian race (*Apis mellifera syriaca*) and Iranian race (*Apis mellifera meda*) in Southeast Anatolia ^{4,5,13} were reported.



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Ruttner (1998) and Güler (1999) defined some ecotypes within each geographical region that serve as transition areas such as, Muğla (Aegean Region) and Borçka-Camili (Eastern and North Eastern Anatolia Region)^{5,6}.

In Ardahan, Artvin, Kars and some neighboring provinces, presence of pure Caucasian bees were noted in first half of the last century, but after mobile apiculture began in 1950s the number of pure bee colonies in this region has gradually decreased. Questions are raised to the existence of any pure Caucasian race in these provinces nowadays¹⁴.

Motherland of Caucasian bees is Central Caucasia, and it has two types, namely *A. m. caucasica* (mountain type) and *A. m. remipes* (lowland type). *A. m. caucasica* is the grey-colored mountain type Caucasian bee which lives in the high valleys of the region; whereas *A. m. remipes* is the yellow-colored lowland type Caucasian bee that lives in the low areas of the region. In Turkey, mountain-type Caucasian bee (*A. m. caucasica*) can be found in pure and hybrid forms in the plateaus of Eastern Anatolia as well as in close proximity to the Caucasian region¹⁵. This race is also one of the economic honeybee races and widely apicultured in Turkey and around the globe¹⁶.

Fıratlı and Budak¹⁷ noted that the widespread mobile apiculture has altered the genetic structure of Anatolian bee populations leading a dramatic increase in admixed populations. It has been claimed that pure Caucasian races can solely be found in isolated areas¹⁸ such as, Posof district of Ardahan Province and Camili basin in Borçka district of Artvin Province¹⁹. Increasing mobile beekeeping in Anatolia and commercial distribution of hybrid queen bees marked at pure Caucasian have collectively caused a high degree of hybridization among ecotypes adapted different ecogeographic regions leading extinction of unique genetic properties of the local landraces¹⁸.

The objectives of this study were (i) to determine the relative abundance of *Apis mellifera* races gathered from Kars Plateau, (ii) to conduct their morphometric analysis, and (iii) to analyze their proteins structures. We hope this study will contribute to conservation activities for *Apis mellifera caucasica* in the area.

MATERIAL and METHODS

During June-July period 2009, 310 honeybee samples were taken from the entrance of hives from 32 different colonies in Kars Plateau (Table 1). In addition, 10 samples of *Apis mellifera caucasica* were taken from Ardahan province. All of the samples were transferred to the laboratory alive in special insect cages, were placed in eppendorf tubes, fixed at -20°C, and stored there until they were used. Carl Zeiss Jena brand stereo-microscope was used for morphological measurements and pictures.

In order to infer degree of admixture between races, pictures of big stains and striping in the first and second tergite of abdomen were taken. The identification of *A. mellifera* races was performed according to Güler et al.⁶ and Güler¹⁴.

As a part of morphometric characterization of the collected samples, general body size, length of fore wing, width of fore wing, the number of hamulus in the hind wing, width of hind leg femur, metatarsus length of hind leg, and metatarsus width of hind leg were measured. Furthermore, dappling (brown spots) and striping (yellow-dark yellow) in first and second tergites of abdomen were also examined.

Means and standard deviations of all the morphological traits measured were computed for pure Caucasian race and hybrids separately from each location using SAS 9.2²⁰. The difference of means for each morphological trait between *A. mellifera caucasica* and hybrid samples of *A. mellifera* were inferred via student t-test using SPSS^{21,22}.

Abdomen, wing and leg parts of each sample was removed for electrophoretic analysis, and remaining head and thorax parts were homogenized in eppendorf tubes by adding 250 µl of pure water. Then, samples were centrifuged at 12.000 rpm for 15 min. at +4°C. After supernatant was obtained, it was transferred into tubes²³ and was kept at -20 °C until electrophoresis procedure. Total protein was determined according to biuret method²⁴. SPS and PAGE procedures were performed according to Laemmli and O'Farrell method^{25,26}. Protein molecule weights were calculated according to the method of Weber et al.²⁷.

Table 1. Localities along with the elevations in Kars and its districts where bee samples were collected from

Tablo 1. Kars ve ilçelerinde arı örnekleri toplanan lokaliteler ve yükseklik değerleri

Localities	Kars	Akyaka	Arpaçay	Susuz	Digor	Sarıkamış	Selim	Kağızman
The Number of Samples	30	50	50	50	20	40	30	40
Elevation	1750	1477	1675	1650	1450	2200	1830	1400

RESULTS

A total of 310 specimens were collected during the study. The most dominant race was hybrid form of *A. mellifera* (71.6%, n=222) followed by *A. mellifera caucasica* (26.5%, n=82) and *A. mellifera remipes* (1.9%, n=6) (*Table 2*).

None of the samples collected from Ardahan, accepted as pure Caucasian race (*A. mellifera caucasica*), displayed bands on their tergites, but only a few had spots. The most of the samples taken from other colonies had dapples and in a few of them striping was observed. The six samples that showed striping were excluded from the statistical analyses due to the low sample size. The number of samples collected from each district along with the number of stained and non-stained samples were given in *Table 2*.

In addition to morphological analysis, pictures of brown dapples from mountain-type Caucasian race and other colonies were taken and shown in *Fig. 1*. Samples taken from Ardahan gene center and Queen production center had similar characteristics to the mountain-type Caucasian race bees (*Fig. 1a, b*). However, very weak brown stains were detected in 1st and 2nd tergites of some samples (*Fig. 1c*).

In the analysis of samples taken from Kars Plateau, most of the samples showed brown-yellow stains in their 1st and 2nd tergites (*Fig. 1d, e and f*) similar to the

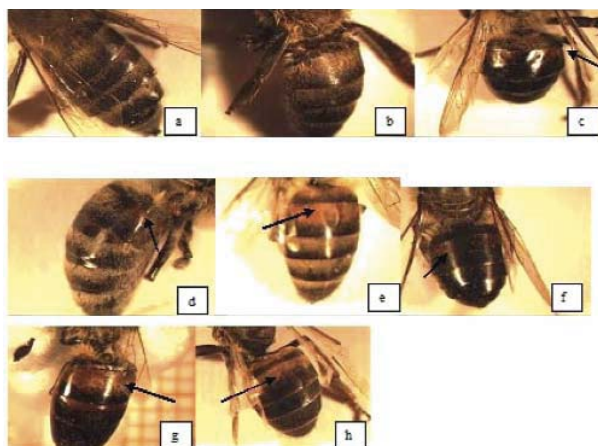


Fig 1. Pictures of the first and the second tergite dappling and striping (a, b: tergite color of mountain-type Caucasian race; c, d and e: dappling in the 1st and 2nd tergites of bees in Kars Plateau; d, e and f: dappling in the 1st and 2nd tergites of bees in Kars Plateau; g, h: striping in the 1st and 2nd tergites of bees in Kars Plateau)

Şekil 1. 1. ve 2. tergit beneklenme ve bantlaşma fotoğrafları (a, b: Kafkas ırkı dağ tipi tergit rengi; c, d, e: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki beneklenme; d, e, f: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki beneklenme; g, h: Kars Platosu'ndaki arıların 1 ve 2. tergit'lerindeki bantlaşma)

mountain-type Caucasian race. In addition, it was found out that most of the samples had yellow stripes in their 1st and 2nd tergites (*Fig. 1g, h*).

The results of the morphological analysis of *A. mellifera caucasica* are given in *Table 3* and that of hybrid form of *A. mellifera* in *Table 4*.

Table 2. The color of first tergite circle of all of the samples collected from Ardahan and Kars Plateau

Tablo 2. Ardahan ve Kars Platosu'ndan toplanan tüm örneklerin 1. tergit halkasının rengi

Localities	Ardahan	Kars	Akyaka	Arpaçay	Susuz	Digor	Sarıkamış	Selim	Kağızman
The number of samples	10	30	50	50	50	20	40	30	40
Non-stained	10	15	11	2	16	0	9	14	15
Stained	0	15	39	48	34	20	31	16	25

Table 3. Means (mm) and standard deviations of the tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, and metatarsus width of hind leg of samples belonging to pure Caucasian (*A. mellifera caucasica*) race collected from different district of Kars Province.

Tablo 3. Saf Kafkas (*A. mellifera caucasica*) bireylere ait örneklerin vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği değerleri ile bu değerlerin ortalaması (mm) ve standart sapma değerleri

Locality	Tarsi L.	No. of Hamulus	Wing L	Wing W	Metatarsus L	Metatarsus W	Femur L	Tibia L
Akyaka	1.12±0.07	21.91±1.81	0.92±0.03	0.30±0.01	0.20±0.01	0.13±0.02	0.30±0.01	0.31±0.01
Ardahan	1.25±0.05	21.20±1.48	0.98±0.07	0.33±0.02	0.20±0.00	0.14±0.01	0.30±0.00	0.30±0.00
Arpaçay	1.12±0.01	19.50±3.54	0.90±0.06	0.30±0.01	0.21±0.01	0.14±0.01	0.32±0.01	0.30±0.01
Kağızman	1.29±0.05	21.93±1.83	0.97±0.03	0.30±0.01	0.20±0.01	0.13±0.02	0.30±0.01	0.30±0.01
Merkez	1.23±0.04	21.07±1.03	0.95±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.31±0.01
Sarıkamış	1.19±0.02	21.44±1.24	0.95±0.04	0.32±0.01	0.20±0.01	0.12±0.01	0.30±0.01	0.31±0.01
Susuz	1.21±0.08	20.69±2.15	0.98±0.03	0.32±0.02	0.20±0.01	0.12±0.02	0.31±0.01	0.31±0.02
Selim	1.20±0.05	21.21±1.12	0.95±0.04	0.30±0.01	0.20±0.01	0.12±0.01	0.29±0.01	0.29±0.02
General	1.22±0.07	21.28±1.65	0.96±0.04	0.31±0.02	0.20±0.01	0.13±0.01	0.30±0.01	0.30±0.01

Table 4. Means (mm) and standard deviations of the tarsi length, fore wing length, fore wing width, number of hamulus hind wing, femur length of hind leg, tibia length of hind leg, metatarsus length of hind length, metatarsus width of hind leg of samples belonging to hybrid samples of *A. mellifera* collected from different district of Kars Province.

Tablo 4. Melez bireylere (*A. mellifera* melez formları) ait örneklerin vücut uzunluğu, ön kanat uzunluğu, ön kanat genişliği, arka kanat çengel sayısı, arka bacak femur uzunluğu, arka bacak tibia uzunluğu, arka bacak metatarsus uzunluğu, arka bacak metatarsus genişliği değerleri ile bu değerlerin ortalaması (mm) ve standart sapma değerleri

Locality	Tarsi L.	No. of Hamulus	Wing L	Wing W	Metatarsus L	Metatarsus W	Femur L	Tibia L
Akyaka	1.12±0.05	23.18±1.64	0.92±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.31±0.01
Ardahan	1.12±0.05	21.67±1.34	0.96±0.03	0.31±0.01	0.20±0.01	0.13±0.03	0.30±0.01	0.30±0.01
Arpaçay	1.21±0.05	21.60±1.35	1.00±0.02	0.31±0.02	0.20±0.01	0.13±0.02	0.30±0.01	0.30±0.01
Kağızman	1.30±0.04	22.52±1.50	0.97±0.02	0.30±0.01	0.21±0.01	0.13±0.01	0.30±0.01	0.30±0.01
Merkez	1.22±0.03	20.53±1.51	0.94±0.03	0.30±0.02	0.21±0.01	0.13±0.01	0.31±0.02	0.31±0.01
Sarıkamış	1.18±0.03	21.61±1.63	0.95±0.03	0.31±0.01	0.20±0.01	0.13±0.01	0.30±0.01	0.31±0.02
Susuz	1.21±0.07	21.21±1.43	0.97±0.03	0.32±0.02	0.20±0.01	0.13±0.02	0.32±0.01	0.32±0.02
Selim	1.20±0.04	21.50±1.41	0.95±0.05	0.30±0.01	0.20±0.01	0.12±0.02	0.29±0.02	0.28±0.01
General	1.18±0.08	21.85±1.64	0.95±0.03	0.31±0.02	0.20±0.01	0.13±0.02	0.30±0.01	0.31±0.02

All samples were either grouped as pure Caucasian or hybrid bees, and the means of each group for each trait along with p values of t-tests given in [Table 5](#). According to these results, when morphological characteristics of Caucasian samples and hybrid samples were compared, the only statistically significant differences were observed in tarsus length, number of hamulus, and femur length. Body of pure Caucasian samples were longer than hybrid ones, whereas they had fewer hamulus and shorter femurs.

So as to determine variation in total protein amount of mountain-type pure Caucasian race bees, the electrophoregrams were also conducted. Gel electrophoresis obtained as a result of electrophoregram is given in [Fig. 2](#).

Significant similarities were observed when comparing the electrophoregrams of pure Caucasian samples taken from Ardahan and from Kars province.

When we compared the electrophoregrams of the pure Caucasian race samples for Ardahan to the hybrids from Kars province, we observed that 77 kD, 114 kD, and 107 kD protein stripes of hybrid samples were

thinner compare to pure Caucasian. However, hybrid samples produced thicker bands in 38 kD and 65 kD proteins.

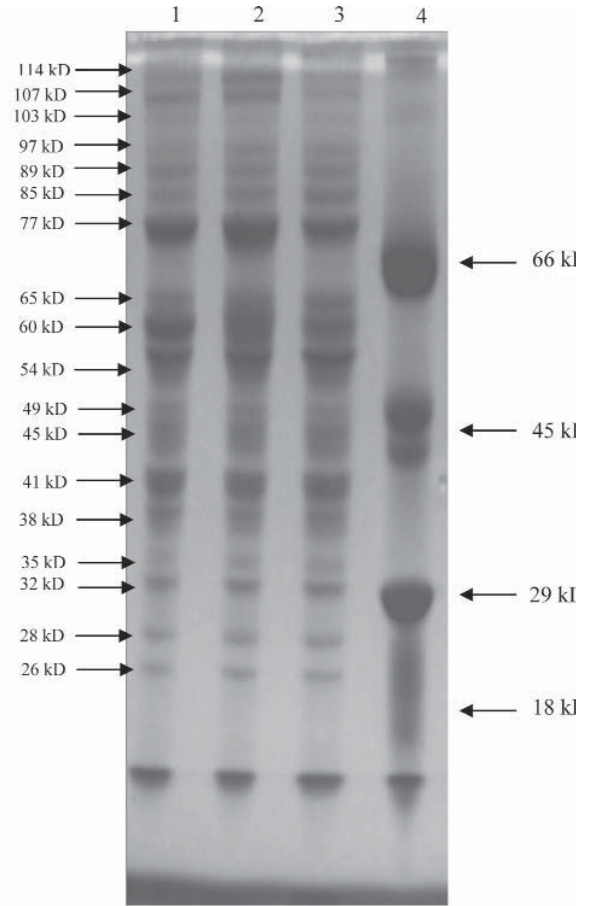


Fig 2. The Protein electrophoregram of 1- Ardahan Caucasian sample, 2-Kars Caucasian sample, 3- Kars hybrid sample, and 4- Standard protein

Şekil 2. 1- Ardahan Kafkas Örneği, 2- Kars Kafkas örneği, 3- Kars Melez örneği, 4- Standart protein

Table 5. Means (mm) of morphological traits measured from hybrid and pure Caucasian samples and P values of the difference between two groups as obtained with T-test

Tablo 5. Melez ve saf Kafkas bireylerinin ortalama (mm) ölçüleri ve her gurubun farklılığının T testi ile test edilmesine ait ($P<0.05$) değerleri

Characters	Caucasian	Hybrid	P Value
Tarsi length	1.25	1.18	<0.001
Number of humulus	21.20	21.70	0.006
Wing length	0.98	0.95	0.758
Wing width	0.325	0.306	0.769
Metatarsus length	0.200	0.203	0.108
Metatarsus width	0.135	0.129	0.096
Femur length	0.300	0.303	0.043
Tibia length	0.300	0.305	0.510

DISCUSSION

In the present study, morphological analysis of the samples taken from Ardahan gene center and Queen production center reveal that most of the samples showed similarities with the mountain-type Caucasian race bees (Fig. 1a, b). However, in the first and second tergite, weak brown spots were detected in some samples (Fig. 1c). The results are partially similar to İnci's data²⁸.

The high abundance of hybrid samples (71.6% of the total catch) in our study is similar to Bodenheimer's finding that the research area is a sub-passage region. Identification of yellow-abdomen circled subjects, albeit a few, in the research makes us believe that, as Çakır reports, *A. m. remipes* living in Elazığ area hybridized with the bees in the region due to mobile apiculture activity²⁹.

In the light of all these data, it can be asserted that colonies in and around Kars have been hybridized apparently and colonies in Ardahan gene center and Queen production station are gradually hybridized towards Caucasian race lowland-type or yellow-trans-Caucasian (*A. m. remipes*) race.

At the same time, it is a reality that although this region was announced as the center of origin, some hybridization has been observed due to mobile apiculture and lack of sufficient legal controlling mechanisms. Ardahan area was under protection with Artvin Province, but due to insufficient legal controlling mechanisms mobile apiculture and individual queen production stations were not controlled, which can make hybridization of the colonies in the region inevitable.

It has been found out that local apicultural colonies in the centre of Kars and its districts were not pure mountain-type Caucasian race, which was claimed before²⁷; morphological analysis of samples showed that most of these bees were hybrids. The only reason for this phenomenon can be the existence of significant mobile apiculture in the districts (especially mobile apiculturists in Elazığ and Bingöl regions where *A. m. remipes* is dominant). Morphological analysis of the samples taken from Kars and its districts showed subjects with yellow stripes on 1st and 2nd tergites (albeit a little), and the existence of brown-yellow stains and dapples in 1st and 2nd tergites of most of the samples mean that hybridization still continues.

The conventional belief is that the mobile apiculture has negative effects on local bee races in Kars Plateau.

This study indicates the relative abundance of bee races of the region and has potential to contribute to the preservation efforts of local bee races of the Kars Plateau.

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Effects of Partial Substitution of Fish Meal with Tuna Liver Meal on the Fatty Acid Profile of Nile Tilapia Fry, *Oreochromis niloticus*

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Summary

This study was designed to evaluate the effect of diets containing different levels of fish meal (FM) and tuna liver meal (TLM) on the growth performance and fatty acid profiles of Nile tilapia fry, *Oreochromis niloticus* (initial mean weight: 2.29 ± 0.07 g fish⁻¹). Each diet replacing 0, 10, 20, 30, 40, and 50% FM by TLM was randomly allocated to triplicate groups of fish in aquaria for 13 weeks. Results demonstrated that final weight, specific growth rate and protein efficiency ratio of fish fed diets replaced up to 30% TLM were not significantly ($P > 0.05$) different from fish fed the control diet, except for TLM levels of ≥ 40 . There were no significant differences in protein and ash contents in whole-body, while dry matter and lipid contents were significantly affected ($P < 0.05$) by dietary treatments. Increasing level of TLM resulted in a gradual increase in the average level of saturated fatty acid in fish flesh as compared to the control group. This increase was characterized by a significant ($P < 0.05$) increment in the level of 16:0, which is the most common fatty acid. Diets containing TLM up to 30% showed a significant increase ($P < 0.05$) in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid from the n-6 group, mainly due to 18:2n-6. Levels of MUFA and PUFA in fish flesh decreased with increasing dietary TLM levels of more than 40%. Level of n-3 in fish flesh was not significant different ($P > 0.05$) between experimental groups. Furthermore, the increase of dietary TLM did not significantly affect the n-3: n-6 ratio in fish flesh.

Keywords: *Oreochromis niloticus*, Fish meal, Tuna liver meal, Fatty acids

Balık Ununun bir Kısmı Yerine Kullanılan Orkinos Karaciğer Ununun Nil Tilapia Yavrularında, *Oreochromis niloticus*, Yağ Asidi Profili Üzerine Etkileri

Özet

Bu çalışma, balık unu ve orkinos karaciğer ununu farklı oranlarda içeren yemlerin Nil tilapia (*Oreochromis niloticus*, başlangıç ağırlık: 2.29 ± 0.07 g balık⁻¹) yavrusunun büyüme performansı ve yağ asidi profili üzerine etkisini belirlemek için gerçekleştirilmiştir. Balık ununun %0, 10, 20, 30, 40 ve 50'si oranında orkinos karaciğer unu ilave edilen yemlerle akvaryumdaki balıklar üç tekrarlı olarak 13 hafta süreyle beslenmiştir. Elde edilen sonuçlara göre %30'a kadar orkinos karaciğer unu içeren yemlerle beslenen balıkların son ağırlık, spesifik büyüme ve protein etkinlik oranı kontrol grubuna göre istatistiksel olarak önemsiz, ancak diğer gruplara göre önemli bulunmuştur ($P < 0.05$). Yemlerdeki orkinos karaciğer unundaki artış kontrol grubu ile karşılaştırıldığında balık etindeki doymuş yağ asidi miktarının artışına neden olduğu belirlenmiştir. Bu durum 16:0'ın düzeyindeki önemli artıştan kaynaklanmaktadır ($P < 0.05$). Yüzde 30'a kadar orkinos karaciğer unu içeren yemler ile beslenen balıkların etinde tekli doymamış yağ asidi (MUFA) ile çoklu doymamış yağ asidi (PUFA) olan n-6 grubunun artışına neden olmuştur. n-6 grubunun artışına 18:2n-6 önemli katkı sağlamıştır ($P < 0.05$). Balık etindeki MUFA ve PUFA düzeyleri, yemdeki orkinos karaciğer ununun %40'ın üzerine çıktığında azalma göstermiştir. Deneme gruplarında n-3 düzeyi istatistiksel olarak önemsiz bulunmuştur. Ayrıca n-3: n-6 oranı yemlerdeki orkinos karaciğer ununun artışıyla önemli bir değişikliğe uğramamıştır.

Anahtar sözcükler: *Oreochromis niloticus*, Balık unu, Orkinos karaciğer unu, Yağ asidi

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INTRODUCTION

The main protein source in aquafeeds is fish meal (FM). However, not only its rising price but changing supply is an important restrictive for the expansion of aquaculture industry¹. Thus, finding alternative protein sources for replacing partial or complete of the fish meal in fish feeds is crucial in order to sustain aquaculture industry. Many studies have been made to use alternative protein sources such as plant proteins²⁻⁶ and animal proteins⁷⁻¹² for reducing fish meal in aquafeeds. As animal or plant protein sources are used to substitute fish meal in fish feeds, it will become increasingly important to ensure that these diets are balanced in view of amino acid and fatty acid compositions. This will ensure not only that the diet satisfies the fish's requirement for these essential highly unsaturated fatty acids (HUFAs) but also that these fatty acids are retained in sufficient amounts in the fish flesh. However, there concerns that extensive substitute of fish meal in fish feeds could adversely affect the marketability of the fish because of consumer perception of altered taste of reduced health benefit due to changed HUFAs content¹³⁻¹⁶.

One of the encouraging animal protein sources is tuna liver meal (TLM) which is a by-product of the Tuna fish rearing industry. Generally, TLM is cheaper than FM because of its reputation as a by-product. Therefore, it may still be considered as an ingredient for use in aquaculture larvae diets. TLM has been tested in carp fry (*Cyprinus carpio*) by Gümüş¹¹, and the use of TLM to substitute FM as protein sources has supplied good growth and feeding efficiency value in fish. However, as far as we know, there is not much information concerning the effects of different levels of dietary TLM on the growth performance and fatty acid profile of the fish, when TLM has been tested in Nile tilapia fry (*Oreochromis niloticus*) in a similar study. The purpose of this study was to evaluate the growth performance and fatty acid profile changes in Nile tilapia fry when partially replacing FM with TLM as an alternative protein source.

MATERIAL and METHODS

The experiment was carried out in the Laboratory of Fisheries Programme of Ortaca Vocational School, Muğla, Turkey, from 15 October 2008 to 15 January 2009. Experimental fish were obtained from the same Laboratory. Prior to start of the experiment, the fry were fed with control diet twice a day for 2 weeks to acclimate to the experimental conditions. At the start of the experiment, 18 glass aquaria (65-L) were each randomly stocked with 20 fry with an average weight of 2.29 ± 0.07

g fish⁻¹. The aquaria were filled with dechlorinated tap water throughout the study and two-third of the aquarium water was changed daily. Each aquarium was supplied with compressed air by air stones from a central compressor. Each diet was assigned to triplicate groups of fish. Fish were fed to apparent satiation twice daily for 13 weeks.

Tuna liver meal (TLM) and other ingredients were from the same sources and experimental diets were prepared as described by Gümüş¹¹. Six isonitrogenous (crude protein 42%, on dry matter basis), isolipidic (16%, on dry matter basis) and isoenergetic (18 kJ g⁻¹) diets replacing 0 (control diet), 10, 20, 30, 40 and 50% of dietary FM by TLM were formulated (Table 1). The digestible energy (DE) content of the diet was estimated using the DE values 20.9 kJ g⁻¹ protein, 37.7 kJ g⁻¹ lipid and 14.6 kJ g⁻¹ carbohydrate¹⁷; fibre was not included in the calculation. Diets were made based upon the results of the chemical composition of the ingredients. Prior to experimental diet preparation, all dry ingredients were ground in a hammer mill and passed through a 0.5 mm mesh sieve and thoroughly mixed and blended thereafter with additional 100 ml of water per kg diet to make a paste. The resulting mixture was then pelleted with a meat grinder to obtain a 2-mm die. The pellets were dried about 24 h in an oven at 70°C. After drying, the diets were broken up and sieved into proper pellet size (0.8-1 mm diameter) and stored at -20°C until use.

During the experimental period, the water temperature ranged from 24°C to 26°C, dissolved oxygen from 4.8 to 5.5 mgL⁻¹, pH from 7.8 to 8.4. Water quality parameters remained within the acceptable ranges for Nile tilapia fry growth during the experimental period.

At the beginning and at the end of the experiment, all fry in each aquarium were anaesthetized under moderate anaesthesia (ethanol solution of clove oil, 20% v:v) and then weighed. A sample of 10 fish at the beginning of feeding experiment and 10 fish at termination were collected and stored frozen (-20°C) for proximate carcass composition and fatty acid analysis.

Proximate composition analyses of diets and fish body were performed following standard methods of AOAC¹⁸. Samples of diets and fish were dried in an oven at 105°C for 24 h to determine dry matter. Protein was determined by measuring nitrogen (Nx6.25) using the Kjeldahl method; lipid by ether extraction using the soxhlet method; ash by combustion at 550°C in a muffle furnace for 24 h; crude cellulose after an alkali and acid digestion, and nitrogen-free extract (NFE) by the difference [NFE=100-(moisture+protein+lipid+ash+ fibre)].

Table 1. Ingredient composition of the experimental diets**Tablo 1.** Deneme yemlerindeki hammadde kompozisyonu

Ingredients (%)	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
FM ¹	17.14	15.42	13.71	11.99	10.28	8.57
TLM ²	0	4.04	8.09	12.13	16.18	20.23
Corn meal ³	5.74	5.74	5.74	5.74	5.74	5.74
Corn gluten ⁴	7.23	7.23	7.23	7.23	7.23	7.23
Soybean meal ⁵	46.9	46.9	46.9	46.9	46.9	46.9
Starch ⁶	10.27	10.12	10.06	9.93	9.90	9.83
Fish oil ⁷	11.12	8.95	6.7	4.52	2.24	0
Vitamin premix ⁸	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix ⁹	0.3	0.3	0.3	0.3	0.3	0.3
L-methionine ¹⁰	0.5	0.48	0.46	0.44	0.42	0.4
Iodized salt	0.1	0.1	0.1	0.1	0.1	0.1
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

¹ **FM:** fish meal,² **TLM:** tuna liver meal. Tuna liver was obtained from Tuna Cage Farm, Ak-Tuna, Gazipaşa, Antalya, Turkey and TLM was prepared at the laboratory of fisheries faculty of Akdeniz University^{3,4-10} obtained from Kilic feed manufacture factory Co. Ltd.⁸ Vitamin premix (mg or IU kg⁻¹ of diet): Vit-A 7000 IU; Vit-D₃, 1000 IU; Vit-E, 100; Vit-K₃, 4.8; Vit-B₁, 12; Vit-B₂, 20; Vit-B₆, 12; Vit-B₁₂, 0.04; Vit-C, 200; Niacin, 120; Folic acid, 3.2; Calcium D- Pantothenate, 30; Biotin, 0.4; Inositol, 200; Endox D Dry, 100⁹ Mineral premix (mg kg⁻¹ of diet): Iron, 18; Copper, 3.6; Manganese, 36; Cobalt, 3; Zinc, 45; Iodine, 1.2; Selenium, 0.24; Magnesium, 90

The contents of fatty acids in experimental diets and fish body were determined by Gas Chromatography (GC). For this purpose, total lipids were extracted by using a modified Bligh and Dyer method ¹⁹, and then saponified and methylated for fatty acid quantification ²⁰. The fatty acid was extracted with *n*-hexane and converted to methyl esters before injecting to gas chromatograph for analysis. Fatty acid methyl esters (FAMES) were analyzed by Gas Chromatography (GC). Gas chromatography was performed on a QP 5050 Pelkin Elmer Aut System XLGC (GCndFID) using column CP SIL 88 FOR FAME (50 m x0.32 mm inner diameter; 0.25 µm film), FID detector at 250°C, automatically injector at 240°C and helium as the carrier gas. Column oven temperature conditions were as follows: initial temperature of 60°C was held for 4 min and then increased 13°C min⁻¹ to 175°C, then held constant for 27 min and finally increased 4 °C min⁻¹ to 250°C, where it was held constant for 15 min. A quantity of 1 µL of the samples was injected on the column, at a split rate of 1:70. Authentic fatty acid methyl esters were used for peak identification. The results of the individual fatty acids were expressed as percentages of total identified FAMES.

The results are presented as mean±SD. Data analysis was performed by one-way analysis of variance (ANOVA) using SPSS 15.0 (SPSS INC. Chicago, IL, USA). Differences among the means were compared using Duncan's multiple range test at a 5% probability level ²¹.

RESULTS

The proximate composition and fatty acid profiles of TLM and each of the main plant and animal protein sources are given in [Table 2](#). The crude lipid content of TLM was higher (65.23%) than that of FM (9.76%), whereas the level of crude protein (30.10%) was lower than that of FM (73.79%). The fatty acid composition of the ingredients indicated that TLM contained highest of monounsaturated fatty acid (MUFA; 40.9%) but contained moderate of saturated fatty acid (SFA; 33.81 %). Amount of polyunsaturated fatty acid (PUFA; 22.4%) in TLM was lower than that of FM and SBM containing considerable amounts of PUFA (39.19 % and 46.32, respectively). On the other hand, SBM did not contain EPA (20:5n-3) or DHA (22:6n-3). TLM contained large amount of n-6, but its n-3 was lower than those of FM. The highest level of n-6 (45.55%) was measured in SBM.

The proximate composition and fatty acid profiles of experimental diets containing different levels of TLM are given in [Table 3](#). The crude protein, crude lipid and digestible energy of all experimental diets were 42%, 16% and 18 kJ g⁻¹, respectively. The fatty acid composition SFA, MUFA and PUFA of the experimental diets ranged from 26.84 to 33.47, 31.75 to 34.11 and 26.63 to 37.65, respectively. Total n-3 of experimental diets followed the similar values as total n-6 fatty acids and ranged from 12.83 to 19.01 and 13.80 to 18.64, respectively. The n-3/n-6 ratio also ranged from 0.85 to 1.12.

Table 2. Proximate composition (% on dry matter basis) and fatty acid profiles (% of total fatty acids) of the main protein sources used in the experimental diets

Tablo 2. Deneme yemlerinde kullanılan temel protein kaynaklarının ham değerleri (% kuru ağırlık) ve yağ asidi profilleri (% toplam yağ asidi)

Parameter	TLM ¹	FM ¹	SBM ¹
Crude protein	30.10	73.79	53.58
Crude lipid	65.23	9.76	3.99
Crude fiber	0.79	0.76	4.31
Dry matter	90.94	92.47	90.70
Ash	2.88	14.83	7.71
NFE ²	1.00	0.86	30.41
Fatty acids³			
14:0	2.72	4.48	1.53
15:0	0.55	0.35	-
16:0	21.98	27.48	23.14
17:0	0.32	0.58	-
18:0	8.24	7.28	7.12
16:1n-7	4.03	4.41	1.04
18:1n-7	4.11	4.86	2.56
18:1n-9	22.55	9.28	16.04
20:1n-9	10.21	-	-
18:2n-6	1.63	0.91	40.14
18:3n-6	0.82	0.4	5.41
20:4n-6	5.53	1.34	-
18:3n-3	1.22	1.05	0.77
20:5n-3	10.54	16.02	-
22:5n-3	0.20	0.95	-
22:6n-3	2.66	18.52	-
SFA	33.81	40.17	31.79
MUFA	40.9	18.55	19.64
PUFA	22.4	39.19	46.32
n-3	14.42	36.54	0.77
n-6	7.98	2.65	45.55
n-3/n-6	1.80	13.78	0.01

Values are mean of triplicate analysis. ¹ TLM: Tuna liver meal; FM: Fish meal; SBM: Soybean meal. ² NFE: nitrogen-free extract. ³ -, not detected; SFA (saturated fatty acid) included 12:0, 14:0, 15:0, 16:0, 17:0 and 18:0; MUFA (monounsaturated fatty acid) included 16:1, 18:1 and 20:1; PUFA (polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6

Nile tilapia fry fed the experimental diets differed significantly ($P < 0.05$) in mean final weight, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) (Table 4). The final weight of fish fed diets including up to 30% TLM was significantly higher ($P < 0.05$) than those of fish fed diets including TLM higher than 30%. This trend was significantly noticeable ($P < 0.05$) for SGR and PER, an obvious decrease was observed as the TLM replacement occurred more than 30%. The FCR value of the fish fed diet with 50%TLM level was poorer than those fed the other diets. No significant differences in condition factor were confirmed on the experimental groups as the level of TLM increased in the diet ($P > 0.05$).

The final proximate composition and fatty acid profiles

of the carcass of fish fed the experimental diets are presented in Table 5. No significant differences were found in the protein and ash contents of fish fed with diets containing different proportion of TLM ($P > 0.05$), whereas, lipid and dry matter contents were affected significantly by dietary treatments ($P < 0.05$). Fatty acid profiles of the fish carcass varied with dietary treatment. Fish fed the TLM diets showed significantly elevated values for the overall SFA. The total short-chain MUFA values did not change, but 18:1n-9 value increased in carcass of fish, whereas PUFAs decreased in increasing of TLM level in diets, compared with that of fish fed control diet, particularly 18:2n-6 and 20:5n-3, giving a low value for total PUFA. Total n-6 fatty acids were lowest in fish fed the diets containing 20 or 30% of TLM level. On the other hand, the ratio of n-3 to n-6 fatty acids was highest in muscle of fish fed the diet including 50% of TLM level (Table 5).

DISCUSSION

The FM replacement with TLM significantly affected the growth response of Nile tilapia fry ($P < 0.05$). When replacing FM more than 30%, final weight, SGR and PER were significantly lower than those of the other groups, except for control group. However, the FCR value of fish fed TLM level of 50% was poorer than those of fish fed the others. These results indicated that TLM could be used to substitute FM by up to 30% without adverse effects on growth performance and feed utilization for Nile tilapia fry, and higher replacement levels resulted in growth reduction or poor feed utilization. This conformed well to the results of Gümüş¹¹ reported that no significant differences in growth performance of carp fry (*Cyprinus carpio*), fed with diets including up to 20% TLM. The results of the present study are in conformity with the previous studies in the literature where the liver meal was shown to be good for growth and survival cyprinids or freshwater prawn post-larvae²²⁻²⁵.

Partial replacement of FM with TLM did not influence the whole-body crude protein and ash contents of Nile tilapia fry, while lipid and dry matter contents were affected by dietary treatments. Similar results were also obtained in the study realized on carp fry, *Cyprinus carpio* by Gümüş¹¹. As different from our findings, Yang²⁶ in gibel carp, and Gümüş¹² in Nile tilapia found out that no significant changes were observed in whole body lipid and dry matter content resulted from the different replacement of FM with various feed ingredients.

Since animal or plant protein sources become more widely used to replace fish meal in fish diets¹, the lipid content of diet is important as a source of dietary energy,

Table 3. Proximate composition (% on dry matter basis) and fatty acid profiles of experimental diets (% of total fatty acids)**Tablo 3.** Deneme yemlerinin ham değerleri (% kuru ağırlık) ve yağ asidi profilleri (% toplam yağ asidi)

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
Moisture	5.62	4.89	5.16	5.03	5.68	4.97
Crude protein	42.41	42.06	42.55	42.07	42.44	42.00
Crude lipid	16.18	16.20	16.03	15.95	16.07	16.10
Ash	7.99	7.68	7.06	6.92	6.71	6.64
Crude fiber	2.40	2.39	2.42	2.43	2.47	2.47
NFE ¹	30.99	31.64	31.91	32.61	32.30	32.77
DE(kJ g ⁻¹) ²	18.08	18.09	18.07	18.08	18.06	18.05
Fatty acids ³						
14:0	5.63	5.86	4.18	4.37	3.61	3.03
15:0	0.30	-	0.30	0.31	0.35	0.37
16:0	17.87	17.20	18.2	21.35	21.73	22.8
17:0	-	0.26	0.32	0.38	0.46	0.51
18:0	3.04	3.76	5.03	5.18	6.29	6.76
16:1n-7	7.58	7.07	5.54	5.39	4.01	3.52
18:1n-7	3.20	3.26	3.59	3.62	3.66	3.77
18:1n-9	11.72	12.7	15.2	16.79	18.93	19.20
20:1n-9	9.25	9.34	9.04	8.65	7.0	6.28
18:2n-6	11.32	9.12	9.12	9.10	9.05	8.59
18:3n-6	1.39	1.32	1.32	1.26	1.15	1.11
20:4n-6	5.93	5.63	5.53	4.57	4.50	3.80
18:3n-3	1.37	1.37	1.21	1.14	1.01	0.80
20:5n-3	11.69	11.65	11.6	9.18	8.14	8.10
22:5n-3	0.68	0.50	0.44	0.40	0.35	0.31
22:6n-3	5.27	4.43	4.34	4.30	4.12	3.42
SFA	26.84	27.08	28.03	27.22	28.83	32.77
MUFA	31.75	32.37	33.37	34.45	33.6	32.47
PUFA	37.65	33.93	33.56	29.95	29.74	26.63
n-3	19.01	17.95	17.59	15.02	13.62	12.63
n-6	18.64	16.07	15.97	14.93	14.7	13.50
n-3/n-6	1.019	1.116	1.101	1.006	0.926	0.935

Values are mean of triplicate analysis. ¹ **NFE**: nitrogen-free extract. ² Estimated according to NRC ¹⁷. ³ -, not detected; **SFA** (saturated fatty acid) included 14:0, 15:0, 16:0, 17:0 and 18:0; **MUFA** (monounsaturated fatty acid) included 16:1, 18:1 and 20:1; **PUFA** (polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6

Table 4. Growth performance and feed utilization of Nile tilapia fry (*Oreochromis niloticus*) fed the experimental diets**Tablo 4.** Deneme yemleri ile beslenen Nil tilapia yavrularının (*Oreochromis niloticus*) büyüme performansı ve yem kullanımı

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
IBW (g)	2.21±0.06	2.36±0.18	2.24±0.07	2.32±0.02	2.29±0.05	2.28±0.07
FBW (g)	18.64±1.11 ^{ab}	20.84±2.77 ^a	18.78±3.03 ^{ab}	20.57±1.18 ^{ab}	15.95±0.62 ^{bc}	12.81±0.02 ^c
SGR (%/d)	3.04±0.04 ^a	3.09±0.10 ^a	2.97±0.15 ^a	3.17±0.05 ^a	2.76±0.01 ^b	2.46±0.05 ^c
FCR	1.12±0.13 ^a	1.01±0.04 ^a	1.12±0.27 ^a	1.02±0.08 ^a	1.13±0.04 ^a	1.52±0.03 ^b
PER	1.82±0.28 ^{ab}	2.10±0.13 ^{ab}	2.16±0.00 ^a	1.95±0.16 ^{ab}	1.76±0.05 ^b	1.31±0.02 ^c
CF	1.92±0.005	1.92±0.13	1.86±0.014	1.89±0.37	1.90±0.57	1.90±0.17

^{a-c} Values in the same row with different superscripts are significantly different from each other ($P<0.05$)

¹ **IBW** = initial body weight; **FBW** = final body weight; **L** = Body length; **SGR** (specific growth rate) = $(\ln \text{FBW} - \ln \text{IBW}) / (\text{time days} \times 100)$; **FCR** (feed conversion ratio) = $\text{Feed intake} / (\text{FBW} - \text{IBW})$; **PER** (protein efficiency ratio) = $(\text{FBW} - \text{IBW}) / (\text{protein intake})$; **CF** (condition factor) = $\text{FBW} / \text{L}^3 \times 100$

Table 5. Body composition (% of wet wt.) and fatty acid profiles (% of total fatty acids) of Nile tilapia fry (*Oreochromis niloticus*) fed the experimental diets**Tablo 5.** Deneme yemleri ile beslenen Nil tilapia yavrularının (*Oreochromis niloticus*) vücut kompozisyonu (yaş ağırlığın %) ve yağ asidi profilleri (toplam yağ asidinin %)

Parameter	%TLM Replacing FM in Diets					
	0 (Control)	10	20	30	40	50
Protein	19.73±0.23	19.37±0.06	19.27±0.79	19.70±0.48	19.68±0.29	19.86±0.18
Lipid	4.23±0.24 ^{ab}	3.74±0.11 ^b	3.53±0.11 ^b	4.75±0.46 ^{ab}	3.92±1.02 ^{ab}	5.43±0.82 ^a
Ash	2.14±0.02	2.09±0.22	2.03±0.47	1.65±0.01	1.88±0.32	1.90±0.04
Dry matter	26.11±0.50 ^{ab}	25.17±0.47 ^b	24.80±0.15 ^b	26.38±1.03 ^{ab}	25.39±1.06 ^{ab}	27.12±0.74 ^a
Fatty acids¹						
14:0	0.31±0.29 ^b	0.14±0.05 ^b	0.11±0.01 ^b	2.03±2.69 ^{ab}	3.78±1.27 ^a	3.20±0.19 ^{ab}
15:0	0.33±0.02 ^b	0.32±0.04 ^b	0.17±0.02 ^c	0.18±0.02 ^c	0.21±0.01 ^c	0.47±0.03 ^a
16:0	17.56±0.08 ^b	18.37±1.11 ^{ab}	18.76±1.88 ^{ab}	17.94±1.63 ^b	20.31±0.65 ^{ab}	21.94±2.15 ^a
17:0	0.12±0.03 ^c	0.14±0.05 ^c	0.1±0.0 ^c	0.2±0.02 ^{bc}	0.26±0.06 ^{ab}	0.34±0.0 ^a
18:0	9.12±1.47	7.67±2.72	9.62±0.56	6.94±1.8	6.95±0.26	9.15±2.86
16:1n-7	6.25±1.06	4.87±1.89	3.78±0.39	4.96±1.74	5.33±1.41	3.79±0.65
18:1n-7	4.36±0.69	4.60±0.37	3.44±1.09	4.06±0.31	4.31±0.75	4.73±0.33
18:1n-9	20.43±0.38 ^b	19.86±2.65 ^b	22.22±1.23 ^{ab}	25.92±1.3 ^a	23.81±2.14 ^{ab}	22.38±0.28 ^{ab}
20:1n-9	1.15±0.1	2.27±1.47	0.93±0.07	1.27±0.08	1.67±0.39	1.71±0.0
18:2n-6	10.9±0.83 ^{ab}	12.50±1.4 ^{ab}	14.95±2.53 ^a	14.64±3.17 ^a	8.13±0.14 ^b	8.99±0.85 ^b
18:3n-6	0.45±0.09 ^{ab}	0.4±0.16 ^{ab}	0.33±0.07 ^b	0.48±0.17 ^{ab}	0.64±0.03 ^a	0.67±0.04 ^a
20:4n-6	1.22±0.14	1.49±0.05	1.46±0.1	1.2±0.22	1.39±0.33	1.38±0.46
18:3n-3	0.40±0.04	0.26±0.05	0.28±0.0	0.31±0.10	0.37±0.09	0.33±0.04
20:5n-3	5.39±0.82 ^a	4.19±0.93 ^{ab}	2.89±0.22 ^b	4.15±1.08 ^{ab}	3.94±0.41 ^{ab}	4.41±0.18 ^{ab}
22:5n-3	1.09±0.56	1.62±0.28	1.46±0.29	1.36±0.01	1.28±0.42	1.07±0.24
22:6n-3	3.53±1.49	5.56±0.92	5.73±0.91	3.67±1.38	4.04±2.20	4.89±2.53
SFA	27.44±1.67 ^b	26.65±1.65 ^b	28.76±1.30 ^b	27.31±0.74 ^b	31.52±0.43 ^{ab}	35.12±5.24 ^a
MUFA	32.2±0.86 ^{ab}	31.61±2.7 ^{ab}	30.38±1.85 ^b	36.21±2.82 ^a	35.12±3.93 ^{ab}	32.62±0.70 ^{ab}
PUFA	23±2.06 ^{ab}	26.03±1.52 ^{ab}	27.11±1.11 ^a	25.82±3.40 ^{ab}	19.8±3.39 ^b	21.76±2.30 ^{ab}
n-3	10.42±1.18	11.64±0.22	10.36±1.44	9.49±0.17	11.13±5.06	10.71±2.64
n-6	12.57±0.88 ^{ab}	14.39±1.29 ^{ab}	16.74±2.56 ^a	16.33±3.22 ^a	9.63±2.94 ^b	11.05±0.33 ^b
n-3/n-6	0.82±0.03	0.81±0.05	0.63±0.18	0.59±0.10	0.94±0.24	0.97±0.26

Values are mean (±SD) of triplicate analysis. ^{a-c} Values in the same row with the different superscripts are significantly different (P<0.05). ¹

SFA (saturated fatty acid) included 14:0, 15:0, 16:0, 17:0 and 18:0; **MUFA** (monounsaturated fatty acid) included 16:1, 18:1 and 20:1; **PUFA** (polyunsaturated fatty acid) included n-3 and n-6; n-3 fatty acid included 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3; n-6 fatty acid included 18:2n-6, 18:3n-6, 20:4n-6

but is also fundamental for the supply an adequate amount of essential fatty acids (EFA) ²⁷. This will ensure not only that the diet satisfies the fish's requirement for these essential HUFA but also that these fatty acids are retained in sufficient amounts in the flesh of the fish. Findings suggest that EPA (20:5n-3), DHA (22:6n-3) and probably arachidonic acid (ARA, 20:4n-6) are essential for most marine and fresh water fish during development ²⁸, as they are required for the normal physiological functioning and development of fish ²⁹. On the other hand, freshwater fish are known to be highly capable of elongating and further desaturating C18 PUFA, in the form of linoleic acid (18:2n-6) and linolenic acid (18:3n-3) to C20 and C22 PUFA in their natural diet ^{29,30}.

Changes in fatty acid profile introduced by diet or feeding history are mirrored in the lipid stores of fish ³¹⁻³³. FM is an excellent source of essential amino acids. FM from oily fish species may contain up to 8-10 % of residual

fat, which commonly contains from 20 to 35% n-3 HUFA. Hence, if fish meal is included in the diet at a level as low as 30 g/100 g, it will provide from 0.5 to 1% of the dry diet n-3 HUFA, and the general EFA requirements of most commonly farmed finfish species will be fulfilled regardless of the dietary lipid source included in the formulation. If residual fish oil is fully substituted with a complete or partial replacement of the fish meal fraction of the diet, the risk of a net deficiency in EFA is possible, and an appropriate source of n-3 HUFA needs to be included in the diet formulation ²⁷.

FM replacement with TLM was not significantly modifying fatty acid profiles of experimental diets, because residual tuna liver oil was added to the experimental diets in order to counteract the loss of n-3 fatty acids due to FM elimination. However, considerable differences were observed in fatty acid profiles of flesh of fish fed diets progressively replaced of FM with TLM,

and some fatty acids increased or decreased in the flesh fish. FM replacement with different proportions of TLM caused a decline of n-3 and n-6 fatty acid levels of the experimental diets that had to be compensated with other sources rich in this type of fatty acids. This was remarkable in PUFA levels, in terms of negative correlation with the inclusion levels of TLM in the diet. Thus for example, accumulation of PUFA in fish flesh proved considerably higher in experimental groups fed with diets containing TLM up to 20 or 30% than in that of the control group, but lower when replacing FM with TLM more than 40%. Similar result was also obtained with MUFA. On the other hand, with respect to the whole fish fatty acid profile, as TLM increasingly replaces FM, SFA tend to increase significantly, and this effect is mainly due to palmitic and stearic acid included high levels of TLM.

In contrast to marine fish, carp and tilapia have been reported to be able to bio-convert larger quantities of linolenic acid to EPA and DHA^{34,35}. This ability for *de novo* synthesis of EFA is consistent with a range of empirically determined requirement data for a range of freshwater species. The HUFA content of cultured fish has often been reported to be lower because of lowered fish meal usage in the diet^{13,14}. On the other hand, carp and tilapia are generally regarded as omnivorous fish and are considered to require greater amounts of n-6 fatty acids than n-3 fatty acids in their diet³⁶. High levels of n-3 PUFA have been reported to depress the growth of tilapia³⁷⁻³⁹ and carp²⁶. However, the level of total n-3 not changed in fatty acid profiles of flesh in fish fed TLM diets, suggesting that Nile tilapia fry have an ability to synthesize HUFA from 18- carbon precursors. However, the dietary fatty acid analysis in fish flesh showed that the n-6 decreased with increasing dietary TLM levels more than 40%, which suggested n-6 deficiency at higher TLM levels and resulted in retardation of lipid metabolism⁴⁰. In other words, fish fed diets with low concentrations of total n-6 had low concentrations of total n-6 in fish flesh. These results were supported by some findings that a strong correlation between the fatty acid compositions of each test diet and the composition of the fish flesh was reported by previously for many different fish species⁴¹⁻⁴⁶.

The results of the present study indicated that dietary replacement of FM with TLM was significantly modifying Nile tilapia fry fatty acid profile. While FM replacement with TLM more than 40% caused a decline in MUFA and PUFA, up to 30% FM replacement was not deprived of MUFA and PUFA acids in flesh of fish fed experimental diets that have to be compensated with residual tuna liver oil rich in this type of fatty acids were added in order to counteract the loss of n-3 fatty acids due to FM

elimination. Further research needs to be conducted using TLM as a FM replacer to determine acceptable fatty acid profiles for larger fish sizes.

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Growth, Slaughter Yield and Proximate Composition of Rainbow Trout (*Oncorhynchus mykiss*) Raised Under Commercial Farming Condition in Black Sea

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Summary

Growth characteristics, slaughter yield and proximate composition of rainbow trout reared under commercial condition in Black Sea were investigated for one production season. The fish were weighted and sampled for quality assessment every month during the production period (December-May), and feed consumption was recorded daily. During the experiment, bimonthly body weight increased from 206 g to 1011 g, specific growth rate varied between 0.4 and 4.79, thermal growth coefficient varied between 0.04 and 0.35. Condition factor, visceral index and hepatosomatic index was altered by feed intake and water temperature. The degree of caecal fat score was highest during harvest month, May. Although carcass yield didn't differ significantly during farming period, fillet yield increased in the last three months. Protein content of fillet lowered from 83% to 70% by the production period. The fillet fat content increased from 10-11% to 15-16% during the experiment, and the most pronounced fat increase occurred in May. Fillets partitioned into six chunks: cranial, intermediate, caudal and their ventral, dorsal subdivisions. Results show small differences in the protein, fat and moisture content of these sections.

Keywords: Rainbow trout, Growth, Slaughter yield, Proximate composition

Karadeniz'de Ticari Koşullar Altında Yetiştirilen Gökkuşığı Alabalığı (*Oncorhynchus mykiss*)'nda Büyüme, Karkas Verimi ve Vücut Kompozisyonu

Özet

Karadeniz'de ticari koşullar altında yetiştirilen gökkuşığı alabalığında büyüme karakteristikleri, karkas verimi, fileto verimi, vücut kompozisyonu, bir üretim dönemi boyunca izlenmiştir. Üretim dönemi boyunca (Aralık-Mayıs) her ay örnekleme yapılarak balıklarda gerekli ölçüm ve analizler yapılmıştır. Deneme sonunda, başlangıçta 206 gram ağırlığa sahip balıkların 1011 grama ulaştığı belirlenmiştir. Spesifik büyüme oranı 0.4 ile 4.79 arasında, termal büyüme katsayısı 0.04 ile 0.35 arasında değişim göstermiştir. Kondisyon faktörü, iç organ oranı, hepatosomatik indeks değerleri su sıcaklığına ve günlük verilen yeme göre değişim göstermiştir. Çekal yağ skoru hasat ayında (Mayıs) en yüksek düzeye ulaşmıştır. Karkas verim zamanına bağlı olarak değişim göstermemesine karşın fileto verimi balık büyüdükçe artmıştır. Başlangıçta %83 olan fileto protein oranı hasat zamanında %70'e düşmüştür. Deneme süresince fileto yağ oranı %10-11'den %15-16'ya çıkmıştır. Kranial, orta, kaudal ve bunların alt parçaları dorsal ve ventral olmak üzere altı parçaya ayrılan filetoda, biyokimyasal değerler küçük farklılıklar göstermiştir.

Anahtar sözcükler: Gökkuşığı alabalığı, Büyüme, Karkas verimi, Fileto verimi, Vücut kompozisyonu

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important finfish species for Turkish aquaculture industry, and it has been cultured in Black Sea, Turkey

since 1990s¹. Increasing numbers of off-shore cage farm has been established in Black Sea for trout culture and trout production in the Black Sea has considerably



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increased in recent years. Rainbow trout are transferred directly from inland freshwater to the brackish Black Sea waters without any adverse effects. In this environment, trout require 5-6 months to grow from 20-200 g to the size of at least 200-1000 g¹.

It is known that rearing fish under controlled conditions can alter their growth, slaughter yield, proximate composition, and other parameters of flesh. The feed and water quality plays a significant role in both growth and proximate composition of fish². It is well known that brackish water enhances growth of trout³⁻⁵ and high growth rates have been reported in rainbow trout cultured in cage conditions in the brackish Black Sea water⁶⁻⁹. Growth of rainbow trout was defined very well in estuarine condition¹⁰⁻¹³ and was studied by several authors^{4,7,9} in Black Sea condition.

Although body composition is a good indicator of the physiological condition of a fish¹⁴ there is limited data on body composition and flesh quality of rainbow trout in estuarine Black Sea condition. Body composition and flesh quality are also essential characteristics of farmed fish for human consumption. They influence consumer acceptance and the ability to refine fish material¹⁵.

The aim of the present study was to investigate growth, slaughter yield and proximate composition of rainbow trout (*Oncorhynchus mykiss*) farmed in offshore cage conditions in South-Eastern Black Sea Coast, Turkey.

MATERIAL and METHODS

Experimental Fish and Fish Rearing

Experimental fish (206.38±48.3 g mean weight and 1st years old) were transferred into floating high density polyethylene (HDPE) cages (16 m diameter and 10 m depth) located in South-East Black Sea Coast, Rize-Turkey (41° 02' 06.36" and 41° 02' 20.56" North longitudes and 40° 32' 10.91" and 40° 32' 26.20" East latitudes) from fresh water cages (16 m diameter and 10 m depth) located in Kuzguncuk Dam Lake, Erzurum Turkey (40° 12' 04.30" and 40° 12' 08.15" North longitudes and 41° 03' 07.10" and 41° 03' 12.18" East latitudes) in November 2005. Fish were stocked into the sea cages with a stocking rate of 10 kg per cubic meter. Fish were fed *ad-libitum* two times daily with commercial extruded trout feed (45% crude protein, 18% crude fat, 10% moisture, 12% ash, 3% crude fibre) when meteorological condition was suitable.

Fish Sampling and Biometric Data Collection

Starting from December 2005 and following each

month (From February 2006 to May 2006) 15 fish were randomly sampled. Before bleeding of fish, the weight and length of the whole fish was recorded and the condition coefficient ($CF = (BW \times 100) / L^3$) of each fish calculated. After bleeding, fish was sexed and filleted. The sex of the fish was classified into two categories: males, females. Male fish were omitted due to insufficient male samples. The caecal fat score was immediately determined upon opening the body cavity. The pyloric caeca was scored according to the amount of fat surrounding the intestine with scores ranging between 0 (individual pyloric caeca clearly visible with no adherent fatty deposits) and 4 (all pyloric caeca completely obscured by visceral fat deposits). After gutting and removal of the kidney (but not gills), the fish were reweighed and the carcass yield ($CY = \text{carcass weight} / \text{fish weight} \times 100$) were calculated. Sample fish were filleted by butterfly filleting method with fillet board. The pin-bone, column and fins were removed, and fillet yield was calculated ($FY = \text{fillet weight} / \text{fish weight} \times 100$). The following indices were also calculated: visceral index ($VI = \text{viscera weight} / \text{fish weight} \times 100$); hepatosomatic ($HSI = \text{liver weight} / \text{fish weight} \times 100$); visceral fat ($VFI = \text{weight of the visceral fat} / \text{fish weight} \times 100$).

The specific growth rate, was calculated from the equation: $(SGR) = ((W_2^{1/3} - W_1^{1/3}) \times t^{-1}) \times 100$, where W_2 is the final weight, W_1 is the initial weight and t is time between W_1 and W_2 in days. The thermal growth coefficient (TGC) was calculated as $(W_2^{1/3} - W_1^{1/3}) \times (\Sigma T)^{-1}$ ¹⁶, where W_2 is the final weight, W_1 is the initial weight and ΣT is the sum of day degrees. The TGC was multiplied by 100 in the table to simplify the numbers.

To measure the proximate quality of fillets, fins and vertebrae were removed. Then fillet were divided into six parts: dorsal part of cranial site (Cr-D), ventral part of cranial site (Cr-V), dorsal part of intermediate site (Int-D), ventral part of intermediate site (Int-V), dorsal part of caudal site (Ca-D) and ventral part of caudal site (Ca-V) (Fig 1). Each part of fillets from all fish was pooled. The pooled fillet parts were ground and homogenized in a blender and then proximate composition of the fillets was analyzed.

Analytical Methods

Proximate composition of diets and fish muscle was analyzed using standard methods¹⁷. Muscle was collected from the sectioned part of trout flesh as shown in Fig. 1. Samples, from pooled fillet parts of specimens were analyzed in triplicate, for proximate composition. Moisture was determined by drying the sample (105°C for 24 h) to a constant weight. Crude protein ($N \times 6.25$) was measured using the Kjeldahl

method after acid digestion. Crude fat was estimated by Soxhlet exhaustive extraction technique using petroleum ether (40-60°C BP) as solvent. Ash was determined by incinerating the dried sample at 550°C for 12 h. Organic matter was determined indirectly, by difference from the ash content.

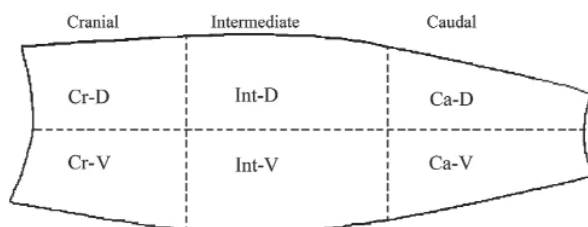


Fig 1. Partitioned fillet site for proximate composition analysis. Cr-D indicates dorsal part of cranial site, Cr-V indicates ventral part of cranial site, Int-D indicates dorsal part of intermediate site, Int-V indicates ventral part of intermediate site, Ca-D indicates dorsal part of caudal site, Ca-V indicates ventral part of caudal site of fillet

Şekil 1. Vücut kompozisyonu analizi için bölünmüş fileto parçaları. Cr-D kranial bölge dorsal kısmı, Cr-V kranial bölge ventral kısmı, Int-D orta bölge dorsal kısmı, Int-V orta bölge ventral kısmı, Ca-D kaudal bölge dorsal kısmı, Ca-V kaudal bölge ventral kısmı göstermektedir

Statistical Analysis

Proportions, as well as proximate composition were arcsine-transformed before statistical analysis. Both the transformed and the untransformed data were subject to analysis of variance (ANOVA) for biometric data, yields of fish and proximate composition. The individual comparisons of groups were obtained using Duncan's multiple range tests for biometric variables. All statistical computation was performed using the SPSS software package (Ver. 10.0 Chicago, IL, USA).

RESULTS

Higher SGR (% BW/day) is recorded in March, April and May compared to those recorded in January and February (*Table 1*). These increases and decreases were correlated with water temperature ($R^2=0.753$). The thermal growth coefficient (TGC) was also used to assess growth, because TGC incorporates both fish size and temperature effects in the growth trajectory. Thermal growth coefficient (TGC) was more reliable than SGR between groups. The lowest TGC was calculated in February and the highest TGC was calculated in March.

Body weight of fish slowly increased during the first three months (December, January and February), but the differences were not significant. However for the last three months body weight increased and reached up to 1011.4 g (*Table 2*) in May 2006. The increase in total fish length was lowered in January (26.66) and February (26.77), but increased thereafter in the following months.

The condition factor decreased from a mean of 1.34 in March to 1.44 in February. The condition factor tended to increase significantly more rapidly in subsequent

Table 1. Bimonthly recorded sea water temperature, daily mean feed intake, SGR and TGC values

Tablo 1. Aylık kaydedilmiş deniz suyu sıcaklığı, yemleme oranı, SGR ve TGC değerleri

Variables	January	February	March	April	May
T (°C)	9.68	9.55	10.2	14	16.83
Feed intake (biomass day %)	0.96	0.16	1.86	2.22	1.54
SGR	1.65	0.40	3.54	4.79	3.39
TGC	0.17	0.04	0.35	0.34	0.20

Table 2. Total length (TL), Weight (W), condition factor (CF), visceral index (VI), hepatosomatic index (HSI), gonadosomatic index (GSI), caecal fat score (CFS), fillet yield (FY) and carcass yield (CY) of cultivated rainbow trout in Eastern Black Sea (Data expressed as mean values $\pm$ S.D.)

Tablo 2. Karadeniz koşullarında yetiştirilen gökkuşağı alabalıklarında, toplam boy (TL), ağırlık (W), kondüsyon faktörü (CF), iç organ indeksi (VI), hepatosomatik indeks (HSI), gonadosomatik indeks (GSI), çekum yağ oranı (CFS), fileto verimi (FY) ve karkas verimi (CY) (veriler ortalama $\pm$ S. Sapma olarak verilmiştir)

Variables	December	January	February	March	April	May
TL (cm)	24.53 $\pm$ 1.81 ^a	26.66 $\pm$ 1.54 ^a	26.77 $\pm$ 2.17 ^a	31.79 $\pm$ 2.28 ^b	35.63 $\pm$ 3.00 ^c	40.22 $\pm$ 3.98 ^d
W (g)	206.93 $\pm$ 50.05 ^a	263.30 $\pm$ 46.11 ^a	278.10 $\pm$ 65.70 ^a	436.80 $\pm$ 95.62 ^b	734.60 $\pm$ 167.86 ^c	1011.4 $\pm$ 257.29 ^d
CF	1.37 $\pm$ 0.11 ^a	1.37 $\pm$ 0.10 ^a	1.44 $\pm$ 0.11 ^{ab}	1.34 $\pm$ 0.10 ^a	1.59 $\pm$ 0.11 ^c	1.53 $\pm$ 0.25 ^{bc}
VI (%)	13.38 $\pm$ 1.55 ^a	14.20 $\pm$ 2.88 ^a	14.29 $\pm$ 1.55 ^a	13.61 $\pm$ 1.43 ^a	18.55 $\pm$ 4.61 ^b	16.88 $\pm$ 2.05 ^b
HSI (%)	1.026 $\pm$ 0.12 ^a	1.288 $\pm$ 0.16 ^b	1.509 $\pm$ 0.28 ^c	1.492 $\pm$ 0.34 ^{bc}	1.830 $\pm$ 0.24 ^d	1.439 $\pm$ 0.20 ^{bc}
GSI (%)	0.085 $\pm$ 0.032 ^{ab}	0.056 $\pm$ 0.023 ^a	0.064 $\pm$ 0.035 ^{ab}	0.142 $\pm$ 0.097 ^c	0.086 $\pm$ 0.032 ^{ab}	0.113 $\pm$ 0.054 ^b
CFS	2.8 $\pm$ 1.08 ^{ab}	3.1 $\pm$ 0.57 ^b	2.7 $\pm$ 1.57 ^{ab}	1.8 $\pm$ 1.68 ^a	3.0 $\pm$ 1.15 ^b	3.5 $\pm$ 0.85 ^b
FY (%)	67.34 $\pm$ 3.03 ^{ab}	66.06 $\pm$ 3.03 ^a	68.38 $\pm$ 2.37 ^{abc}	69.94 $\pm$ 1.85 ^{bc}	69.89 $\pm$ 4.10 ^{bc}	70.96 $\pm$ 2.55 ^c
CY (%)	75.41 $\pm$ 2.39 ^b	72.33 $\pm$ 3.11 ^a	74.90 $\pm$ 1.80 ^b	75.85 $\pm$ 1.65 ^b	74.05 $\pm$ 4.08 ^{ab}	75.48 $\pm$ 2.37 ^b

Values expressed in the same row with different letter indicate differences are statistically significant ($P<0.05$)

months, April and May 2006. The highest condition factor was recorded as 1.59 and 1.53 in May and April, respectively. Similarly, a higher visceral index (VI) was found in April (18.55%) and May (16.88%). HSI was also high in April due to high feeding rate. The highest GSI (0.142%) was calculated in March samplings. The lowest caecal fat score was recorded as 1.8 in March and the highest was recorded as 3.5 in May.

Concerning fillet yield, FY was raised gradually from beginning of experiment to last month May. Fish had showed a fillet yield of 67.34% in December, and 70.96% in May that is harvest stage. On the other hand carcass yield was lowered on January (72.33%), but it was detected invariable in other sampling months.

Mean crude protein content in the whole fillet (Fig. 2) decreased from February 2006 ($86.72 \pm 3.02\%$) to May 2006 ($70.22 \pm 3.43\%$) ($P < 0.05$). General trend, crude protein was lowered in all parts of fillet after February.

The higher mean crude protein rate was detected as $90.26 \pm 3.43\%$ in the dorsal part of caudal site of trout on February, and the lowest crude protein rate was detected in $60.96 \pm 1.72\%$ during experiment. Ventral part of cranial and intermediate sections performed lower rate crude protein than dorsal parts.

Mean crude fat of whole fillet (WF) was decreased for three months, from December (11.81%) to February (9.19%). After February mean crude fat was increased up to highest level on May (16.64%). Ventral part of cranial site and intermediate site had a higher level of crude fat than both dorsal part of those parts and caudal site (Fig 2).

Mean crude ash content lowered in whole fillet month by month. Crude ash content of whole fillet was calculated sequentially 1.4%, 1.29%, 1.33%, 1.29%, 1.27%, 1.30% from December to May (Fig. 2). Higher crude ash content was detected for the dorsal part of

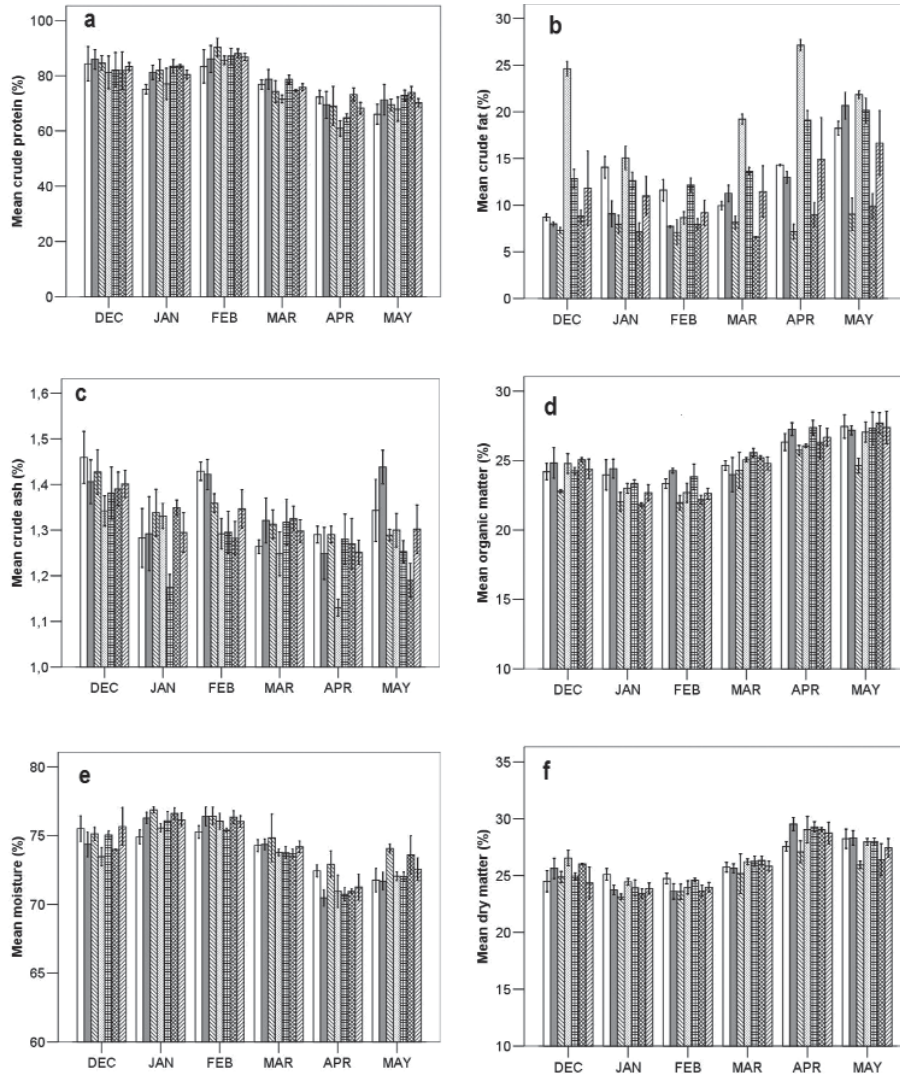


Fig 2. Mean crude protein (a), mean crude fat (b), mean crude ash (c), mean organic matter (d), mean moisture (e) and mean dry matter (f) content of partitioned fillet site and whole fillet in Black Sea reared rainbow trout. (□) indicates dorsal part of cranial site, (▨) dorsal part of intermediate site, (▩) dorsal part of caudal site, (▧) ventral part of cranial site, (▦) ventral part of intermediate site, (▤) ventral part of caudal site and (▥) whole fillet. Error bars represent mean $\pm$ S.E.

Şekil 2. Karadeniz’de ticari şartlar altında yetiştirilen gökkuşuğu alabalığı filetosunda kısımlara göre ortalama ham protein (a), ortalama ham yağ (b), ortalama ham kül (c), ortalama organik madde (d), ortalama nem (e) ve ortalama kuru madde (f) oranları. (□) kranial bölge dorsal kısmı, (▨) orta bölge dorsal kısmı, (▩) kaudal bölge dorsal kısmı, (▧) kranial bölge ventral kısmı, (▦) orta bölge ventral kısmı, (▤) kaudal bölge ventral kısmı ve (▥) tüm filetoyu göstermektedir. Hata çubukları ortalama $\pm$ S.H’yı göstermektedir

sections than ventral sites. Unlike crude ash, organic matter content of whole fillet was slowly reduced from December to February and thereafter it was increased from February to May (Fig. 2).

Water content in the whole fillet was found as 75.65%, 76.14%, 76.02% and 75.65% in December, January, February and March, respectively. However, a decrease was recorded in April (71.25%) and May (72.56%) (Fig. 2). Unlike moisture, dry matter content of whole fillet was slowly decreased from December to February and then, increased from February to May (Fig. 2). This increase after February was significant ($P<0.05$) between months.

DISCUSSION

Weight gain varied significantly between sampling months. This was related to water temperature and feeding regime that was arranged according to water temperature. Temperature was changed between 9-16°C. Growth of fish is dependent to changes in water temperature, diet, fish size, and seasonal period of the year and is usually measured in terms of weight or length of the fish¹⁸. Specific growth rate (SGR) is an index used to compare actual mass gain to predicted mass gain of salmonids in aquaculture¹⁹. The calculation of SGR assumes that the cube root of mass increases linearly over time and that the effect of temperature on growth is linear between 5 and 15°C. But in this study, SGR was changed between 0.4 and 4.79 during farming activities. In previous studies, SGR rate of rainbow trout was reported between 1.02-1.11⁶, 3.36⁴, 1.35-1.79²⁰ or between 0.79-3.26⁷ in Black Sea condition. Our results were in close agreement with those of the previous reports by those authors. Thermal growth coefficient (TGC) was used to assess growth, because TGC incorporates both fish size and temperature effects in the growth trajectory. TGC was calculated between 0.04 and 0.35 and strong linear correlation was detected with SGR ($R^2=0.84$).

Slaughter parameters of fish such as VI were significantly different ($P<0.05$) in the last two months of the experiment than the initial four months of trial. Unlike CFS, HSI was recorded significant higher ($P<0.05$) for the last four month period compared to those recorded during the initial two months. Carcass yield was almost constant during the course of the experiment, except January data, which was significantly lower ($P<0.05$) than the other months. Fillet yield was highest in May. This was linked directly to the higher VI index of the rainbow trout from this group. Similarly, Mathis et al.²¹ reported that the VI index was one of the parameters

that were correlated with filleting yield in perch. Condition factor followed the same trend, increasing by months except March. This was linked to higher water temperature and higher feed consumption.

Within sampling periods, crude protein content of whole fillet was lower in periods with higher water temperature (April and May). The dorsal part (Int-D, Ca-D) had higher protein content than the ventral parts in general (Fig. 2). There are reports on variation in body constituents of fish reared at different temperatures²². In the present study with rainbow trout, higher protein content was found in lower water temperature (except January). However, no effect of temperature or ration was noted on body protein content in *Oncorhynchus nerka*²³ and *Salmo trutta*²⁴. Cr-V and Int-V were significantly different from other partitioned fillet sites for crude fat except last sampling months (Fig. 2). In general fat content of ventral parts were detected higher than dorsal parts. Andrews and Stickney²⁵ observed that in channel catfish, an increase in temperature led to increased lipid content. In rainbow trout fillets the lipid content tends to increase following the cranio-caudal direction, whereas this trend seems to be less pronounced in the dorso-ventral direction²⁶. Fat content of whole fillet and portioned fillet parts are straightly related with condition factor. Crude ash and organic matter content of whole fillet related inversely. Organic matter content of fillet increased until harvest month, May. Lower organic matter content was detected in dorsal part of caudal site.

Water content of whole fillet was reduced month by month until fish harvest. Teskeredzic and Pfeifer²⁷ reported that the fish reared in brackish water condition has lower water content, higher fat deposit than reared in fresh water condition. Crude fat content and moisture of fillet related inversely. Similarly, some scientist reported these two components are inversely related in fish flesh^{2,28}.

Results of these studies showed that remarkable differences in proximate composition emerged between the dorsal and ventral fillet portions of farmed rainbow trout under commercial condition, even though these differences were not of equal importance for all of the components considered.

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Population Structures and Some Growth Properties of Three Cyprinid Species [*Squalius cephalus* (Linnaeus, 1758); *Tinca tinca* (Linnaeus, 1758) and *Alburnus escherichii* Steindachner, 1897] Living in Camkoru Pond (Ankara-Turkey) ^[1]

Deniz INNAL * 

[1] This study is summarized from the author's MSc Thesis and presented as 3 posters in XIV. Symposium of Fisheries, Muğla - Turkey

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Summary

This study concerns an investigation of the population structures and some growth properties of European Chub [*Squalius cephalus* (Linnaeus, 1758)], Tench [*Tinca tinca* (Linnaeus, 1758)] and Anatolian Bleak (*Alburnus escherichii* Steindachner, 1897) living in Camkoru Pond (Çamlidere- Ankara- Sakarya Basin). For this purpose the population densities, age and sex compositions, growth in length and weight, length-weight relationships of fish species have been obtained and compared with other studies. The densities of fish species are (38.78%) Tench; (31.19%) European Chub; (25.69%) Anatolian Bleak. The maximum ages for Tench and Anatolian Bleak were VIII, and for European Chub was X. The length and weight relationship for European Chub, Anatolian Bleak and Tench populations was as follows: $W = 0.0134 L^{3.0126}$, $W = 0.0066 L^{3.2501}$ and $W = 0.0144 L^{3.0066}$. Student's t test showed that differences in length and weight between females and males of the same age groups of species were statistically insignificant ($P > 0.05$)

Keywords: *Tinca tinca*, *Squalius cephalus*, *Alburnus escherichii*, Population structures, Growth properties, Camkoru Pond

Çamkoru Göleti (Ankara-Türkiye)'nde Yaşayan Üç Cyprinid Türünün [*Squalius cephalus* (Linnaeus, 1758), *Tinca tinca* (Linnaeus, 1758) ve *Alburnus escherichii* Steindachner, 1897] Populasyon Yapısı ve Bazı Büyüme Özellikleri

Özet

Bu çalışma Çamkoru Göleti (Çamlidere-Ankara-Sakarya Havzası)'nde yaşayan Kefal [*Squalius cephalus* (Linnaeus, 1758)], Kadife [*Tinca tinca* (Linnaeus, 1758)] ve İnci (*Alburnus escherichii* Steindachner, 1897) balığının populasyon yapısı ve bazı büyüme özelliklerinin araştırılması ile ilişkilidir. Bu amaçla, balık türlerinin populasyon yoğunluğu, yaş ve eşey kompozisyonu, boy ve ağırlık büyümesi ve boy-ağırlık ilişkisi saptanmış ve diğer çalışmalarla karşılaştırılmıştır. Balık türlerinin yoğunluğu; Kadife (38.78%); Kefal (31.19%); İnci (25.69%)'dir. Kadife ve İnci balığı için maksimum yaş VIII, Kefal için X'dur. Kefal, İnci ve Kadife populasyonunun boy - ağırlık ilişkisi sırasıyla $W = 0.0134 L^{3.0126}$, $W = 0.0066 L^{3.2501}$ ve $W = 0.0144 L^{3.0066}$ 'dir. Türlerin aynı yaş gruplarında erkek ve dişi bireyler arasında boy ve ağırlık açısından fark, t-testiyle önemsiz ($P > 0.05$) olarak gözlenmiştir.

Anahtar sözcükler: *Tinca tinca*, *Squalius cephalus*, *Alburnus escherichii*, Populasyon yapısı, Büyüme özellikleri, Çamkoru Göleti

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INTRODUCTION

The Tench (*Tinca tinca*), European Chub (*Squalius cephalus*) and Anatolian Bleak (*Alburnus escherichii*) belong to the family Cyprinidae, which contains numerous species of fish present in Turkey ¹. *S. cephalus* and *A. escherichii* are the native fish species of Camkoru Pond. After the reservoir construction *T. tinca*, *Cyprinus carpio* and *Oncorhynchus mykiss* were introduced into Camkoru Pond. Although stocking programmes of alien fish species have been applied into this pond there is no published data about population structures and biological properties of fish species.

Many investigations have been made to determine the various biological properties of Chub ²⁻⁷ and Tench ⁸⁻¹⁴, but there is only one study about Anatolian Bleak ¹⁵.

The aim of the study; the population densities, age and sex compositions, growth in length and weight, length-weight relationships of fish species (*S. cephalus*, *A. escherichii*, *T. tinca*) have been obtained and compared with other studies.

MATERIAL and METHODS

The study was carried out on the determination of population structures and some growth properties of *Squalius cephalus*, *Alburnus escherichii* and *Tinca tinca* population in Camkoru Pond (Camlidere-Ankara) located on the Kadinboğazi Creek, 105 km northwest of Ankara with a surface area of 7 ha. Fish species were caught monthly with nets of various mesh sizes (10, 17, 23, 30, 40 and 50 mm) between August 2002 and August 2003. The abundance of species was ascertained according to Sisli ¹⁶. Abundance % = $[Ni/Nt] \times 100$, (Ni: The number of specimens of the species; Nt: The total number of specimens). After being caught, the fish samples were transported to the

laboratory where their size (fork length; cm, referred to as LF in the text and weight; g, referred to as W in the text) were measured and weighed to the nearest 1.0 mm and 0.1 g, respectively. Age determination was carried out according to the method of Lagler ¹⁷ (The roman numerals indicate a subjective classification of ages). Proportional growth in weight and length between the ages were calculated from the Relative growth (R) equations; $RW = [(W_{t+1} - W_t)/W_t] \times 100$, $RFL = [(FL_{t+1} - FL_t)/FL_t] \times 100$ respectively, where W_{t+1} are the weight in grams and FL_{t+1} length in cm at age t+1, W_t is weight in grams and FL_t is length in cm at age t. Length-weight relationship was described by the following equation: $W = aL^b$. Where: W: mass in grams; L: fork length in centimeters. a: constant; b: constant described as isometric or allometric growth type. Differences between growth in males and females within the same age groups were tested by Student's t test ($P > 0.05$).

RESULTS

Fish Species and Abundance

Origin of species and the frequency-abundance data are given in [Table 1](#).

Six fish species, 1199 specimens were captured from Camkoru Pond. *Tinca tinca* (38.78%) was determined to have the greatest population size followed by *Squalius cephalus* (31.19%), *Alburnus escherichii* (25.69%), *Gobio gobio* (3.34%), *Cyprinus carpio* (0.92%) and *Oncorhynchus mykiss* (0.08%).

The Sex and Age Compositions

The age group and sex distribution of species are shown in [Table 2](#).

The ages of the captured specimens of *S. cephalus* ranged from I to X years and the 3rd group was dominant

Table 1. Origin of species and the frequency-abundance data

Tablo 1. Türlerin kaynağı ve yoğunluk-bolluk verisi

Species	Family	Origin of Species	Introduction			Number of Individuals		
			Purpose	Year	Number	Total	%	Rank
<i>Squalius cephalus</i>	Cyprinidae	Native	-			374	31.19	2
<i>Alburnus escherichii</i>	Cyprinidae	Native	-			308	25.69	3
<i>Gobio gobio</i>	Cyprinidae	Native	-			40	3.34	4
<i>Tinca tinca</i>	Cyprinidae	Introduced	Accidental	1993	200	465	38.78	1
<i>Cyprinus carpio</i>	Cyprinidae	Introduced	Recreational Fishing	2001	5000	11	0.92	5
				2002	6000			
				2003	5000			
<i>Oncorhynchus mykiss</i>	Salmonidae	Introduced	Recreational Fishing	1997	8000	1	0.08	6
				2001	6000			

Table 2. The sex and age compositions of species**Tablo 2.** Türlerin yaş ve eşey kompozisyonları

AGE	<i>Squalius cephalus</i>						<i>Alburnus escherichii</i>						<i>Tinca tinca</i>					
	Female		Male		F+M		Female		Male		F+M		Female		Male		F+M	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
I	-	-	-	-	25	8.14	-	-	-	-	-	-	-	-	-	-	41	13.1
II	10	3.26	6	1.95	16	5.21	2	0.66	1	0.33	3	1	37	11.8	32	10.2	69	22
III	114	37.1	61	19.9	175	57	81	26.9	74	24.6	155	51.5	51	16.2	39	12.4	90	28.7
IV	28	9.12	15	4.89	43	14	16	5.32	19	6.31	35	11.6	30	9.55	47	15	77	24.5
V	11	3.58	6	1.95	17	5.54	23	7.64	26	8.64	49	16.3	11	3.5	6	1.91	17	5.41
VI	6	1.95	2	0.65	8	2.61	23	7.64	20	6.64	43	14.3	4	1.27	3	0.96	7	2.23
VII	9	2.93	2	0.65	11	3.58	7	2.33	7	2.33	14	4.65	5	1.59	3	0.96	8	2.55
VIII	3	0.98	2	0.65	5	1.63	-	-	2	0.66	2	0.66	3	0.96	2	0.64	5	1.59
IX	3	0.98	-	-	3	0.98	-	-	-	-	-	-	-	-	-	-	-	-
X	4	1.3	-	-	4	1.3	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	188	61.2	94	30.6	307	100	152	50.5	149	49.5	301	100	141	44.9	132	42.1	314	100

Table 3. The minimum, maximum and average fork length (cm)-weight (gr) of the age groups of *S. cephalus***Tablo 3.** *S. cephalus*'ta yaş gruplarının minimum, maksimum ve ortalama çatal boy ve ağırlık değerleri

AGE	N	Female				N	Male				t - test		N	Female + Male			
		L (cm)		W (gr)			L (cm)		W (gr)		L (cm)	W (gr)		L (cm)		W (gr)	
		(min-max)	S	(min-max)	S		(min-max)	S	(min-max)	S				(min-max)	S	(min-max)	S
I		-	-	-	-	-		-	-	-	-	25	9.19±0.55 (8-10.1)	0.1	9.95±1.25 (8.3-12.7)	-	
II	10	13.42±1.19 (11.3-14.6)	0.4	37.08±10.09 (18.2-46.3)	3.19	6	13.75±1.58 (11-14.9)	0.7	38.03 ±14.84 (18.9-50.2)	6.06	0.37 (P>0.05)	0.89 (P>0.05)	16	13.54±1.30 (11-14.9)	0.3	37.44±11.61 (18.2-50.2)	2.9
III	114	17.06±1.25 (14-20.2)	0.1	70.24±15.30 (46.5-110.3)	1.43	61	16.66±1.17 (14.9-20)	0.2	64.64±12.97 (48-105)	1.66	0.46 (P>0.05)	0.39 (P>0.05)	175	16.92±1.23 (14-20.2)	0.1	68.28±14.73 (46.5-110.3)	1.11
IV	28	20.54±2.29 (18.2-24.7)	0.4	126.31±38.31 (84-192)	7.24	15	21.00±2.26 (17.9-24.1)	0.6	129.93±39.75 (83-187)	10.3	0.53 (P>0.05)	0.78 (P>0.05)	43	20.70±2.26 (17.9-24.7)	0.3	127.57±38.38 (83-192)	5.85
V	11	24.33±0.89 (23.4-26.1)	0.3	207.32±29.31 (179-262.3)	8.84	6	24.03±1.18 (22.2-25.5)	0.5	200.68±21.28 (172.5-226)	8.69	0.61 (P>0.05)	0.6 (P>0.05)	17	24.22±0.98 (22.2-26.1)	0.2	204.98±26.25 (172.5-262.3)	6.37
VI	6	26.67±1.47 (24.9-29)	0.6	266.45±29.96 (230.2-295.5)	12.2	2	27.35±0.64 (26.9-27.8)	0.5	290.00±5.66 (286-294)	4.0	0.41 (P>0.05)	0.12 (P>0.05)	8	26.83±1.31 (24.9-29)	0.5	272.3±27.65 (230.2-295.5)	9.77
VII	9	29.92±1.55 (27.2-32.4)	0.5	361.67±45.36 (290.5-435.5)	15.1	2	31.00±2.83 (29-33)	2.0	381.50±72.83 (330-433)	51.5	0.69 (P>0.05)	0.77 (P>0.05)	11	30.12±1.70 (27.2-33)	0.5	365.27±47.34 (290.5-435.5)	14.3
VIII	3	33.27±0.78 (32.4-33.9)	0.5	491.67±25.70 (462-507)	14.8	2	33.45±1.91 (32.1-34.8)	1.4	457.50±57.28 (417-498)	40.5	0.92 (P>0.05)	0.55 (P>0.05)	5	33.34±1.11 (32.1-34.8)	0.5	478±38.74 (417-507)	17.3
IX	3	35.30±0.6 (34.7-35.9)	0.4	584.33±13.05 (572-598)	7.54	-	-	-	-	-	-	-	3	35.30±0.6 (34.7-35.9)	0.4	584.33±13.05 (572-598)	7.54
X	4	37.38±0.87 (36.3-38.4)	0.4	764.75±66.14 (702-845)	33.1	-	-	-	-	-	-	-	4	37.38±0.87 (36.3-38.4)	0.4	764.75±66.14 (702-845)	33.1

in the population. The fish population was 61.24% female and 30.62% male. The ages of captured specimens of *T. tinca* ranged from I to VIII and the 3rd group was dominant in the population. The fish population was 44.90% female and 42.04% male. The ages of captured specimens of *A. escherichii* II to VIII and the 3rd group was dominant in the population. The fish population was 50.50% female and 49.50% male.

Length and Weight Composition

The minimum, maximum and average fork lengths (cm) - weights (gr) with confidence intervals (CI) at 95% significance levels and standard error (S) for male, female of the age groups of species are given in [Tables 3, 4](#) and [5](#). Length-weight relationships of species are given in [Fig. 1](#).

Determined minimum and maximum fork length values of *S. cephalus* were 8 and 38.4 cm. Among the European Chub caught 66.12% were 12-20 cm, 9.45% were shorter than 12 cm and 24.43% were longer than 20 cm. Weights of European Chub ranged between 8.3 gr and 845 gr, the majority being (44.95%) 60-120 gr.

Fork Lengths of Anatolian Bleak ranged between 7.5 cm and 16.4 cm. Among the Anatolian Bleak caught 44.85% were 9-11 cm, 9.64% were shorter than 9 cm, and 45.51% were longer than 11 cm. Weights of Anatolian Bleak ranged between 7.1 gr and 56.8 gr, the majority being (59.80%) 7-17 gr.

Fork Lengths of Tench ranged between 8.2 cm and 32.5 cm. Among the Tench caught 71.97% were 12-22

cm, 15.28% were shorter than 12 cm, and 12.75% were longer than 22 cm. Weights of Tench ranged between 8.2 gr and 453 gr, the majority being (54.15%) 40-160gr .

The differences in length and weight between males and females was statistically insignificant ($P>0.05$) in all age groups of three species.

The parameter b of the fishes ranged from a minimum of 3.0066 for *T. tinca* to a maximum of 3.2501 for *A. escherichii*.

Growth in Length and Weight

The relative increase in length and weight of European Chub, Anatolian Bleak and Tench from Camkoru Pond during the study are given in Table 6.

Table 4. The minimum, maximum and average fork length (cm)-weight (gr) of the age groups of *A. escherichii*

Tablo 4. *A. escherichii*'de yaş gruplarının minimum, maksimum ve ortalama çatal boy ve ağırlık değerleri

AGE	N	Female				N	Male				t - test		N	Female + Male			
		L (cm)		W (gr)			L (cm)		W (gr)		L (cm)	W (gr)		L (cm)		W (gr)	
		(min-max)	S	(min-max)	S		(min-max)	S	(min-max)	S				(min-max)	S	(min-max)	S
I		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II	2	7.65±0.21 (7.5-7.8)	0.15	7.15±0.07 (7.1-7.2)	0.05	1	7.5 -	-	7.1 -	-	-	-	3	7.60±0.17 (7.5-7.8)	0.1	7.13±0.06 (7.1-7.2)	0.03
III	81	9.68±0.74 (8-11.6)	0.08	10.49±1.79 (7.3-14.8)	0.2	74	9.65±0.64 (8.1-11)	0.07	10.39±1.90 (7.1-14.5)	0.22	0.8 (P>0.05)	0.74 (P>0.05)	155	9.67±0.69 (8-11.6)	0.06	10.45±1.84 (7.1-14.8)	0.15
IV	16	11.44±0.72 (10.4-12.6)	0.18	17.01±5.48 (12.6-28.5)	1.37	19	11.62±0.47 (10.7-12.4)	0.11	18.03±4.16 (13.2-27.3)	0.95	0.39 (P>0.05)	0.54 (P>0.05)	35	11.54±0.60 (10.4-12.6)	0.1	17.56±4.76 (12.6-28.5)	0.8
V	23	13.09±0.48 (12.1-13.7)	0.1	30.64±3.47 (21.2-36.5)	0.72	26	13.27±0.47 (11.5-13.8)	0.09	30.87±3.79 (17-36.2)	0.74	0.18 (P>0.05)	0.83 (P>0.05)	49	13.18±0.48 (11.5-13.8)	0.07	30.76±3.61 (17-36.5)	0.52
VI	23	14.30±0.33 (13.5-14.8)	0.07	40.27±4.39 (35-48)	0.91	20	14.13±0.29 (13.5-14.7)	0.07	38.19±3.75 (32-43.8)	0.84	0.07 (P>0.05)	0.1 (P>0.05)	43	14.22±0.32 (13.5-14.8)	0.05	39.30±4.19 (32-48)	0.64
VII	7	15.13±0.46 (14.4-15.7)	0.17	43.90±4.19 (40.9-52.1)	1.59	7	15.31±0.54 (14.5-15.8)	0.2	44.91±4.96 (39-52.3)	1.88	0.5 (P>0.05)	0.69 (P>0.05)	14	15.22±0.49 (14.4-15.8)	0.13	44.41±4.45 (39-52.3)	1.19
VIII		-	-	-	-	2	16.30±0.14 (16.2-16.4)	0.1	55.10±2.40 (53.4-56.8)	1.7	-	-	2	16.30±0.14 (16.2-16.4)	0.1	55.10±2.40 (53.4-56.8)	1.7

Fig 1. Length-weight relationships of species

Şekil 1. Türlerin boy-ağırlık ilişkisi

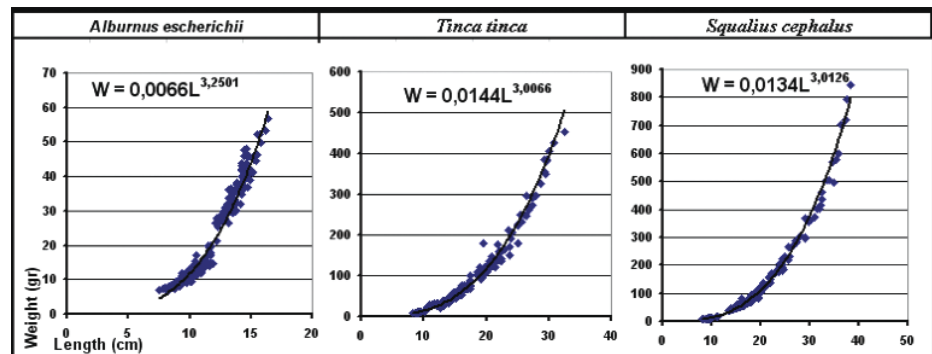


Table 5. The minimum, maximum and average fork length (cm)-weight (gr) of the age groups of *T. tinca***Tablo 5.** *T. Tinca*'da yaş gruplarının minimum, maksimum ve ortalama çatal boy ve ağırlık değerleri

AGE	N	Female				N	Male				t - test		N	Female + Male			
		L (cm)		W (gr)			L (cm)		W (gr)		L (cm)	W (gr)		L (cm)		W (gr)	
		(min-max)	S	(min-max)	S		(min-max)	S	(min-max)	S				(min-max)	S	(min-max)	S
I		-	-	-	-	-	-	-	-	-	-	41	9.16±0.47 (8.2-10.3)	0.07	10.61±1.58 (8.2-14.3)	0.25	
II	37	12.86±0.86 (10.7-14.7)	0.16	31.48±6.09 (21.3-50.2)	1	32	12.42±0.73 (10.6-14.5)	0.13	28.41±5.38 (23.0-48.6)	0.95	0.92 (P>0.05)	0.93 (P>0.05)	69	12.65±0.82 (10.6-14.7)	0.1	30.06±5.93 (21.3-50.2)	0.71
III	51	16.50±1.89 (13.9-19.5)	0.26	70.25±24.20 (38.7-112.3)	3.39	39	16.08±1.68 (13.5-19.4)	0.27	66.85±21.96 (37.8-116)	3.51	0.28 (P>0.05)	0.49 (P>0.05)	90	16.32±1.80 (13.5-19.5)	0.19	68.78±23.19 (37.8-116)	2.44
IV	30	20.23±0.95 (17.5-22.3)	0.17	123.64±16.38 (98-180.3)	2.99	47	19.95±1.10 (18.5-22.3)	0.16	117.00±15.04 (96.4-146.3)	2.19	0.25 (P>0.05)	0.8 (P>0.05)	77	20.06±1.05 (17.5-22.3)	0.12	119.59±15.81 (96.4-180.3)	1.8
V	11	23.47±1.40 (21.9-25.2)	0.42	183.99±30.79 (142.5-225.9)	9.28	6	23.45±0.82 (21.8-23.9)	0.34	181.55±20.76 (150.3-211.4)	8.48	0.97 (P>0.05)	0.85 (P>0.05)	17	23.46±1.20 (21.8-25.2)	0.29	183.13±26.99 (142.5-225.9)	6.55
VI	4	26.43±0.73 (25.7-27.4)	0.36	268.23±31.31 (233.7-298.2)	15.7	3	26.23±0.67 (25.5-26.8)	0.38	253.37±6.48 (248.4-260.7)	3.74	0.73 (P>0.05)	0.42 (P>0.05)	7	26.34±0.65 (25.5-27.4)	0.25	261.86±23.82 (233.7-298.2)	9
VII	5	28.72±0.96 (27.4-29.6)	0.43	333.18±45.65 (274.2-385.5)	20.4	3	27.83±0.67 (27.1-28.4)	0.38	312.33±26.39 (282-330)	15.2	0.18 (P>0.05)	0.44 (P>0.05)	8	28.38±0.93 (27.1-29.6)	0.32	325.36±38.81 (274.2-385.5)	13.7
VIII	3	30.6±1.71 (29.2-32.5)	0.98	414.1±35.24 (384.3-453)	20.4	2	30.3±0.85 (29.7-30.9)	0.6	403.2±31.11 (381.2-425.2)	22	0.81 (P>0.05)	0.74 (P>0.05)	5	30.48±1.29 (29.2-32.5)	0.58	409.74±29.98 (381.2-453)	13.4

Table 6. The relative increase in length and weight values of species**Tablo 6.** Türlerin boy ve ağırlık değerlerinde relatif artış

AGE	<i>Squalius cephalus</i>						<i>Alburnus escherichii</i>						<i>Tinca tinca</i>					
	Female		Male		F+M		Female		Male		F+M		Female		Male		F+M	
	RFL	RW	RFL	RW	RFL	RW	RFL	RW	RFL	RW	RFL	RW	RFL	RW	RFL	RW	RFL	RW
I	-	-	-	-	0.47	2.76	-	-	-	-	-	-	-	-	-	-	0.38	1.83
II	0.27	0.89	0.21	0.7	0.25	0.82	0.27	0.47	0.29	0.46	0.27	0.47	0.28	1.23	0.29	1.35	0.29	1.29
III	0.2	0.8	0.26	1.01	0.22	0.87	0.18	0.62	0.2	0.74	0.19	0.68	0.23	0.76	0.24	0.75	0.23	0.74
IV	0.18	0.64	0.14	0.54	0.17	0.61	0.14	0.8	0.14	0.71	0.14	0.75	0.16	0.49	0.18	0.55	0.17	0.53
V	0.1	0.29	0.14	0.45	0.1	0.33	0.09	0.31	0.06	0.24	0.08	0.28	0.13	0.46	0.12	0.4	0.12	0.43
VI	0.12	0.36	0.13	0.32	0.12	0.34	0.06	0.09	0.08	0.18	0.07	0.13	0.09	0.24	0.06	0.23	0.08	0.24
VII	0.11	0.36	0.08	0.2	0.11	0.31	-	-	0.06	0.23	0.07	0.24	0.07	0.24	0.09	0.29	0.07	0.26
VIII	0.06	0.19	-	-	0.06	0.22	-	-	-	-	-	-	-	-	-	-	-	-
IX	0.06	0.31	-	-	0.06	0.31	-	-	-	-	-	-	-	-	-	-	-	-
X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

The age composition in present study of European Chub, Anatolian Bleak and Tench populations was as follows: I-X; II-VIII and I-VIII. Because of the large mesh size (>10 mm), fish less than I year old for European Chub and Tench populations and II year old for Anatolian Bleak populations were not represented.

The age composition in previous studies of European Chub populations was as follows: Işıklı Lake, I-V ¹⁸; Topçam Dam Lake, I-VII ³; Sır Dam Lake, I-VI ¹⁹; Sarıyar

Dam Lake, I-X ²⁰. The life span of the European Chub population in the Camkoru Pond was longer than the other studies except Sarıyar Dam Lake. The age composition in previous studies of Tench populations was as follows: Kesikköprü Dam Lake, I-VI ⁹; Bayındır Dam Lake, I-V ²¹. Kayaboğazı Dam Lake I-VII ²². Porsuk Dam Lake, I-VIII ²³, Abant Lake, II-IX ¹³; Hirfanlı Dam Lake I-VI ²⁴. The age composition of Anatolian Bleak populations in Sakarya Basin reported as I-V ¹⁵; These differences in the age distribution of the populations may be due to sampling differences, fishing activity, trophic status and ecological characteristics of the water bodies.

Overall, the percent female and male population in each age class of European Chub was as follows for females and males, respectively: Age 1., 8.14% (Immature); age 2., 3.26% (female) and 1.95% (male); age 3., 37.1% and 19.9%; age 4., 9.12% and 4.89%; age 5., 3.58% and 1.95%; age 6., 1.95% and 0.65%; age 7., 2.93% and 0.65%; age 8., 0.98% and 0.65%; age 9., 0.98% (female); age 10., 1.95% (female). Females were more numerous in all age groups of the European Chub populations and males were not sampled in age groups IX and X. It can be stated from these results that male individuals of the European Chub population are shorter lived than females.

The percent female and male population in each age class of Anatolian Bleak was as follows for females and males, respectively: Age 2., 0.66% and 0.33%; age 3., 26.9% and 24.6%; age 4., 5.32% and 6.31%; age 5., 7.64% and 8.64%; age 6., 7.64% and 6.64%; age 7., 2.33% and 2.33%; age 8., 0.66% (male). Males were more numerous in IV and V age groups of the Anatolian Bleak populations.

The percent female and male population in each age class of Tench was as follows for females and males, respectively: Age 1., 13.1% (Immature); age 2., 11.8% and 10.2%; age 3., 16.2% and 12.4%; age 4., 9.55% and 15%; age 5., 3.5% and 1.91%; age 6., 1.27% and 0.96%; age 7., 1.59% and 0.96%; age 8., 0.96% and 0.64. Females were more numerous in II, III, V, VII and VIII age groups of the Tench populations.

Of all the European Chub examined, 25 were immature, 188 were female, and 94 were male. The sex ratio was 1.0 females to 0.5 male in the European Chub populations in present study. The sex composition differed between the ages, with a higher percentage of females in the all age classes. It was determined that female strongly dominated in the population of European Chub in the Camkoru Pond. There were significant differences in the ratio of females to males in the European Chub populations. The unequal sex ratio for mature fish respects different survival rates for males and females. It is not clear which factors might be responsible in the fluctuation of male and female population distribution of *S. cephalus* in Camkoru Pond. The sex ratio (female:male) in previous studies of European Chub populations was as follows: Tödürge Lake, 1:0.42²⁵ Işıklı Lake Chub, 1:1.46¹⁸; Topçam Dam Lake, 1:0.37³; Sır Dam Lake 1:0.82¹⁹; Karakaya Dam Lake, 1: 0.57⁴; İkizcetepeler Dam Lake, 1:1.41⁷. The sex ratio of European Chub populations in the Tödürge Lake, Karakaya Dam Lake and Camkoru Pond are quite similar.

Of all the Anatolian Bleak examined, 152 were

female, and 149 were male. The sex ratio was 1.0 females to 0.98 male in this population. Of all the Tench examined, 41 were immature, 141 were female, and 132 were male. It was 1.0 females to 0.94 male in the Tench populations. The almost equal ratio of females to males at relatively similar sizes in two fish populations (*A. escherichii* and *T. tinca*) indicate that equal numbers of two sexes are born and enter the population.

The sex ratio (female:male) in previous studies of Tench populations was as follows: Kesikköprü Dam Lake, 1:0.94⁹; Bayındır Dam Lake, 1:1.04²¹; Kayaboğazı Dam Lake, 1:0.95²²; Abant Lake 1:0.83¹³; Hirfanlı Dam Lake 1:1.42²⁴ Porsuk Dam Lake, 1:0.96²³. The sex ratio of Tench populations in the Kesikköprü Dam Lake, Kayaboğazı Dam Lake, Porsuk Dam Lake and Camkoru Pond are quite similar. The sex ratio differs from one population to another of the same species and may vary from year to year in the same population. The reasons of this variation may be different and numerous: the seasonal aspect, the feeding and maturation periods, different growth rates in males and females, mortality difference for each sex and perhaps the climatical and biological conditions.

The Fork length of the European Chub population in Camkoru Pond ranged from 8 cm to 38.4 cm. The mean fork length of all samples was 18.51 cm. Lengths for ages I, II, III, IV, V, VI, VII, VIII, IX and X were 9.19; 13.54; 16.92; 20.70; 24.22; 26.83; 30.12; 33.34; 35.30 and 37.38 cm respectively. The fork lengths in age groups II, IV, VI, VII and VIII were higher for males, and in III and V age groups for females. The mean female body length was 19.71 cm and individual length ranged from 11.3 to 38.4 cm. The mean male body length was 18.57 cm and individual length ranged from 11 to 34.8 cm. The fork length of the age groups of European Chub populations in the Sarıyar Dam Lake²⁰ and Camkoru Pond are quite similar.

The mean fork length of all samples of Anatolian Bleak examined was 11.37 cm and individual fish ranged in length from 7.5 cm to 16.4 cm. Lengths for ages II, III, IV, V, VI, VII and VIII were 7.60; 9.67; 11.54; 13.18; 14.22; 15.22 and 16.30 cm respectively. The fork lengths in age groups IV, V and VII were higher for males, and in II, III and VI age groups for females. The mean female body length was 11.31 cm and individual length ranged from 7.5 to 15.7 cm. The mean male body length was 11.44 cm and individual length ranged from 7.5 to 16.4 cm. The fork length of the IV age group of Anatolian Bleak populations in the Sakarya 3 basin (11.40 cm)¹⁵ and Camkoru Pond are quite similar.

The Fork length of the Tench population in Camkoru Pond ranged from 8.2. cm to 32.5 cm and The mean fork

length of all samples was 16.73. Lengths for ages I, II, III, IV, V, VI, VII and VIII were 9.16; 12.65; 16.32; 20.06; 23.46; 26.34; 28.38 and 30.48 cm respectively. The fork lengths in all age groups were higher for females. The mean female body length was 17.90 cm and individual length ranged from 10.7 to 32.5 cm. The mean male body length was 17.84 cm and individual length ranged from 10.6 to 30.9 cm. The fork length of the III and IV age groups of Tench populations in the Abant Lake ¹³ and Camkoru Pond are quite similar.

The total weights of the European Chub population in Camkoru Pond ranged from 8.3 gr to 845 gr and the total weights for ages I, II, III, IV, V, VI, VII, VIII, IX and X were 9.95; 37.44; 68.28; 127.57; 204.98; 272.3; 365.27; 478; 584.33; 764.75 gr respectively. The weights in age groups II, IV, VI and VII were higher for males, and in III, V and VIII age groups for females. The mean body weight of all samples was 114.78 gr. The mean female body weight was 134.74 gr and individual weight ranged from 18.2 to 845 gr. The mean male body weight was 102.73 gr and individual weight ranged from 18.9 to 498 gr. The body weight of the III and IV age groups of European Chub populations in the Topçam Dam Lake and Camkoru Pond are quite similar.

The total weights of the Anatolian Bleak population in Camkoru Pond ranged from 7.1 gr to 56.8 gr and the total weights for ages II, III, IV, V, VI, VII and VIII were 7.13; 10.45; 17.56; 30.76; 39.30; 44.41 and 55.10 gr respectively. The weights in age groups IV, V and VII were higher for males, and in II, III and VI age groups for females. The mean body weight of all samples was 20.48 gr. The mean female body weight was 20.23 gr and individual weight ranged from 7.1 to 52.1 gr. The mean male body weight was 20.74 gr. and individual weight ranged from 7.1 to 56.8 gr. The body weight III of the IV age group of Anatolian Bleak populations in the Sakarya 3 basin (11.13 and 15.78 gr) ¹⁵ and Camkoru Pond are quite similar.

The total weights of the Tench population in Camkoru Pond ranged from 8.2 gr to 453 gr and the fork lengths for ages I, II, III, IV, V, VI, VII and VIII were 10.61; 30.06; 68.78; 119.59; 183.13; 261.86; 325.36 and 409.74 gr respectively. The weights in all age groups were higher for females. The mean body weight of all samples was 88.29 gr. The mean female body weight was 102.57 gr and individual weight ranged from 21.3 to 453 gr. The mean male body weight was 97.17 gr and individual weight ranged from 23 to 425.2 gr.

The length and weight values according to age show great variation between different habitats these differences are attributes to different age readings by the different

researchers, feeding habitats, population density, ecological conditions, parasite load and sexual maturity age as well as the differences in habitat.

The slope (b) values of the length weight regression of both sexes combined of European Chub, Anatolian Bleak and Tench populations was as follows: 3.0126; 3.2501 and 3.0066. The b value, showing the type of growth in fish, has been shown to range from 2 to 4 ²⁶. When the b value for the length-weight relationship in a fish population is 3 or very close, it can be classified as isometric; however, if this value is considerably different than 3, it can be classified as allometric. For the European Chub, the b value was reported to be 3.12 in Topçam Dam Lake ³, 3.04 in Işıklı Lake ¹⁸, 3.19 in Sır Dam Lake ¹⁹. For the Tench, the b value was reported to be 3.08 in Abant Lake ¹³, 3.17 in Bayındır Dam Lake ²¹, 3.17 in Kesikköprü Dam Lake ⁹; 2.63 in Hirfanlı Dam Lake ²⁷; 3.01 in Mogan Lake ²⁸. The length-weight relationship in fish may vary according to species, age and sexes. Geographic location and associated environmental conditions, such as seasonality (date and time of capture), stomach fullness, disease and parasite loads, can also affect the value of b ^{26,29}. The slope (b) values of the length weight regression of both sexes combined of Anatolian Bleak and European Chub populations were significantly different from 3. Length-weight relationship showed that the Tench populations in Camkoru pond grows isometric; Anatolian Bleak and European Chub populations grow allometric (Weight increased allometrically with length).

The highest relative increases in length were in age group I of European Chub and Tench populations and II age group of Anatolian Bleak in Camkoru Pond. The relative increases in length among II, IV and VII age groups of European Chub; only V age groups of Anatolian Bleak; V and VI age groups of Tench were higher for females than males. The highest relative increases in weight were in age group I of European Chub and Tench populations and IV age group of Anatolian Bleak in Camkoru Pond. The relative increases in weight among II, IV, VI and VII age groups of European Chub; II, IV and V age groups of Anatolian Bleak; III, V and VI age groups of Tench were higher for females than males. As can be seen in Table 6. The relative increases in length decreased gradually with age in three fish species but the relative increases in weight decreased gradually with age in two fish species (European Chub and Tench).

Six fish species, 1199 specimens were captured from Camkoru Pond. *Tinca tinca* (38.78%) was determined to have the greatest population size followed by *Squalius cephalus* (31.19%), *Alburnus escherichii* (25.69%), *Gobio gobio* (3.34%), *Cyprinus carpio* (0.92%) and *Oncorhynchus*

mykiss (0.08%). Some native fish species living in inland waters of Turkey have become endangered due to a combination of introduction of exotic fishes, aquaculture, overexploitation, excessive water abstraction (Innal, pers. observation). The introduction of species into an aquatic system has been shown to directly, or indirectly, impact aquatic macrophytes, water quality and aquatic fauna including plankton, benthic macroinvertebrates, fishes and wildlife.

The introduction of *Tinca tinca* into Camkoru Pond was met with great success by showing adaptation to the pond's ecological characteristics but no such success was met with the introduction of *Cyprinus carpio* and *Oncorhynchus mykiss*. In order to increase the population density of native fish species (European Chub and Anatolian Bleak), It will be necessary to control the Tench population in Camkoru Pond.

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Tozlayıcı Arıların Yonca (*Medicago sativa* L.) Klon Hatlarının Meyve ve Tohum Tutmasına Etkisi

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Özet

Bu araştırma tozlayıcı arıların yonca (*Medicago sativa* L.) klon hatlarının meyve ve tohum bağlaması üzerine etkisinin saptanması amacıyla 2004–2007 yılları arasında yürütülmüştür. Araştırma ile ilgili tarla denemeleri taban koşullarındaki deneme alanında bölünmüş parseller deneme desenine göre 3 tekrarlamalı olarak kurulmuştur. Deneme materyali olarak farklı çeşitler içerisinde seçilen 8 klon hattı kullanılmıştır. Arıların polinasyondaki etkilerini saptamak amacıyla, kafes kapatılarak içerisinde arı girişi önlenen bitkiler ve arıların ziyaretine serbest bırakılan bitkiler üzerinde, salkımda bakla, baklada tane sayıları ile meyve bağlama (%) ve bitki başına ortalama tohum verimleri saptanmıştır. Üç yıllık araştırma bulguları, yonca klon hatları arasında incelenen özellikler açısından önemli farklılıklar olduğunu göstermiştir. Arılardan tecrit edilen kafes içindeki yonca klon hatlarının salkımdaki bakla sayısı ve meyvedeki tohum sayısı sırasıyla 1.0 ve 1.3 adet, meyve bağlama oranı %5.9, bitki başına ortalama tohum verimi ise 0.8 g olarak saptanmıştır. Serbest tozlanma koşullarında ise salkımdaki bakla sayısı ve meyvede tohum sayısı sırasıyla 8.1 ve 3.4 adet, meyve bağlama oranı %47.6, bitki başına tohum verimi 12.7 g olarak saptanmıştır. İncelenen özellikler açısından yonca klon hatlarının kafes içi ve kafes dışı uygulamaları arasındaki önemli farkın büyük oranda polinatör arılardan kaynaklandığı saptanmıştır. Araştırma bulguları, polinatör arıların yoncanın tohum tutma, meyve bağlama ve tohum veriminde çok önemli olduklarını ortaya koymuştur.

Anahtar sözcükler: Polinatör arı, Yonca, Klon, Kafes, Meyve bağlama

Effect of Pollinator Bees on Pod and Seed Setting of Alfalfa (*Medicago sativa* L.) Clones

Summary

This research was conducted to determine effect of pollinator bees on pod and seed setting of alfalfa clones throughout 2004-2007. The experiment was conducted as randomized split plot design with three replications under low land conditions. The eight alfalfa clonal lines, derived from different cultivars were used as experimental material. To determine effects of bee pollination, number of pods per raceme, seed number per pod, pod setting percentage and seed yield per plant were determined on both caged and open pollinated plants of the clonal lines. According to average of three years results, there were significant differences among alfalfa clonal lines in terms of above mentioned components. Under caged conditions, mean seed number per pod, pod number per raceme, pod setting percentage and seed yield per plant were determined as 1.3, 1.0, 5.9 and 0.8 g, respectively while under open pollination they were 8.1, 3.4, 47.6% and 12.7 g, respectively. The big differences in pod and seed setting could be attributed to the presence of bees. It was concluded that pollinator bees are essential in the seed production of alfalfa.

Keywords: Pollinator bee, Alfalfa, Clone, Cage, Pod setting

GİRİŞ

Yonca dünyada ve ülkemizde yetiştirilen en önemli baklagil yem bitkilerinden birisidir. Bu bitki, tarımı yapılan hemen tüm yem bitkilerden daha yüksek bir yem

değerine sahiptir. Birim alana protein verimi de yüksek olan yoncanın kuru ve yeşil otu her türlü hayvan için lezzetli ve besleyicidir ¹. Ülkemizde son yıllarda yonca

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ekim alanlarında önemli oranda artışlar görülmektedir. Fakat bu artışı karşılayacak tohum üretimi yeterince yapılamamaktadır. Bu nedenle her yıl yurt dışından önemli miktarda tohum ithali yapılmaktadır². Üretimin ülke ihtiyacını karşılamaktan uzak olması; yonca tohum üretim alanlarının yetersizliği ve dekara tohum veriminin potansiyel verime göre oldukça düşük düzeylerde kalmasından kaynaklanmaktadır. Potansiyel verime ulaşmada uygun yetiştirme teknikleri yanında, tozlamada etkili olan arılardan yeterli şekilde faydalanılması gerekir. Rincker ve ark.³ başarılı bir yonca tohum yetiştiriciliği için uygun iklim koşullarının ve polinatör arıların önemini vurgulamışlar, elverişli koşullarda yonca tohum veriminin 211 kg da⁻¹'a kadar yükseldiğini belirtmişlerdir. Yoncanın yabancı çiçek tozuyla döllenmeye gereksinim duyduğu ve maksimum tohum verimi için gerekli koşullardan birisinin de optimum tozlanma olduğu birçok araştırmada belirtilmiştir^{4,7}.

Ülkemizin değişik yörelerinde 30 cins ve 5 familyaya ait 150 civarında arı türünün yonca çiçeklerini ziyaret ettiği; bunlardan *Andrena ovulata*, *Melitturga clavicornis*, *Melitta leporina*, *Megachile rotundata*, *Bombus argilaceus* ve *Apis mellifera* türlerinin yoncanın tozlaşmasında önemli oldukları belirtilmiştir. Ülkemizin yonca bitkisinin ana vatanı olmasına karşın tozlayıcı arılar üzerine yapılan çalışmaların son derece sınırlı olduğu vurgulanmıştır⁶. Tozlayıcı arıların Erzurum koşullarında meyve ve tohum bağlamaya etkileri Özbek⁸ tarafından araştırılmıştır. Araştırmacı kafes teli ile tecrit edilmiş yonca parsellerinde ortalama meyve bağlama oranının (%5.50), serbest şartlardaki meyve bağlama oranına (%64.50) göre çok düşük düzeyde kaldığını, meyvedeki tohum sayısının kafes içinde 1.42 adet, kafes dışında ise 4.34 adet olduğunu, arıların yoncanın tohum bağlamasında çok etkili olduklarını ortaya koymuştur.

Sulama, gübreleme gibi kültürel tedbirlerle toplam çiçek sayısının artırılması, yaprak kesici arı kolonilerinin üretim parsellerine yerleştirilmesi ile tozlanan çiçek sayısının ve buna bağlı olarak tohum veriminin yükseldiği belirtilmiştir⁹. Özbek¹⁰, yaprak kesici arı (*M. rotundata*)'nın Türkiye faunasında oldukça yaygın olduğunu, yonca tohum üretim bölgelerimizde kullanılması gerektiğini, çünkü bal arılarının yonca tozlanmasında sınırlı düzeyde etkili olduğunu, tozlanmada etkili olan diğer yaban arılarının da ülkemizde yoğunluklarının giderek azaldığı, buna bağlı olarak yonca tohum üretiminin de çok düşük düzeylerde kaldığını belirtmiştir. Richards ve Kevan¹¹, yaprak kesici arı türünün yoncanın tozlanmasında en etkili arı türü olduğunu, kültüre alındığını, uygun kullanımla bu türün yonca tohum verimini 20 kat artırabileceğini belirtmişlerdir. Yapılan bir araştırmada, yoncada salkım başına meyve sayısı ve meyve

başına tohum sayısının sırasıyla 13.3 ve 6.5 adet, tohum veriminin ise 26.8 kg da⁻¹ olduğu, araştırmada meyvede tohum sayısının benzer çalışmalardan önemli derecede yüksek bulunmasının deneme parsellerinin yanında bal arısı kovanlarının bulunmasından kaynaklandığı açıklanmıştır¹³. Çukurova ekolojik koşullarında yonca parsellerinde özellikle yaprak kesici arıların yaygın olduğu ve etkili şekilde polinasyon yaptıkları, yonca tohum veriminin yıllara ve sıra aralığına göre değişmek üzere 39.3-82.2 kg da⁻¹ arasında değiştiği belirtilmiştir¹⁵.

Yonca çiçeğinde tripping olayından önce tozlanmayı engelleyen en önemli unsur, stigma üzerinde bulunan kütikula tabakası olup, bu membran stigma salgısı ile polen arasında engel oluşturarak polinasyonu engellemektedir. Buna karşılık bir miktar polen tanesinin stigmatik kütikula üzerine preslenerek stigma salgısı ile temas geçtiği ve %20'ye kadar tozlanma olabildiği saptanmıştır¹⁶. Yaprak kesici arıların polen toplayarak beslenmesi için çok sayıda yonca çiçeğini ziyaret ettiği ve fırlatma yaptığı, bal arılarının ise daha çok nektar topladığı, tripping olayından sakındığı belirtilmiştir¹⁷. Yeni Zelanda'da yürütülen bir araştırmada, yoncanın salkımda bakla sayısının sıra arası, tohum miktarı ve yıllara göre değişmek üzere 4.2-4.5 adet, meyvede tohum sayısının 2.9-3.4 adet, tohum veriminin ise 102.3-209.4 kg ha⁻¹ arasında değiştiği belirtilmiştir¹⁸.

Yonca çeşitleri arasında tohum verimi ve meyvedeki tohum sayısının yıllara bağlı olarak önemli derecede etkilendiği, salkımdaki bakla sayısının 5.6-15.1 adet arasında değiştiği, salkımdaki tohum sayısının 2.4-4.2 adet, tohum veriminin ise 20.2-55.7 kg da⁻¹ arasında değiştiği Rincker¹⁹ tarafından belirtilmiştir. Farklı yonca genotipleri ile farklı yıl ve lokasyonlarda yürütülen bir çalışmada, genotiplerin tohum verimlerinin ortalama 801 kg ha⁻¹, meyvede tohum sayısı ve salkımda bakla sayısının sırasıyla 4.6 ve 16.6 adet olduğu saptanmıştır²⁰. Farklı yonca çeşitlerinin serbest tozlanma koşullarında salkımda çiçek, salkımda bakla, baklada tane ve bitki başına tohum veriminin genotipler ve yıllara göre değiştiği, ilk yıl ortalama tohum verimlerinin 751 kg ha⁻¹ olmasına karşılık, 2. yıl verimin 354 kg ha⁻¹'a düştüğü belirtilmiştir²¹. Güney Kaliforniya'da yüksek sıcaklığın yonca çiçeğinin daha kolay fırlama olmasını sağladığı ve bal arılarının bölgede bu amaç için yaygın ve etkili şekilde kullanıldığı belirtilmiştir. Kuzey Amerika ve Kanada'da yonca tohumculuk endüstrisinin gelişmesi bal arılarına kıyasla polinasyonda çok daha etkin olan yaprak kesici arıların keşfiyle ve bunların ithal edilerek veya üretilerek kontrol edilebilir düzeyde kullanılmasıyla arttığı belirtilmiştir^{7,12}.

Yoncada meyve bağlama ve tohum tutmanın tamamen arı faaliyetlerine bağlı olduğunun belirlenmesinden

sonra, değişik ülkelerde bu konuda birçok araştırma yapılmış ve tozlanmada etkili olan arılardan faydalanılması yoluna gidilmiştir. Bu çalışma yoncanın tozlanmasını sağlayan arıların yoncanın meyve bağlama ve tohum tutmasına etkilerinin saptanması amacıyla yürütülmüştür.

MATERYAL ve METOT

Bu araştırma, 2004-2007 yılları arasında taban koşullardaki araştırma alanında yürütülmüştür. Araştırma alanının topografyası düz konumlu, toprakları organik madde bakımından zayıf, kireç yönünden zengin, pH yönünden hafif alkali tepkimeli, tuzluluk yönünden tuzsuz ve killi yapıdadır²². Fosfor bakımından orta düzeyde, elverişli bakır (Cu) orta, mangan (Mn) ise yeterli seviyededir. Araştırmanın yürütüldüğü deneme alanının içinde bulunduğu Adana iline ait uzun yıllar ortalaması ve denemenin yürütüldüğü yıllar ile ilgili iklim verileri **Tablo 1**'de verilmiştir. **Tablo 1** incelendiğinde, uzun yıllar ortalamasına göre Adana'daki yıllık toplam yağış miktarı 650.7 olup, en fazla yağış (123.8 mm) Aralık ayında, en az yağış (5.6 mm) ise Ağustos ayında düşmektedir. En yüksek nispi nem (%68) Temmuz ayında, en düşük nispi nem (%60) Ekim ayındadır. En sıcak ay ortalaması 28.1°C ile Ağustos ayında, en soğuk ay ortalaması 9.3°C ile Ocak ayında ortaya çıkmaktadır. Bitki gelişmesi, çiçeklenme ve meyve bağlama yönünden çok önemli olan Mart-Haziran aylarındaki toplam yağış miktarı 2005, 2006, 2007 ve uzun yıllarda sırasıyla 171, 79, 247 ve 185 mm olarak gerçekleşmiştir. 2006 yılına ait değer (79 mm) diğer yıllardan önemli derecede düşük olduğu görülmektedir.

Deneme "Çukurova Bölgesinde Yonca Islah Araştırmaları" TA/2003/04/no'lu proje kapsamında tesis edilen yonca kaynak populasyonu içerisinde seçilen genotiplerle 2004 yılında tesis edilen klon parselleri üzerinde

kurulmuştur. Her parseli 80x80 cm aralıkla yetiştirilen 12 bitkiden oluşan 9.6 m uzunluğundaki bir sıra oluşturmuştur²³.

Araştırmada kullanılan 1. klon Pioneer-5929, 2. ve 3. klon Çukurova Tarımsal Araştırma Enstitüsü populasyonu, 4. klon Kalender, 5 ve 6. klon Mesa-sırsa, 7 ve 8. klonlar ise Calfa çeşidi içerisinde seçilen genotiplerden oluşmuştur.

Parsellerde ilk biçim ot elde etmek amacıyla Nisan ayı içerisinde yapıldıktan sonra, bitkiler çiçeklenmeye başlamadan önce her klon hattında tesadüfen seçilen 3 bitki ebatları 0.8 x0.8x1 m olan ve üzerine dokuma sıklığı 1.8x1.6 mm aralıklı naylon sineklik teli geçirilmiş tel kafesle kapatılmıştır. Bu şekilde kafes içerisine tozlanmada etkili olabilecek her türlü arı, sinek ve böcek girişi önlenmiştir. Aynı parseldeki diğer üç bitki arıların ziyaretine serbest bırakılmıştır. Kafesler etiketlenerek hasat zamanına kadar bu şekilde bırakılmıştır.

Parseller üzerinde yapılan incelemede *Halictus*, *Dasypoda*, *Megachile*, *Melitta* cinslerine ait arı türlerinin yonca çiçeklerinde önemli derecede polinasyon yaptıkları saptanmıştır. Deneme parsellerinin yakınında çok sayıda arı kovani bulunmasına rağmen, bal arıları ilkbahar aylarında daha çok çevrede yaygın olarak bulunan ak üçgül (*Trifolium repens*), Acem üçgülü (*Trifolium resupinatum*), İskenderiye üçgülü (*Trifolium alexandrinum*), meryem diken (Silybum marianum), yaban hardalı (*Sinapis arvensis*), arı otu (*Phaselia tanacetifolia*) ve tarla tilki kuyruğu (*Alopecurus myosuroides*) gibi çeşitli bitkileri sıkça ziyaret ettikleri saptanmıştır. Yaz mevsimine doğru belirlenen bitkiler çiçeklenme dönemini tamamladıktan sonra bal arılarının yonca çiçeklerini daha çok ziyaret ettikleri fakat bu dönemde bile polinasyonda yaban arıları kadar etkili olmadıkları gözlemlenmiştir. Temmuz ayı içerisinde bitkiler tohum olgunluğuna ulaştıklarında her sıra par-

Tablo 1. Adana ilinin 2005, 2006, 2007 ve uzun yıllarına ait sıcaklık, yağış ve nispi nem değerleri *

Table 1. Temperature, precipitation and relative humidity data at Adana province for the 2007, 2008, 2009 and long term average *

Ay	Sıcaklık Ortalaması				Yağış				Nispi Nem (%)			
	2005	2006	2007	UY **	2005	2006	2007	UY **	2005	2006	2007	UY **
Ocak	10.2	8.9	9.4	9.3	51.0	36.0	34.0	109.4	66.0	62.0	72.5	65.0
Şubat	9.8	10.4	10.0	10.0	75.0	131.0	127.0	88.6	64.0	73.0	72.0	65.0
Mart	13.4	13.6	13.3	12.9	61.0	46.0	75.0	65.4	72.0	76.0	74.5	65.1
Nisan	17.5	17.7	15.6	17.3	52.0	9.0	115.0	53.0	68.0	71.0	71.0	67.1
Mayıs	21.0	21.8	23.0	21.6	42.0	20.0	32.0	46.6	67.0	69.0	70.0	66.2
Haziran	24.2	25.1	25.5	25.4	16.0	4.0	25.0	20.3	72.0	73.0	70.7	65.0
Temmuz	27.4	27.2	30.0	27.7	8.0	41.0	0.0	6.3	79.0	78.0	68.0	68.0
Ağustos	28.1	28.6	28.7	28.1	24.0	0.0	0.0	5.6	76.0	79.0	72.3	68.0
Eylül	24.9	25.6	25.5	25.4	28.0	37.0	0.0	15.4	69.0	67.0	62.3	63.0
Ekim	19.8	20.6	21.9	21.3	37.0	157.0	32.0	43.0	60.0	71.0	64.0	60.0
Kasım	14.5	14.2	14.3	14.8	65.0	92.0	31.0	73.3	66.0	65.0	65.0	63.0
Aralık	12.6	10.3	9.3	10.5	64.0	0.0	116.6	123.8	69.0	58.0	60.8	67.0
Ort/Top	18.6	18.7	18.9	18.7	523.0	573.0	563.3	650.7	68.8	67.9	68.3	65.2

* Adana Meteoroloji Bölge Müdürlüğünden alınmıştır, ** UY: Uzun Yıllar Ortalaması

selden kafes içindeki ve dışındaki 3'er bitki biçme makasıyla biçilerek laboratuara taşınmıştır. Arılardan tecrit edilmiş bitkilerle arıların ziyaretine serbest bırakılmış bitkiler arasında meyve ve tohum bağlamadaki farklılığı saptamak amacıyla her bitkiden tesadüfen seçilen 20 adet çiçek salkımı alınmıştır ²⁰. Çiçek salkımlarındaki brakteler ve meyveler sayılarak ⁸ salkımdaki ortalama çiçek sayısı, meyve sayısı ve meyvede tohum sayısı ve bitki başına tohum verimleri saptanmıştır. Meyve bağlama %'si ise salkımdaki meyve sayısının salkımdaki çiçek sayısına bölünmesi ve oranlanması ile hesaplanmıştır.

İstatistik Analizler

Araştırmada elde edilen verilere üç yıl tekrarlanan bölünmüş parseller deneme desenine göre MSTAT-C istatistik paket programı yardımıyla varyans analizi uygulanmıştır.

BULGULAR

Araştırmanın sürdürüldüğü üç yılda incelenen klon hatlarının kafes içi ve kafes dışı koşullarda saptanan salkım başına bakla sayısı ortalamaları *Tablo 2*'de verilmiştir. Açıkta tozlanan klon hatlarının 3 yıllık ortalama bakla sayısının 8.1 adet olduğu, tozlayıcılardan tecrit edilmiş kafes içindeki bitkilerdeki bakla sayısının ise 1.0 adet olduğu tespit edilmiştir. Araştırmanın sürdürüldüğü üç yılda incelenen klon hatlarının kafes içi ve kafes dışı koşullarda saptanan salkım başına bakla sayısı ortalamaları yıldan yıla önemli farklılık göstermiştir.

İncelenen klon hatlarında izolasyonlu ve izolasyonsuz koşullarda üç yılda saptanan meyve bağlama oranı yıllara bağlı olarak önemli farklılık göstermiştir (*Tablo 3*). Üçüncü yılda meyve bağlama oranı birinci ve ikinci yıl-

Tablo 2. Yonca klon hatlarının kafes içi ve serbest tozlanma koşullarında salkımda bakla sayısı (adet)

Table 2. Mean numbers of pods per raceme for alfalfa clonal lines under caged or open pollinated conditions

Klon Hatları	2005			2006			2007			Genel Ortalama		
	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.
1	0.2 h ¹ *	9.0 d	4.6 E ⁺	0.1 h	7.9 a	4.0A-C ⁺	1.0 ij	7.5 bc	4.2C ⁺	0.4 f	8.1 b	4.3CD ⁺
2	2.3 f	7.9 e	5.1 DE	0.3 h	7.7 a	4.0A-C	1.3 g ⁺	4.2 f	2.8F	1.3 de	6.6 c	4.0D
3	0.8 g ⁺	10.8 b	5.8B-D	1.8 g	7.0 bc	4.4A	0.6 j	8.6 a	4.6A	1.1 e	8.8 a	4.9AB
4	1.1 gh	13.6 a	7.4 A	2.8 f	5.9 e	4.4A	1.1 h-j	5.3 e	3.2E	1.7 d	8.3 b	5.0A
5	0.1 i	12.8 a	6.5 B	0.2 h	7.3 ab	3.8BC	0.7 j	7.0 cd	3.8D	0.3 f	9.1 a	4.7AB
6	1.6 fg	10.4 bc	6.0 BC	0.5 h	6.4 c-e	3.5CD	1.8 g	6.7 d	4.3BC	1.3 de	7.8 b	4.6BC
7	0.4 h ¹	10.3 bc	5.4 CD	0.1 h	6.2 de	3.2D	0.6 j	7.0 b-d	3.8D	0.3 f	7.9 b	4.1D
8	1.6 fg	9.5 cd	5.5 CD	1.6 g	6.8 b-d	4.2AB	1.6 g-h	7.6 b	4.6AB	1.6 d	8.0 b	4.8AB
Ort.	1.0 b ¹	10.5 a	5.8 A ²	0.9b ¹	6.9 a	3.9 B	1.1b ¹	6.7 a	3.9B	1.0 b ¹	8.1 a	4.6

* Aynı yılda benzer küçük harf ile gösterilen klon hattı X kafes uygulama kombinasyon ortalamaları Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

* Aynı sütun içerisinde benzer büyük harf ile gösterilen ortalamalar Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

¹ Aynı yılda benzer harf ile gösterilen kafes içi-dışı ortalamaları istatistiksel olarak birbirinden farklıdır

² Benzer büyük harf ile gösterilen yıl ortalamaları LSD testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

Tablo 3. Yonca klon hatlarının kafes içi ve serbest tozlanma koşullarında meyve bağlama oranları (%)

Table 3. Mean pod setting percentages for alfalfa clonal lines under caged or open pollinated conditions

Klon Hatları	2005			2006			2007			Genel Ortalama		
	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.
1	1.6f *	60.8 b	31.2B ⁺	0.8 h	51.2 a	26.0B ⁺	7.6g ¹	66.4a	37.0A ⁺	3.4 ij	59.5 a	31.4A ⁺
2	6.2 f	32.9 e	19.5F	1.9 h	39.1 cd	20.5CD	5.9 h-j	19.2e	12.6D	4.6 i	30.4 f	17.5F
3	3.7 f	55.9 bc	29.8BC	15.7 f	45.0 b	30.4A	4.3 j	61.2b	32.8B	7.9 h	54.0 b	31.0A
4	5.3 f	66.5 a	35.9A	18.5 f	36.0 de	27.2B	8.7 gh	44.9d	26.8C	10.8 g	49.1 cd	30.0AB
5	2.0 f	56.8 bc	29.4BC	0.7 h	42.2 bc	21.5C	4.5 ij	53.2c	28.9C	2.4 j	50.7 c	26.6CD
6	6.0 f	36.1 e	21.1EF	2.5 h	33.9 e	18.2E	13.1 f	51.6c	32.3B	7.2 h	40.5 e	23.9E
7	1.9 f	52.8 c	27.4CD	0.7 h	36.1 de	18.4DE	3.8 j	54.1c	29.0C	2.1 j	47.7 d	24.9DE
8	7.0 f	41.9 d	24.4DE	8.1 g	43.3 b	25.7B	10.fg ²	60.9b	35.6A	8.4 h	48.7 cd	28.6BC
Ort.	4.2 b ¹	50.5 a	27.3B ²	6.1 b ¹	40.8 a	23.5C	7.3 b ¹	51.5a	29.4A	5.9 b ¹	47.6 a	26.8

* Aynı yılda benzer küçük harf ile gösterilen klon hattı X kafes uygulama kombinasyon ortalamaları Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

* Aynı sütun içerisinde benzer büyük harf ile gösterilen ortalamalar Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

¹ Aynı yılda benzer harf ile gösterilen kafes içi-dışı ortalamaları istatistiksel olarak birbirinden farklıdır

² Benzer büyük harf ile gösterilen yıl ortalamaları LSD testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

lara göre istatistiksel olarak önemli derecede daha yüksek olmuştur. Üç yılda da kafes dışındaki bitkilerde meyve bağlama oranı kafes içindeki bitkilere göre istatistiksel olarak önemli derecede daha yüksek olmuştur. Üç yıllık ortalamaya göre kafes dışındaki bitkilerde %47.6 olan meyve bağlama oranı kafes içindeki bitkilerde %5.9 olmuştur.

İncelenen klon hatlarında üç yılda serbest tozlanma ve kafes ile izole edilmiş koşullarda saptanan bakla başına tane sayısı ortalamaları yıllara bağlı olarak önemli bir farklılık göstermemiştir (Tablo 4). Araştırmının 1. ve 2. yılında 2.4 olan bakla başına tane sayısı 3. yılda 2.3 adet olmuştur. Araştırma sonunda üç yıllık ortalamaya göre, kafes ile izole edilen bitkilerde bakla başına ortalama 1.3 tane saptanırken, kafes ile izole edilmeyen bitkilerde bu değer 3.4 adet olmuştur.

Klon hatlarında kafes içi ve dışı koşullarda üç yılda saptanan bitki başına tohum verimi ortalamaları Tablo 5'te verilmiştir. Tablo 5 incelendiğinde klon hatları bitki başına tohum verimi açısından üç yılda da istatistiksel olarak önemli farklılıklar göstermiştir. İncelenen yonca klon hatlarında kafes içi ve dışı koşullarda üç yılda saptanan bitki başına tohum verimi ortalamaları yıllara bağlı olarak önemli derecede farklılık göstermiştir.

TARTIŞMA ve SONUÇ

Salkımda Bakla Sayısı

Araştırmının birinci yılında saptanan salkım başına bakla sayısı ortalaması (5.8 adet) ikinci ve üçüncü yıllara göre istatistiksel olarak önemli derecede daha yüksek olmuştur. Salkım başına meyve sayısının araştırmanın

Tablo 4. Yonca klon hatlarının kafes içi ve serbest tozlanma koşullarında baklada tane sayısı (adet)

Table 4. Number of seeds per pod of alfalfa clonal lines under caged or open pollinated conditions

Klon Hatları	2005			2006			2007			Genel Ortalama		
	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.
1	1.2fg *	2.6 d	1.9CD ⁺	1.0 e	3.0 bc	2.0CD	0.6 h	2.9 d	1.8E	0.9 ı	2.8 de	1.9 e
2	1.8 e	2.5 d	2.2BC	0.4 f	3.2 ab	1.8BC	1.1 g	2.4 e	1.8E	1.1 hı	2.7 e	1.9 e
3	1.8 e	4.4 b	3.1A	3.0 bc	3.3 ab	3.2A	0.7 h	3.5 c	2.1CD	1.8 f	3.8 b	2.8 b
4	1.1 fg	2.7 d	1.9CD	2.4 cd	3.2 ab	2.8AB	1.1 g	3.0 d	2.1D	1.5 g	3.0 cd	2.3 c
5	0.9 dg	5.3 a	3.1A	1.4 e	3.7 a	2.6B	1.8 f	5.2 a	3.5A	1.4 gh	4.7 a	3.1 a
6	1.5 ef	3.4 c	2.4B	0.9 ef	3.3 ab	2.1C	0.4 h	4.0 b	2.2C	0.9 ı	3.6 b	2.3 c
7	0.1 h	3.4 c	1.8D	1.0 e	2.5 cd	1.7D	1.8 f	3.5 c	2.6B	1.0 ı	3.1 c	2.1 d
8	1.8 e	4.0 b	2.9A	2.3 d	3.1 b	2.7A	1.7 f	3.6 c	2.6B	1.9 f	3.6 b	2.8 b
Ort.	1.3 b ¹	3.5 a	2.4	1.6 b ¹	3.2 a	2.4	1.2 b ¹	3.5 a	2.3	1.3 b ¹	3.4 a	2.4

* Aynı yılda benzer küçük harf ile gösterilen klon hattı X kafes uygulama kombinasyon ortalamaları Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

* Aynı sütun içerisinde benzer büyük harf ile gösterilen ortalamalar Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

¹ Aynı yılda benzer harf ile gösterilen kafes içi-dışı ortalamaları istatistiksel olarak birbirinden farklıdır

² Benzer büyük harf ile gösterilen yıl ortalamaları LSD testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

Tablo 5. Yonca klon hatlarının kafes içi ve serbest tozlanma koşullarında tohum verimi (g/bitki)

Table 5. Mean seed yields of alfalfa clonal lines under caged or open pollinated conditions (g/plant)

Klon Hatları	2005			2006			2007			Genel Ortalama		
	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.	K. içi	K. dışı	Ort.
1	0.3h *	11.2 e	5.7D ⁺	0.2 h	4.5 e	2.3G ⁺	0.2 ı	7.5 f	3.9 F ⁺	0.2 ı	7.7 f	4.0 E ⁺
2	2.4 g	7.2 f	4.8D	0.5 h	7.4 b	3.9CD	1.4 h	7.3f	4.4F	1.4 g	7.3 f	4.4E
3	0.2 h	11.9 e	6.1D	0.2 h	5.3 d	2.7FG	0.2 ı	17.1c	8.6C	0.2 ı	11.4 d	5.8D
4	0.7 gh	16.1 d	8.4C	2.5 f	6.8 c	4.6B	0.6 hı	5.8g	3.2G	1.3 gh	9.6 e	5.4D
5	0.3 h	29.2 a	14.8A	0.2 h	6.2 c	3.2EF	1.0 hı	25.0a	13.0A	0.5 hı	20.1 a	10.3A
6	1.0 gh	15.8 d	8.4C	0.8 h	9.8 a	5.3A	0.2 ı	22.8b	11.5B	0.6 g ¹	16.1 b	8.4B
7	0.3 h	20.5 c	10.4B	0.3 h	6.6 c	3.5DE	0.4 ı	11.2e	5.8E	0.4 ı	12.8 c	6.6C
8	1.8 gh	26.3 b	14.1A	1.7 g	6.7 c	4.2BC	1.0 hı	15.2d	8.1D	1.5 g	16.1 b	8.8D
Ort.	0.9 b ¹	17.3 a	9.1A ²	0.8 b ¹	6.7 a	3.7C	0.6 b ¹	14.0a	7.3B	0.8 b ¹	12.7 a	6.8

* Aynı yılda benzer küçük harf ile gösterilen klon hattı X kafes uygulama kombinasyon ortalamaları Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

* Aynı sütun içerisinde benzer büyük harf ile gösterilen ortalamalar Duncan testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

¹ Aynı yılda benzer harf ile gösterilen kafes içi-dışı ortalamaları istatistiksel olarak birbirinden farklıdır

² Benzer büyük harf ile gösterilen yıl ortalamaları LSD testine göre $P \leq 0.05$ hata sınırları içerisinde istatistiksel olarak birbirinden farklıdır

ikinci ve üçüncü yıllarında birinci yıla göre daha düşük olmasının nedeni, 2004 yılında kök klonu olarak toprağa dikilen bitkilerin yıldan yıla yaşlanması olabilir.

Araştırmanın üç yılında ve üç yıllık ortalamaya göre serbest tozlanan bitkilerde salkım başına meyve sayısı kafes ile izole edilen bitkilere göre istatistiksel olarak önemli derecede daha yüksek olmuştur. Açıkta tozlanan klon hatlarının 3 yıllık ortalama bakla sayısı 8.1 adet iken, tozlayıcılardan tecrit edilmiş kafes içindeki bitkilerdeki bakla sayısı ise 1.0 adet olarak saptanmıştır. Bu konuda yapılan bir çalışmada Özbek⁸ kafes içi şartlarda salkımdaki ortalama bakla sayısını 0.93 adet, serbest tozlanan parsellerde ise 9.36 adet olarak saptamıştır. Özbek'in bulguları⁸ bu çalışmadaki bulgularla uyum içersindedir.

Araştırmanın üç yılında da incelenen klon hatları salkım başına bakla sayısı açısından istatistiksel olarak önemli farklılıklar göstermişlerdir. Ancak, klon hatları arasındaki bu farklılık tozlanmanın açık koşullarda veya kafes içerisinde olmasına bağlı olarak önemli farklılık göstermiştir. Üç yıllık ortalamalara göre, kafes dışında 3 ve 5 no'lu klon hatları diğer hatalara göre istatistiksel olarak önemli derecede daha yüksek salkım başına meyve sayısı ortalaması göstermiştir. Yapılan bir çalışmada yonca genotipleri arasında nektar içeriği bakımından önemli farklar bulunduğu, buna bağlı olarak yüksek veya düşük nektar yoğunluğuna sahip genotiplerin salkım başına meyve sayısı ve tohum miktarlarının sırasıyla yüksek ve düşük bulunduğu saptanmıştır²⁴. Kafes içi koşullarda 4 ve 8 no'lu hatlar 2 ve 6 no'lu hatlar dışındaki hatlardan istatistiksel olarak önemli derecede daha yüksek salkım başına bakla sayısı ortalaması göstermişlerdir. Klonlar arasındaki bu farklılık Viands ve ark.^{16'}nin vurguladığı gibi bazı genotiplerin kendi kendilerini tozlayabilme özelliğinde olmasından kaynaklanabilir.

Meyve Bağlama Oranı (%)

İncelenen klon hatlarının meyve bağlama oranı açısından önemli derecede farklılık gösterdiği, ancak klon hatları arasındaki bu farklılığın kafes içinde veya kafes dışında olmalarına göre değiştiği ortaya çıkmıştır. Nitekim, üç yıllık ortalamaya göre, kafes dışında 1 no'lu klon hattı diğer hatlara göre istatistiksel olarak önemli derecede daha yüksek meyve bağlama oranı göstermesine karşılık, kafes içi koşullarda 3, 6 ve 8 no'lu klon hatları diğer hatlara göre önemli derecede daha yüksek meyve bağlama oranı göstermişlerdir. Kafes dışında doğal tozlanma koşullarında klon hatları arasında salkımda bakla, baklada tane ve meyve bağlama oranları yönünden önemli derecede farklılık olması polinasyonda etkili olan arı türlerinin bazı yonca genotipleri için seçici davranmalarından kaynaklanabilmektedir. Bu konuda Pedersen ve

ark.⁵ yaprak kesici ve bal arılarının, yonca klon hatlarının farklı çiçek rengi ve nektar özellikleri nedeniyle, polinasyonda oldukça seçici davrandıklarını saptamışlardır. Bu çalışmada kafes içi klon hatlarında saptanan meyve bağlama oranları Özbek'in bulguları⁸ ile benzerlik gösterirken, kafes dışı uygulamasındaki değerlerle kısmen benzerlik göstermektedir.

Baklada Tane Sayısı

Klon hatları arasında her üç yılda da bakla başına tane sayısı açısından istatistiksel olarak önemli farklılıklar olduğu, bitkilerin kafes ile izole edilmelerinin bakla başına tane sayısında önemli farklılık yarattığı ve klon hattı x kafes izolasyonu interaksyonunun istatistiksel olarak önemli olduğu ortaya çıkmıştır. Araştırmanın üç yılında da kafes ile izole edilen bitkilerde bakla başına tane sayısı ortalaması izole edilmeyen bitkilere göre istatistiksel olarak önemli derecede daha düşük olmuştur. Üç yıllık ortalamaya göre, kafes ile izole edilen bitkilerde bakla başına ortalama 1.3 tane saptanırken, kafes ile izole edilmeyen bitkilerde bu değer 3.4 olmuştur. İncelenen klon hatları üç yılda da bakla başına tane sayısı açısından istatistiksel olarak önemli farklılıklar göstermiştir. Ancak, klon hatları arasındaki bu farklılıklar bitkilerin kafes ile izole edilip edilmemesine bağlı olarak farklılık göstermiştir. Üç yıllık ortalamaya göre, izolasyonsuz koşullarda 5 no'lu klon hattı diğer klon hatalarına göre istatistiksel olarak önemli derecede daha yüksek bakla başına tane sayısı ortalaması göstermesine karşılık, izolasyonlu koşullarda 3 ve 8 no'lu klon hatları diğer hatlara göre istatistiksel olarak daha yüksek bakla başına tane sayısı göstermişlerdir. Araştırmada saptanan ortalama baklada tane sayısı değerleri Özbek⁸, Askarian ve ark.¹⁸ ile Rumbaugh ve ark.^{25'}nin bulguları ile benzerlik gösterirken, Mermer ve Serin¹³ ile Bolanos ve ark.^{20'}nin bulgularından daha düşük bulunmuştur. Bu konuda Elçi¹⁴ yoncanın meyve ve tohum oluşumunda çevre koşullarının önemini vurgulamış ve tohum veriminde bitki genotipinin önemli faktör olduğunu, farklı yonca klonlarının farklı miktarda tohum ürettiklerini saptamıştır.

Bitki Tohum Verimi (gr/bitki)

Araştırmanın birinci yılında 2 ve 3. yıllara göre önemli derecede daha yüksek tohum verimi elde edilmiştir. Bu durum, özellikle bitkilerin aktif olarak geliştiği Nisan, Mayıs ve Haziran ayları içerisinde düşen yağış miktarının yıllara göre önemli derecede farklılık göstermesi nedeniyle bitkilerde tohum oluşturan sap sayısının farklılık göstermesi ile açıklanabilir. Zira bu konuda yapılan birçok çalışmada salkımda çiçek, meyvede tohum sayısı ve tohum verimlerinin çeşitlere ve yıllara göre önemli derecede değiştiği saptanmıştır^{18,19,21}. Araştırmanın yürütüldüğü üç yılda da kafes dışı koşullarda bitki başına tohum

verimi kafes içi koşullardakine göre istatistiksel olarak önemli derecede daha yüksek olmuş ve üç yıllık ortalama-ya göre kafes dışı koşullarda 12.7 g olan bitki başına tohum verimi, kafes içi koşullarda 0.8 g olmuştur.

İncelenen klon hatları bitki başına tohum verimi açısından üç yılda da istatistiksel olarak önemli farklılıklar göstermiştir. Ancak, klon hatları arasındaki bu farklılık bitkilerin kafes ile izole edilip edilmemesine göre değişim göstermiştir. Nitekim, üç yıllık ortalama-ya göre kafes dışı koşullarda 5 no'lu klon hattı diğer hatlara göre önemli derecede daha yüksek bitki başına tohum verimi ortalaması göstermesine karşılık, kafes içi koşullarda 2 ve 8 no'lu klon hatları 4 ve 6 no'lu hatlar dışındaki hatlarda daha yüksek bitki başına tohum verimi ortalaması göstermişlerdir. Serbest tozlaşma koşullarında bitki başına ortalama tohum verimi bulgumuz, Rumbaugh ve ark.'nın²⁵ farklı lokasyon ve klon hatlarında saptadıkları bulgular (2.8-22.9 g) ile kısmen benzerlik göstermektedir.

Sonuç olarak, ele alınan ölçütler incelendiğinde yonca klon hatlarının kafes içi ve kafes dışı uygulamaları arasındaki tohum tutma, meye bağlama ve tohum verimindeki çok önemli farkın büyük oranda tozlayıcı arılardan kaynaklandığı ve tozlayıcı arıların bu bakımdan önemi ortaya çıkmıştır. Birçok araştırmada belirtildiği gibi normal üretim koşullarında (açık tozlanma) bile polinasyon yetersizliği sonucunda elde edilen tohum veriminin potansiyel verimden oldukça düşük bulunduğu bu araştırmada da saptanmıştır. Bu olumsuzluğun azaltılması için birçok ülkede olduğu gibi, yoncanın en önemli tozlayıcısı olan yaprak kesici arı türünün ülkemizde de üretilmesi ve tohumluk üretimi yapan kuruluşlarca tozlanmayı artırma amacıyla kullanması ile tohum üretiminde önemli artışlar sağlanabilecektir.

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Pathological and Microbiological Investigations in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Naturally Infected with *Lactococcus garvieae*

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Summary

During the summer of 2006, an outbreak of lactococcosis took place in a rainbow trout farm in the southern Aegean region of Turkey. Clinically lethargy, erratic swimming, darkness in colour and exophthalmia were observed. Histopathological examination revealed epicarditis, peritonitis, enteritis and panophthalmitis. The gills had lamellar edema, hemorrhage and telangiectasia. Histopathological findings were also found in the liver, kidney, pyloric caeca and swim-bladder. In conclusion, the bacterium isolated from the rainbow trout farm was identified as *Lactococcus garvieae* by biochemically, and its pathological effects on the rainbow trout tissues were widely evaluated firstly in Turkey.

Keywords: *Lactococcus garvieae*, Pathological and microbiological findings, Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

Lactococcus garvieae ile Doğal Enfekte Gökkuşığı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik ve Mikrobiyolojik İncelemeler

Özet

Çalışmada, 2006 yılı yaz mevsiminde Ege Bölgesi'nin güneyinde bulunan bir alabalık işletmesinde görülen laktokokkozis enfeksiyonu tanımlandı. Letarji, düzensiz yüzme, deride renk değişiklikleri ve ekzoftalmus gözlenen klinik bulguları. Histopatolojik incelemede, epikarditis, peritonitis, enteritis ve panoftalmitis görüldü. Solungaçlarda ödem, kanama ve telangiektazi belirlendi. Karaciğer, böbrek, pilorik kese ve hava kesesinde de histopatolojik bulgular mevcuttu. Mikrobiyolojik incelemeler sonucunda etkenin *Lactococcus garvieae* olduğu saptandı. Sunulan bu çalışma ile Türkiye'de ilk kez gökkuşığı alabalıklarında *Lactococcus garvieae* enfeksiyonunun patolojik bulguları kapsamlı olarak değerlendirilmiştir.

Anahtar sözcükler: *Lactococcus garvieae*, Patolojik ve mikrobiyolojik bulgular, Gökkuşığı alabalığı (*Oncorhynchus mykiss* Walbaum, 1792)

INTRODUCTION

The Lactococcosis genus was formerly known as the lactic group of streptococci and consisted of *Streptococcus lactis*, *Streptococcus cremoris* and *Streptococcus diacetylactis*. After the genus was established *Lactococcus garvieae* (*L. garvieae*) was also to this genus added ¹. *L.*

garvieae, previously described as *Streptococcus garvieae*, was originally isolated from cow with mastitis in the United Kingdom ². Since that time, Gram-positive bacterium, *L. garvieae* (junior synonym of *Enterococcus serilicida*) has been isolated from various species of fish in

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different parts of the world including Japan ³, Australia ⁴, South Africa ⁵ and Europe ⁶. The first microbiological description of *L. garvieae* in Turkey was in 1995 ⁷. Since then, the bacterium has caused serious losses of rainbow trout in hatcheries of Turkey ⁸⁻¹⁰.

Bacteriologic and pathologic studies confirmed that *L. garvieae* was the etiological agent of a hemorrhagic septicemia in farmed trout, and the disease characterized with exophthalmos, darkness of the skin and haemorrhages in the internal organs ¹⁰⁻¹⁵. The main histopathological findings in infected fish are epicarditis, peritonitis, enteritis, meningitis and panophthalmitis ^{12,16,17}.

The purpose of this study is to elucidate the pathological and microbiological results of the lactococcosis of naturally infected with *L. garvieae* in rainbow trout.

MATERIAL and METHODS

During the summer of 2006, an outbreak that occurred in a fish farm in the southern Aegean region of Turkey. When the outbreak was determined in the fish farm, the mortality rates in affected fish ranged from 50% to 60%.

Table 1. The API 20 Strep profile for *L. garvieae* isolated from rainbow trout farm

Tablo 1. Alabalık işletmesinden izole edilen *L. garvieae*'nin API 20 Strep testine göre biyokimyasal özellikleri

Properties							
Catalase	-	α -Galactosidase	-	ADH	+	Trehalose	+
Oxidase	-	β -Glukoronidase	-	Ribose	+	Inulin	-
VP	+	β -Galactosidase	-	Arabinose	+	Raffinose	-
Hippurate	-	Alkaline phosphatase	-	Mannitol	+	Amygdalin	-
Esculin	+	Leucine arylamidase	+	Sorbitol	-	Glycogen	-
Pyrrolidonylarylamidase	+	Motility	-	Lactose	-	β -Hemolysis	-

(+; Positive, -; Negative)

The total of 30 dead or euthanased rainbow trout (weight 120 $\pm$ 10 g) collected from the rearing pools were necropsied. Tissue samples (heart, liver, kidney, gills, stomach, pyloric caeca, intestines, spleen, swim-bladder, eyes, brain, skin and skeletal muscles) taken from rainbow trout were fixed in 10% neutral-buffer formalin solution, embedded in paraffin, sectioned in 5 μ m and stained routinely with haematoxylin and eosin (H&E). The selected kidney, liver, spleen, heart, eye and gill sections were also stained by Brown and Brenn staining method for bacteria ¹⁸.

Tissue samples from heart, liver, kidney and spleen were taken for isolation of bacteria, and streaked directly onto tryptic soy agar (TSA, Merck) supplemented with 5% sheep blood. Biochemical tests on samples were carried out in API 20 Strep systems (Biomerieux, France), and the incubation was 72 h at 25°C ¹⁹.

RESULTS

Bacteriological Findings

After incubation for 48 h at 25°C, pure cultures of whitish colonies were observed. All isolates were non-motile, oxidase and catalase negative, and Gram-positive cocci short chain. Based on phenotypic characteristics, the bacterium was identified as *L. garvieae*. The results are presented in [Table 1](#).

Pathological Findings

Lethargie, anorexia and darkness in colour on the skin were observed on the diseased fish. The infected fish were generally found to be swimming weakly and irregularly near the surface of the water.

Macroscopical findings: Redness of fins were determined, hemorrhages at the base of the pelvic and pectoral fins and around the anus were identified. Erosive- ulcerative changes in the skin were only seen in two fish. Uni- or bilateral exophthalmos occurred frequently accompanied by hemorrhages in the ocular



Fig 1. Petechial hemorrhages on the serosal surface of the pyloric caeca (arrows)

Şekil 1. Pilorik kese serozal yüzeyinde peteşiyel kanamalar (oklar)

cavity with the opacity of the cornea. The gill filaments were swollen, hemorrhagic and covered by mucus. The abdomen was distended, and a hemorrhagic serous fluid was found in the abdominal cavity. The petechial hemorrhages were also observed on the mesenterium and serosa of the some organs including stomach, pyloric caeca (Fig. 1) and swim-bladder. The liver was swollen and yellowish-white in colour. The spleen and kidney were generally enlarged and dark in colour. The pyloric caeca and intestines were swollen and they filled with reddish-mucus content.

Microscopical findings: The distribution of the microscopic findings were presented in Table 2. The most common microscopical findings were observed in the heart and peritoneum. The epicardial lesions consisted mainly of inflammatory cells and varying amounts of fibrin and cellular debris (Fig. 2A). Within the myocardium, infiltrations of lymphoid cell (Fig. 2B) and activation of the ventricular endothelial macrophages were also present. Cellular or fibrinocellular peritonitis was generally observed (Fig. 3A). Severe infiltrations of inflammatory cell and fibrin were generally detected in

Table 2. Assessment of the microscopic findings in fish tissues infected with *L. garvieae*

Tablo 2. *L. garvieae* ile enfekte balık dokularında mikroskopik bulguların dağılımı

Organs	Microscopic Findings	Number of Fish (n)			
		0	1	2	3
Heart	epicarditis	4	6	13	7
	infiltration of lymphoid cells in the myocard	15	8	5	2
Periton	peritonitis	12	4	7	7
	adhesion	18	6	3	3
	hemorrhage	10	4	10	6
	clump of bacteria	16	5	7	2
Gill	hemorrhage	8	11	5	6
	lamellar edema	5	10	7	8
	telangiectasia	10	7	9	4
	clump of bacteria	19	4	7	0
	increas numbers of EGCs	16	3	6	5
	hyperplasia of the interlamellar cell	16	7	5	2
	lamellar fusion	20	4	5	1
Eye	hemorrhage	6	7	8	9
	infiltration of inflammatory cell	10	8	7	5
	clump of bacteria	14	8	5	3
Liver	degeneration	12	8	10	0
	infiltration of lymphoid cell	17	2	7	4
	fatty droplets in the hepatocytes	13	6	8	3
Kidney	degeneration	13	7	5	5
	hyalin droplets in the epithel cells of the proximal tubuli	15	8	4	3
	increase in the melanomacrophage centres	14	3	5	8
Pyloric caeca	desquamation of mucosal epithelium	12	6	9	3
	increses of level of mucin	9	7	9	5
	increase number of the EGCs	12	3	10	5
Stomach	increase number of the EGCs	10	4	7	9
Intestine	hyperemia	5	5	8	12
	hemorrhage	6	3	7	14
	infiltration of inflammatory cell	16	7	5	2
Swim-bladder	hemorrhage	11	9	5	5
	infiltration of inflammatory cell	19	3	6	2
Spleen	clump of bacteria	20	4	6	0
Brain	meningitis	29	0	1	0
Skin	erosion - ulcer	28	1	1	0

(0; none, 1; mild, 2; moderate; 3; severe. n; number of fish)

the serosa of the pyloric caeca and mesenterium.

In the eyes, there were hemorrhages and infiltrations of inflammatory cell in the sclera and to a lesser extent in the choroid and retina. Clumps of bacteria were found in the lumens of the blood vessels (Fig. 3B) and hemorrhagic areas, and macrophages also contained similar bacteria. The gills showed vascular changes including edema, hemorrhage and telangiectasia (Fig. 3C). Swelling of epithelial cells of the gills were common present, and some of epithelial cells were separated from the secondary lamellae. Small clumps of gram-positive bacteria were also determined in the blood vessels. Some fish had lamellar hyperplasia and fusion coincided with an increased of eosinophilic granular cells (EGCs) within primary lamellae.

Lesions of the liver included degeneration of the

hepatocytes and perivascular and periductular lymphoid cell infiltrations (Fig. 3D). The sinusoids were dilated, the hepatocytes were contained varying size lipid droplets. In the kidney, degeneration and hyaline droplets in epithelium of the proximal tubuli were generally found. In addition, an increases in melanomacrophage centres were also determined in some fish. In the spleen, the blood vessels were filled up erythrocytes, and some vessels were contained small clumps of gram-positive bacteria.

Histopathological findings were marked in the pyloric caeca to anterior intestines. Sections from the pyloric caeca showed that desquamation and necrosis of mucosal epithelium with increased level of the mucin in the lumen. Large numbers of the EGCs within submucosa of the pyloric caeca were also seen. Severe hyperemia and

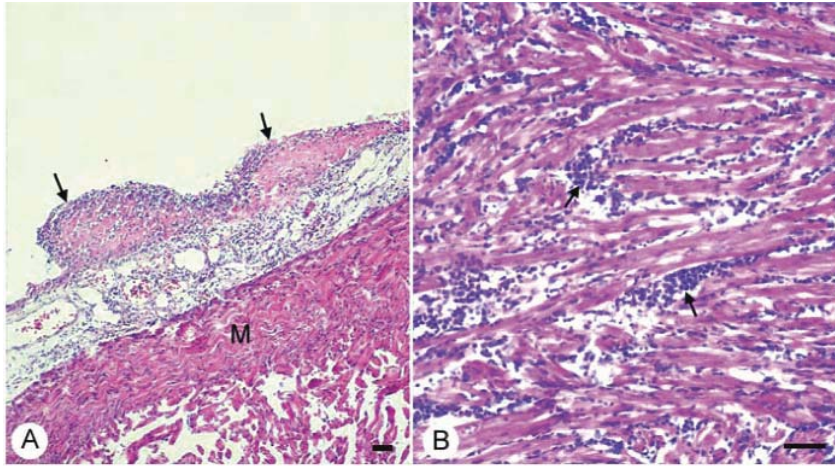
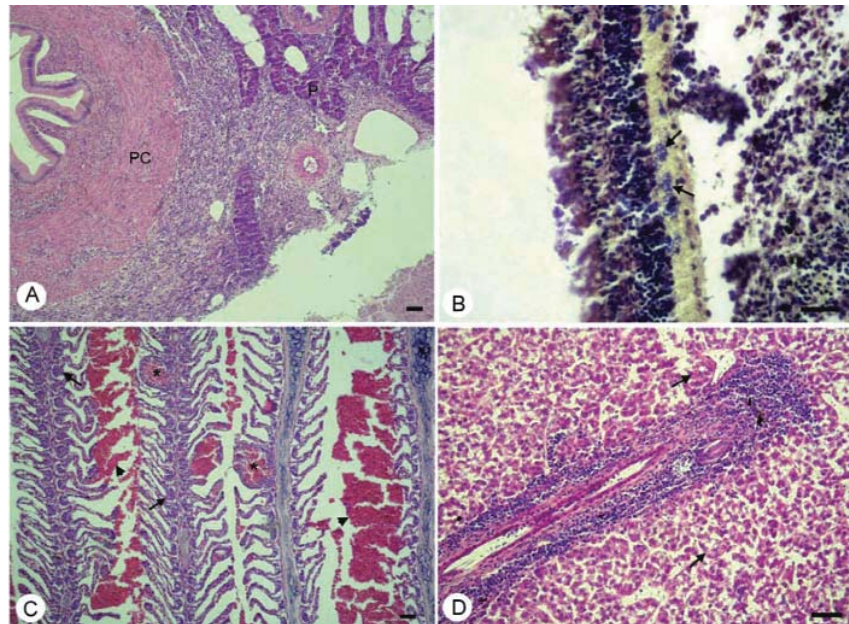


Fig 2. Heart. A. Epicarditis consisted of inflammatory cells and fibrin (arrows), (M: Myocard), HE., Bar= 50µm. B. Lymphoid cell infiltrations in the myocardium (arrows). HE., Bar= 50µm

Şekil 2. Kalp. A. Yangısal hücrelerden ve fibrinden oluşan epikarditis (oklar), (M: Miyokard), HE., Bar= 50µm. B. Miyokardiyumda lenfoid hücre infiltrasyonları (oklar). HE., Bar= 50µm

Fig 3. A. Peritonitis consisted of inflammatory cells. (PC: Pyloric caeca, P: Pancreas). HE., Bar= 50 µm. B. Gram-positive bacteria in the lumens of blood vessel of the eye (arrows), Brown-Brenn, Bar: 30 µm. C. Hemorrhages (arrow-heads), telangiectasia (*) and hyperplasia (arrows) in the gills. HE., Bar=50 µm. D. Degeneration in the hepatocytes (arrows) and perivascular and periductular lymphoid cell infiltrations in the liver. HE., Bar=50 µm

Şekil 3. A. Yangısal hücrelerden oluşan peritonitis. (PC: Pilorik Kесе, P: Pankreas). HE., Bar=50 µm. B. Gözde damar lümenlerinde Gram-pozitif bakteriler (oklar), Brown-Brenn, Bar=30 µm. C. Solungaçlarda kanama (okbaşıları) telangiektazi (*) ve hiperplazi (oklar) HE., Bar=50 µm. D. Karaciğerde hepatositlerde dejenerasyon (oklar) ve perivasküler ve perikanaliküler lenfoid hücre infiltrasyonları. HE., Bar=50 µm



hemorrhages in the villus of the intestines were often determined. Infiltration of inflammatory cells in the lamina propria were also observed in some fish. The findings observed in the stomach were similar but less severe.

The swim-bladder wall was usually edematous. In some cases, there was mild hemorrhages and inflammatory cell infiltrations in the serosa. Meningitis in one fish and erosive-ulcerative dermatitis in two fish were also present.

DISCUSSION

Lactococcosis is one of the infectious diseases with significant economic and sanitary repercussions for trout farms in Mediterranean countries during the summer months ^{6,20-22}. The first description in Europa of *L. garvieae* as a fish pathogen was in 1993 ²⁰. Microbiologically the bacterium was first isolated from a trout farm in 1995, in Turkey ⁷. Since then, the bacterium has causes serious losses of rainbow trout in farms in Turkey as well as in most other European countries ^{8-10,13,22}. For this reason, *L. garvieae* has been considered as the new primary causative agent of rainbow trout in Turkey ⁸⁻¹⁰.

It is reported that outbreaks of lactococcosis in particular are mostly related to poor environmental conditions and other stress factors ^{15,23}. The water temperature and quality are the most important factors influencing the onset of the disease outbreaks ^{15,22,23}. In the present study, the results obtained from the fish farm, strongly support the connection between environmental stress and outbreak of the lactococcosis. Because when the disease was occurred, the water temperature was over 18°C and the pools were also overcrowded.

Clinical signs and gross findings in *L. garvieae* infection in rainbow trout described here are similar to those reported in rainbow trout and other fish ^{10,12-14,16}. But, these lesions are similar to some fish diseases caused by different agents in rainbow trout ²⁴. Therefore, a presumptive diagnosis of the lactococcosis in rainbow trout can not be made on the basis of clinical signs and gross findings.

Lactococcosis is characterized by hemorrhagic septicemia in fish ^{11,12,25}. *L. garvieae*, agent of lactococcosis produces lesions in the vascular endothelium that causing blood extravasation, leading to hemorrhages and petechias at the surface internal organs. Over the external surface, it also intensely affects the most irrigated tissues such as perianal or buccal area and fins ²⁵. Diffuse hemorrhages related to haemorrhagic septicemia

in the external surfaces, eyes and on the serosal surfaces of the fish in the present study are similar to those described by other investigators ^{11,12,16}.

The lesions characterized by epicarditis, peritonitis and panophthalmitis in the present study are comparable those of lactococcosis in fish reported by the others ^{12,17}. Therefore, these lesions in the rainbow trout could be considered as the most characteristic histopathologic findings of the lactococcosis. Fibrinous meningitis associated with the lactococcosis have also been described in fish ^{16,17}, but in the present study, the meningitis was determined in only one fish. In addition, the histopathological findings of the intestine, kidney and spleen manifested in this study are different from those described by some authors ^{12,16,17}, especially with the lack of any extensive erosions with pseudomembrane-like formation of the intestines and any necrosis of the kidney and spleen ^{16,17}. Moreover, periductular and perivascular lymphoid cell infiltrations observed in the liver of some fish have not been described previously for lactococcosis.

The oral route is the main route of transmission of *L. garvieae* have been proposed ^{26,27}, but the results from this study suggest that the gills and eyes are a major site of attachment and the proliferation of *L. garvieae* during infection period. After the bacteria penetrate through the gills and eyes, the spread of blood vessels and finally causing septicemia.

In this study, the isolated bacteria was identified as *L. garvieae* using the API 20 (25°C, 72h) system. The biochemical properties of bacteria isolated from rainbow trout in this study are very similar to those described in other studies ^{9,13,17,19,21}.

It is widely accepted idea that *L. garvieae* is considered as one of the main pathogen of fish, but numerous cases of infections with *L. garvieae* in human were also reported ²⁸⁻³⁰. However, in a recent report ³¹, sporadic human case related to consumption of raw fish foods infected with *L. garvieae* was also described. The relationship between outbreaks of *L. garvieae* and human infections has not been proved until now, and our results cannot support that *L. garvieae* might be considered potentially a zoonotic bacterium to humans.

In the present study, the bacterium isolated from the rainbow trout was identified by biochemically, and its pathological affects on the rainbow trout were firstly evaluated in Turkey. It was also reported that *L. garvieae*, agent of lactococcosis, might cause outbreaks with high mortality in rainbow trout in Turkey especially in hot seasons.

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A Preliminary Survey on Settlement and Recruitment Patterns of Mediterranean Mussel (*Mytilus galloprovincialis*) in Dardanelles, Turkey

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Summary

In this study, the settlement and growth of the Mediterranean mussel, *Mytilus galloprovincialis*, was monitored from September 2002 to February 2004 in Kilya Bay, Dardanelles. Water temperature ranged between 8.3-25°C, the average chlorophyll-a was 0.70 µg L⁻¹. The mussel abundances on the ropes in Kilya Bay, indicated the highest density of mussels in November 2002 (14184 ind. 0.25 m⁻¹) and March 2003 (15485 ind. 0.25 m⁻¹) and lowest in February 2004 (3713 ind. 0.25 m⁻¹). Settlement was significantly different between samplings (P<0.05). At the end of 18 months, 91.36% of spat on rope was smaller than 2.8 mm. The highest percentage of length class was 1.5-2.79 mm with 40.26%. Even though mussel size reached a maximum of 55.7 mm, their density was very low, 0.20%.

Keywords: Mediterranean mussel, *Mytilus galloprovincialis*, Spat, Settlement, Dardanelles

Akdeniz Midyesinin (*Mytilus galloprovincialis*) Çanakkale Boğazı'nda Tutunma ve Stoğa Katılma Durumu Üzerine Bir Ön Çalışma

Özet

Çanakkale Boğazı'na bağlı Kilya Koyu'nda yapılan bu çalışmada, Eylül 2002 - Şubat 2004 tarihleri arasında Akdeniz midyelerinin (*Mytilus galloprovincialis*) yerleşimi ve büyümeleri takip edilmiştir. Su sıcaklıkları 8,3-25°C arasında değişirken, ortalama klorofil-a 0,70 µg L⁻¹ olarak bulunmuştur. Kilya Koyu'ndaki iplerde en yüksek midye yoğunluğu Kasım 2002'de (14184 birey 0,25 m⁻¹) ve Mart 2003'de (15485 birey 0,25 m⁻¹) elde edilirken, en düşük miktar Şubat 2004'te (3713 birey 0,25 m⁻¹) tespit edilmiştir. Yerleşimde örneklemeler arasında önemli derecede farklılıklar bulunmuştur (P<0,05). 18 ayın sonunda, iplerde yerleşmiş olan spatların 91,36%'sının 2,8 mm'den daha küçük olduğu görülmüştür. Boy sınıfları arasında en yüksek oran 40,26% ile 1,5-2,79 mm boy aralığında belirlenmiştir. Midyeler maksimum 55,7 mm boya ulaşmasına rağmen, bu boy grubunda yoğunluğun çok düşük olduğu görülmüştür (0,20%).

Anahtar sözcükler: Akdeniz midyesi, *Mytilus galloprovincialis*, Spat, Yerleşim, Çanakkale Boğazı

INTRODUCTION

Marine mussels, perhaps of all the species of shellfish which are cultivated around the world, most readily demonstrate these characteristics that go to make up the 'ideal candidate for aquaculture'. The natural availability of seed sources without the need of hatchery production has been a significant positive factor in the development of mussel farming ^{1,2}.

Mussel culture has a short history in Turkey. The Mediterranean mussel, *Mytilus galloprovincialis*, is an abundant species in the Marmara Sea in Dardanelles and there is only one mussel farm (longline). The long-line culture depends upon mussel seed collection from artificial collectors and intertidal zone.

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Colonization on natural and artificial substrates by mussels and oysters may occur by settlement of competent pediveliger larvae and/or by settlement of drifting postlarvae^{3,7}. This settlement pattern could have practical importance for the mussel grower, because its knowledge in a given area may improve the chance of collecting mussel seed from nature⁸.

The purpose of this study was to investigate the seasonal settlement differences and growth of mussels, *M. galloprovincialis*, on suspended ropes between September 2002 and February 2004 in Kilya Bay, Dardanelles.

MATERIAL and METHODS

The study was carried out between September 2002 and February 2004 in an area of the north-western of Turkey, Kilya Bay in Dardanelles (40° 12.01'N, 26° 21.30'E) (Fig. 1).

Surface water samples were collected monthly intervals from September 2002 to February 2004. Water temperature was measured to 0.1°C accuracy. Salinity was estimated by Mohr Knudsen method⁹. Seston and chlorophyll-a were determined by filtering over filter paper of 0.45 µm porosity⁹.

Plaited rope collectors were homemade, prepared from old fishing nets for collecting of mussel spats. Old fishing nets were cut 3 equal lines. Then these lines were plaited like a pigtail to create a rope (4 cm in diameter) which was used as a mussel spat collector. Plastic pegs were used at the end of ropes to prevent mussel losses. Twenty four collectors in triplicate (8x3=24) were hung from a raft every 0.5 m in September, 2002. One month

after placement (October 2002), an initial sampling was carried out to evaluate newly settled mussels. Following samplings took place in November 2002, December 2002, January 2003, March 2003, June 2003, October 2003 and February 2004 to observe the settlement and development of mussels on the ropes. Each sampling period, collectors (3 ropes) were taken out to cut 3 cm segments from three parts (upper, middle and down part of rope) of each rope at 0.35-0.45 m depth. Other parts of the ropes were not used to continue experiments.

Mussels were collected from the sampled parts of rope, washed with water under a sieve collecting all mussels smaller than 1 mm. Mussels were sorted into thirteen length classes with the help of a series of sieves with the following mesh size: <0.47, 0.48-0.99, 1.0-1.49, 1.5-2.79, 2.8-4.99, 5.9-9.99, 10-19.9, 20-29.9, 30-39.9, 40-49.9, 50-59.9 mm. Mussel yields were determined by count and measurement. Based on the sample counts the total number of individuals per rope was calculated. Mussels under 5 mm in length was measured under a microscope and the others with a caliper.

Differences in mean numbers of settled mussels were analyzed by using one-way ANOVA and Tukey's test as a post-hoc test. The length frequency distribution for each sampling was tested using χ^2 (Chi square) statistics. All data was carried out using the statistical package SPSS 11.0. Differences were considered significant when $P < 0.05$.

RESULTS

Chlorophyll-a, seston, salinity, and temperature of sea water were recorded in Kilya Bay between

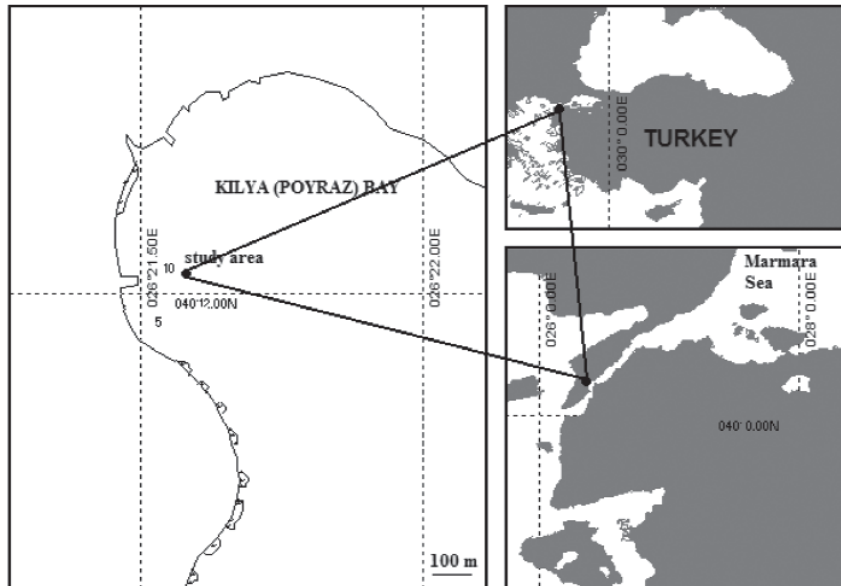


Fig 1. Map of research area in Dardanelles, North-Western Turkey

Şekil 1. Türkiye'nin Kuzeybatısında bulunan Çanakkale Boğazı'ndaki araştırma alanının haritası

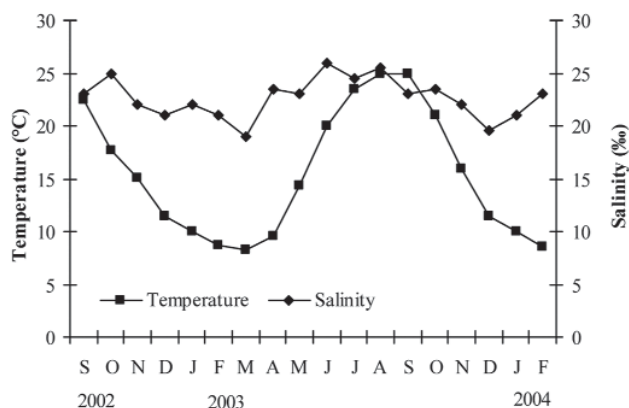
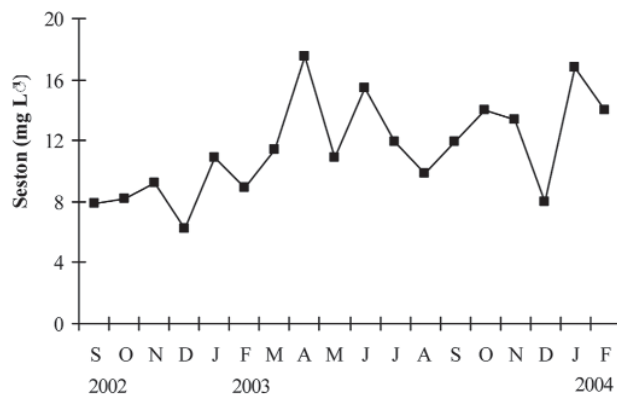
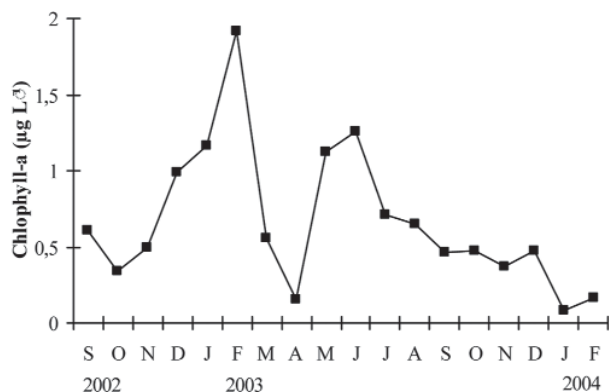
Table 1. Mussel numbers on rope during the experiment**Tablo 1.** Deneme süresince iplerdeki midye sayıları

Months	Total Number of Spat (ind. 0.25m ⁻¹)	Observed Maximum Length (mm)
October 2002	9765±1232	1.54
November 2002	14184±2580	2.02
December 2002	6414±1045	2.09
January 2003	7585±1120	5.9
March 2003	15485±2665	12.5
June 2003	10220±1323	30.4
October 2003	4245±965	48.9
February 2004	3713±528	55.7

September 2002 and February 2004. Minimum water temperature was 8.3°C in March 2003; maximum temperature was 25°C in August and September 2003. Salinity levels in the seawater ranged between 19-25.5 ‰ during the study (Fig. 2).

Seston was minimum (6.2 mgL⁻¹) in December 2002 and maximum (17.5 mgL⁻¹) in April 2003 (Fig. 3). The mean value of chlorophyll-a was 0.70 ±0.1 µgL⁻¹ (Fig. 4).

The mussel abundances on the ropes in Kilya Bay, indicated higher density of mussels in November 2002 and March 2003 (14184 and 15485 ind. 0.25 m⁻¹) and

**Fig 2.** Temperature and salinity levels at the study area**Şekil 2.** Çalışma alanında sıcaklık ve tuzluluk seviyeleri**Fig 3.** Seston changes in Kilya Bay**Şekil 3.** Kilya Koyu'ndaki seston değişimleri**Fig 4.** Chlorophyll-a values in Kilya Bay**Şekil 4.** Kilya Koyu'ndaki klorofil-a değerleri

decreasing to February 2004 (Table 1). Settlement was significantly different between samplings ($P < 0.05$).

Length frequencies of mussel seeds are shown in Fig. 5. With regard to the <0.47 mm length classes, first two months, October and November, showed significantly higher density ($9765 \pm 1232 \text{ ind. } 0.25 \text{ m}^{-1}$ and $14184 \pm 2580 \text{ ind. } 0.25 \text{ m}^{-1}$) than other months. At the last sampling period, although the frequencies of the smallest (<0.47 mm) and the largest (50-59.9 mm) size classes showed 1.69% and 0.26%, the highest percentage (40.26%) was observed with the size class of 1.5-2.79 mm. Only 10 mussels were between 50-59.9 mm.

collector types and environmental conditions (water temperature, food availability, current, etc.)¹¹. Based on the percentage of newly settled mussels smaller than 0.47 mm occurred mainly in October (65.48%), and then decreased to 27.49% in December. On collectors situated in Kilya. The following months were not up 10%. Generally attachment of new spat decreased after January 2003. It can be three reasons for decreasing of newly settled mussels. Either there were not enough larvae for settling, sweeping of larvae by water movements or larvae did not prefer to settle on the ropes covered with mussels. Peteiro et al.¹² remarked sub-

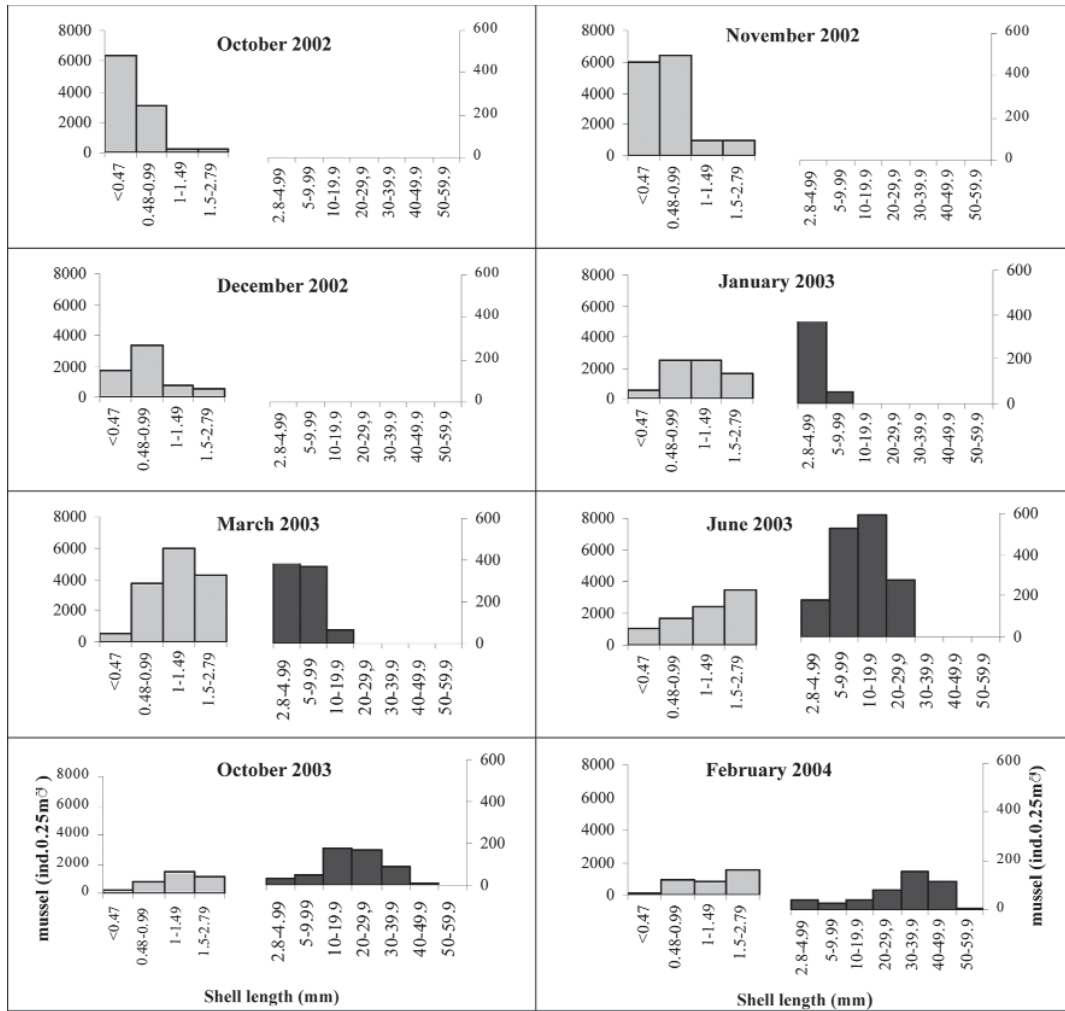


Fig 5. The number of settled mussels on the ropes during the study

Şekil 5. Çalışma süresince iplerdeki yerleşmiş midyelerin sayısı

DISCUSSION

Spat collecting plays an important part in the continuity of suspended mussel cultivation¹⁰. Efficiency of spat collection depends on density of pediveliger,

merged time of collector ropes and advised minimum submersion time of 41 and 46 days for mussel spat settlement. However we observed employed ropes were not favourable for larval settlement for a long time.

Although Dardanelles has mainly rough weather

during winter, the highest density of mussels on March rope can explain with these ropes were not affected by wave and current and kept mainly all attached mussels on its body as extraordinary.

A direct relationship between spawning periods and subsequent settlement of post larval juveniles has been shown for several mussel species^{13,14}. But there is not any published data for reproductive and spawning period of *M. galloprovincialis* in Dardanelles to compare the settlement results.

The total number of spat was 9767 and 14184 ind. 0.25 m^{-1} in October and November. Yıldız¹⁵ and Yıldız et al.¹⁶ reported better settlement in winter months than summer months in Dardanelles. Prior studies¹⁵⁻¹⁷ demonstrated that higher mussel (newly settled) settlement densities were obtained on ropes between February and March (53641 ind. 0.25 m^{-1}) in Kilya Bay. Karayücel et al.⁵ observed 19760 spat m^{-1} at 3 m depth in May and 15187 spat m^{-1} in June in Black Sea. Alfaro and Jeffs¹⁴ recorded that the higher mussel settlement (2028 spat 0.5 m^{-1}) in August and decreased through December (61 spat 0.5 m^{-1}) in Northern New Zealand. Okumus¹⁸ suggested that the seed density is of over 1200 individuals m^{-1} is necessary to produce high yields of cultured mussels. Apparently, seed density of our ropes is very high and need thinning.

Growth of settled spat on ropes can be affected by heavy spat density as well as environmental conditions. Yıldız and Lök¹⁹ reported that 10 mm in shell length of mussel spat reached 29.37 mm in net bags and 48.18 mm on rope without net in one year in Kilya Bay. However settled mussels in this study did not show similar growth pattern. Karayücel and Karayücel²⁰ indicated that shell and tissue growth in mussels is mainly affected by environmental factors although there is also slight genetic effect. The most important hydrological parameters for bivalve growth are water temperature and food availability²¹. Among the hydrological parameters, chlorophyll-a value was observed lesser amount ($0.70 \pm 0.1 \mu\text{g L}^{-1}$) than¹⁹ reported for the same place ($2.14 \pm 1.1 \mu\text{g L}^{-1}$), while other parameters were acceptable for mussel growth. It seems to be chlorophyll value is not enough for mussel growth. The other important factor is heavy density of mussels on ropes. The abundances of attached mussels on ropes are also affecting their food intake and giving slow growth. For this reason, thinning out is necessary to obtain better growth in Kilya Bay. Extremely high densities may limit growth due to overcrowding and competition for available food⁵.

The results of this study indicate that Kilya Bay have

a high potential for the settlement of mussel seed. But further studies such as spawning period, different collector types, hydrological conditions (wave, current, plankton community, etc.) are necessary to understand Kilya Bay for successful mussel culture.

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Investigation of Pathological Findings Infected with *Aeromonas salmonicida* in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) ^[1]

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Summary

Experimental furunculosis caused by *Aeromonas salmonicida* was induced in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) using intraperitoneal injection and immersion challenge methods. A total of 65 (50 experimental and 15 control) rainbow trouts (weight 155 ± 15 g and 20-25 cm in size) were used for this experimental work. *Aeromonas salmonicida* at a concentration of 3×10^5 cfu/ml (0.1 ml per fish) was intraperitoneally injected to twenty-five fish (first group). A second group of 25 fish was immersed in 30 l water inoculated to which 3 ml of bacterial inoculum (3×10^5 cfu/ml) had been added for one hour. Sterile saline solution (0.1 ml/fish) was intraperitoneally injected to control group. Necropsy of the fish was performed, macroscopic and microscopic findings were evaluated. The chronic form of disease was observed in both groups. Anorexia, irregular swimming, hemorrhages at the dorsal, ventral and pectoral base of fins were the main clinic and macroscopic findings. However, in some fish, diffused or focal scale eruption on the dermis from operculum to caudal fin, periocular hemorrhages and exophthalmos were observed. Similar microscopic findings were determined in the both experiment groups. Microscopically, foci of bacteria with no inflammatory response especially in the muscles, gills, heart, stomach, pyloric caeca, intestines, kidney, spleen and liquefactive necrosis in the muscles were the main microscopic findings.

Keywords: *Aeromonas salmonicida*, Rainbow trout, Experimental infection, Pathological findings

Aeromonas salmonicida ile Enfekte Edilen Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik Bulguların İncelenmesi

Özet

Bu araştırma *Aeromonas salmonicida* ile deneysel Furunkülozis'in intraperitoneal yol ve immersiye yöntemiyle oluşturulması amacıyla yapıldı. Bu amaç için, 50 adet deneme grubu, 15 adet kontrol grubu olmak üzere toplam 65 adet, 155 ± 15 g ağırlığında, 20-25 cm büyüklüğünde gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum, 1792) kullanıldı. Intraperitoneal yolla oluşturulan enfeksiyonda (birinci grup) 25 adet balığa 3×10^5 hücre/ml olacak şekilde 0.1 ml bakteri inokulatu verildi. Immersiye yöntemiyle uygulama yapılan diğer gruptaki (ikinci grup) 25 adet balık, 3×10^5 hücre/ml olacak şekilde 3 ml bakteri inokulatu verilen 30 l suda 1 saat bekletildi. Kontrol grubundaki 15 balığa ise 0.1 ml serum fizyolojik intraperitoneal olarak verildi. Bu balıkların sistemik nekropsileri yapıldı, makroskopik ve mikroskopik bulgular değerlendirildi. Her iki deneme grubunda da hastalığın kronik formu şekillendi. Balıklarda klinik ve makroskopik olarak, iştahsızlık, deri renginde koyulaşma, yüzme bozuklukları, dorsal, ventral ve pektoral yüzgeçlerin tabanında kanamalar görüldü. Ayrıca, bazı balıklarda, operkulumdan kuyruk yüzgecine kadar uzanan bölgede pullarda dökülmeler, perioküler kanamalar ve ekzoftalmus dikkati çekti. Her iki deneme grubunda da, mikroskopik olarak benzer bulgular görüldü. Balıklarda mikroskopik olarak kaslarda, solungaçlarda, kalpte, midede, pilorik keselerde, bağırsaklarda, böbreklerde ve dalakta yangısal bir yanıt görülmezsiz bakteri kümeleri ve kaslarda erime nekrozları saptandı.

Anahtar sözcükler: *Aeromonas salmonicida*, Gökkuşağı alabalığı, Deneysel enfeksiyon, Patolojik bulgular

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INTRODUCTION

Furunculosis, caused by *Aeromonas salmonicida*, is a septicæmic and contagious disease characterized by the furuncle formation on the skin with necrotic and hemorrhagic muscle lesions^{1,2}, and leads to outbreaks in many families of fish, especially Salmonidae and Cyprinidae^{3,4}. In Turkey, *Aeromonas salmonicida* was isolated from the three fish hatcheries located in West-Aegean region in 2003⁵. The disease may be seen in peracute, acute, subacute and chronic forms. The peracute form of furunculosis frequently occurs in the fingerling fish. The acute form of furunculosis is common in growing fish and fish usually die within 2-3 days. Subacute and chronic forms are more common in older fish, and at the beginning of infection, deaths occur rarely, but the deaths rate gradually increases⁶.

Aeromonas salmonicida is a gram-negative, aerobic, non-motile, fermentative, non-spore forming, bacillus approximately 1.0 µm x 2.0 µm in size^{1,3,6}. The cell wall of *Aeromonas salmonicida* possesses some virulent factors such as an additional cell envelope protein (A-Layer Protein) and lipopolysaccharid⁷⁻¹⁰. A-Layer Protein is important for virulence. Because *Aeromonas salmonicida*, possesses A-Layer Protein, has the ability to adhere, enter and to survive within macrophages^{7,11,12}. In addition, the extracellular products (ECP) of *Aeromonas salmonicida* consisted of 70-kDa serine protease (extracellular protease, EP) and glycerophospholipid:cholesterol acyltransferase complexed with lipopolysaccharide (GCAT/LPS) play an important role in pathogenesis and virulence¹³⁻¹⁵.

The aim of this study was to investigate macroscopic and microscopic findings in rainbow trouts experimentally infected with *Aeromonas salmonicida* via the intraperitoneal route and immersion challenge methods.

MATERIAL and METHODS

The ethics committee of Adnan Menderes University approved the design and protocol of the experiments (15.01.2004-2004/0013). In this research, a total of 65 (50 fish in experimental groups and 15 fish in control group) rainbow trouts (weight 155±15 g and 20-25 cm in size) obtained from a commercial fish farm with no history of furunculosis, were used. Fish were divided into three groups: First group (25 fish), second group (25 fish) and control group (15 fish). They were acclimatized for 14 days in three separate pools (one for control group and two for experimental groups). Water temperature was maintained at 15-18°C. During the experimental period, external filter (600 l/h) was used in all aquariums.

Dissolved oxygen of the water and pH in all pools were uniform (pH 7.25 and dissolved oxygen 9.1-9.4 mg/l). The fish were fed daily with a commercial feed (No.2 Bağcı). Before the intraperitoneal injections, fish were anaesthetized with 2-phenoxyethanol (0.25%)¹⁶. *Aeromonas salmonicida* (field strain) at a concentration of 3x10⁵cfu/ml (0.1 ml per fish) was intraperitoneally injected to twenty-five fish in the first group. The other twenty-five fish (the second group) were immersed into 30 l water inoculated with 3 ml of bacterial inoculum (3x10⁵ cfu/ml) for one hour. The same procedure was repeated after three days. Sterile saline solution (0.1 ml per fish) was intraperitoneally injected to fifteen fish in the control group. Experiment was performed for 31 days. Necropsy of the fish was performed and tissue samples were collected and fixed in 10% formalin solution, embedded in paraffin, sectioned at 5 µm and stained routinely with Hematoxylin-Eosin (H&E). The selected kidney, liver, spleen, heart and gills were stained by Brown and Brenn staining method for the isolation of bacteria. In addition, to evaluate the presence of lipid in the liver, Oil Red O staining method was used¹⁷.

RESULTS

In this experiment, chronic form of furunculosis was developed in the both groups. In the first group, the deaths began on 6th day and reached to maximum rate on 27-28th day; in the second group, the deaths began on 7th day and reached maximum rate on 23-25th day. The surviving fish were euthanasied on the 31st day of the experiment [total 7 fish (3 fish from first group, 4 fish from second group)]. Fish in the control group were euthanasied and changes were observed comparatively. *Aeromonas salmonicida* was reisolated from the infected fish and no *Aeromonas salminicida* or any other pathogenic bacteria were isolated from fish in the control group.

Clinical signs consisted of anorexia, darkening in the color or depigmentation of the skin and irregular swimming in the both groups.

Macroscopic Findings

In both groups, macroscopically, hemorrhage at the dorsal, ventral and pectoral base of fins, diffused or focal scale eruption on the dermis from operculum to caudal fin were often noted. Furthermore, diffuse hemorrhages in the kidney, stomach and intestines; periocular hemorrhages and exophtalmos; anemia; hyperemia or anemia in the gills; a great reduction in the size of kidney tissue; ascites and petechial hemorrhages on the perivisceral tissue (*Fig. 1*) were determined in some fish. Generally, due to anorexia, the stomach, pyloric caeca and intestines were empty.



Fig 1. Petechial hemorrhages on the perivisceral tissue (arrow)

Şekil 1. Perivisceral dokuda peteşiyal kanamalar (ok)

Microscopic Findings

Similar microscopic findings were determined in the both experiment groups. The main microscopic findings were the foci of bacteria with no inflammatory response in the different tissue and organs, and liquefactive necrosis in the muscles.

The liquefactive necrosis was observed in the muscles (**Fig. 2A**). The sizes and the locations of the necrosis varied among the fish. Some of the necrotic muscle bundles were filled with the destruction products or they were completely empty.

Foci of bacteria with no inflammatory response were observed in the necrotic areas of muscle (**Fig. 2B**); around secondary lamellae in the gills; in the heart; serosal surface of the stomach, pyloric caeca and intestines; in the tubular lumen of the kidney; capsular surface of the spleen and kidney.

In the liver, discrete vacuoles were present in the cytoplasm of the hepatocytes. These vacuoles were

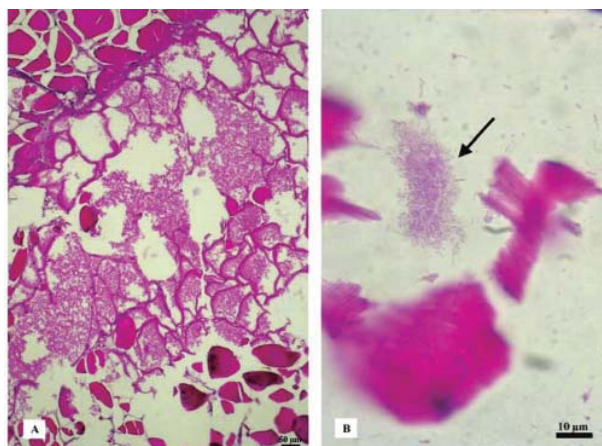


Fig 2. A: The liquefactive necrosis in the muscle tissue. HE. Bar, 50 µm, **B:** Foci of bacteria with no inflammatory response in the muscle tissue (arrow), HE. Bar, 10 µm

Şekil 2. A: Kaslarda erime nekrozu. HE. Bar, 50 µm, **B:** Kaslarda yangısal bir yanıt görülmeksizin bakteri kümeleri (ok), HE. Bar, 10 µm

recognized as fat droplets stained with Oil Red O method in frozen sections.

Hydropic degeneration in the proximal and distal tubules, reduction in the lymphoid cells of hematopoietic tissue (**Fig. 3**), and glomerular atrophy were determined in the kidney. The most important microscopic lesion in the spleen was necrosis in the lymphoid tissue.

The primary and secondary lamellar lesions consisted of edema, adhesion, hyperplasia, hyperemia and telangiectasia in the gills. Marked edema in the secondary lamellae was often present.

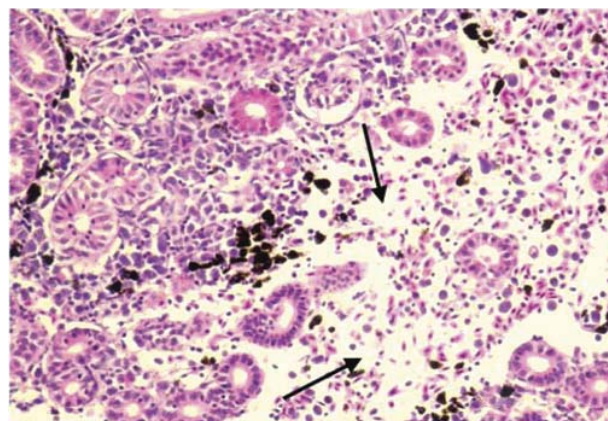


Fig 3. Reduction in the lymphoid cells of hematopoietic tissue in the kidney (arrows), HE. Bar, 50 µm

Şekil 3. Böbrekte hematopoietik dokunun lenfoid hücrelerinde azalma (oklar), HE. Bar, 50 µm

In addition, elevated numbers and degranulation of eosinophilic granular cells (EGC) in the submucosa of the stomach and pyloric caeca were determined. However, same finding was detected in only one fish's gill.

DISCUSSION

In the chronic form of furunculosis anorexia, exophthalmos, darkening in the color of skin, irregular swimming, hemorrhages at the dorsal, ventral and pectoral bases of fins, and furuncle formation at the skin were reported as the main clinic and macroscopic findings ^{6,12,14}. In the present study, anorexia, irregular swimming, darkening in the color of skin, hemorrhages at the bases of fins, scale eruptions were observed following the inoculations. But furuncle formation was not seen.

It has been reported that the 70-kDa serine protease causes of liquefaction necrosis in the muscles ¹³. In addition, on the other hand, GCAT/LPS combination with 70-kDa serine protease induces liquefaction necrosis in the muscles ¹⁸. In the present study, the liquefaction

necroses in the muscles were located especially around foci of bacteria, and we suggested that liquefaction necrosis in the muscles could be related to the extra-cellular products of *Aeromonas salmonicida*.

One of the important microscopic findings of furunculosis is foci of bacteria with no inflammatory response in the dermis, muscle and internal organs ^{1,19}. Recent studies suggest that GCAT/LPS have haemolytic, leucocytolytic and cytotoxic activities ^{6,12,20}. That is because of these effects of GCAT/LPS, destruction of hematopoietic tissue occurs in kidney and spleen. Moreover, a few authors suggested that *Aeromonas salmonicida*, possesses A-Layer Protein, the ability to adhere, enter and, to survive within macrophages bring about phagocytosis inhibition ^{7,11}. Phagocytosis inhibition and hematopoietic tissue destruction constitute foci of bacteria with no inflammatory response in the dermis, muscles and internal organs. In this study, foci of bacteria with no inflammatory response in the muscles, gills, heart, stomach, pyloric caeca, intestines, kidney and spleen, and reduction in the lymphoid cells of hematopoietic tissue of the kidney were seen. As a matter of fact the selected tissue sections were stained by Brown and Brenn staining method for bacteria and positive results were obtained. We suggested that foci of bacteria with no inflammatory response in the muscles, gills and internal organs might be related to A Layer Protein and activity of GCAT/LPS.

In conclusion, in both groups, macroscopic and microscopic findings of this study might be considered important pathological findings of furunculosis in rainbow trout.

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A Field Study on Bio-Ecology of *Hygrotus ahmeti* (Coleoptera: Dytiscidae) in the Eastern Anatolia Region of Turkey

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Summary

Hygrotus (Coelambus) ahmeti Hájek, Fery and Erman, 2005 is endemic for Turkey and it has limited distribution. Aquatic insects were collected once a month from eight different habitats around Erzurum (Çat district) during March-September 2009 period. *H. ahmeti* could be only sampled from one habitat. A total 546 insect specimens belonging to 27 species were sampled in this mountain meadow pond. *H. ahmeti* was a rare species in the study area (Distribution=12.5%). While the species were sampled with very low number during June-August period (one each specimen), it reached its peak (n=10) during September period. Habitat waters were determined as class I waters with regard to various parameters (Na, K, Mg, Ca, Cl, F, NH₄, NO₂, NO₃, SO₄, PO₄, water temperature, dissolved oxygen, pH and conductivity). We believe that these findings will contribute to both determining the diversity of aquatic insects in mountain meadow ponds and bio-ecology of *H. ahmeti*.

Keywords: *Hygrotus ahmeti*, Dytiscidae, Ecology, Aquatic insects, Diversity, Turkey

Doğu Anadolu Bölgesi'nde (Türkiye) *Hygrotus ahmeti* (Coleoptera: Dytiscidae)'nin Biyo-Ekolojisi Üzerine Bir Alan Çalışması

Özet

Hygrotus (Coelambus) ahmeti Hájek, Fery and Erman, 2005 Türkiye için endemik ve sınırlı yayılışa sahiptir. Mart-Eylül 2009 süresince Erzurum (Çat) civarında sekiz farklı habitattan ayda bir sucul böcekler toplandı. *H. ahmeti* yalnızca bir habitattan örneklenebildi. Dağ çayırı tipi bu habitatta 27 türe ait toplam 546 böcek toplandı. *H. ahmeti*, çalışma alanında nadir bir türdü (Yayılım=%12.5). Haziran-Ağustos süresince oldukça düşük sayıda (1'er birey) örneklenen tür, Eylül periyodunda en yüksek populasyon yoğunluğuna (n=10) ulaştı. Habitat sularının birçok parametre (Na, K, Mg, Ca, Cl, F, NH₄, NO₂, NO₃, SO₄, PO₄, su sıcaklığı, çözünmüş oksijen, pH ve iletkenlik) açısından I. sınıf su kalitesinde olduğu belirlendi. Bu bulguların, hem *H. ahmeti*'nin biyo-ekolojisine hem de dağ çayırı su birikintilerinde sucul böcek çeşitliliğinin belirlenmesine katkı sağlayacağını düşünüyoruz.

Anahtar sözcükler: *Hygrotus ahmeti*, Dytiscidae, Ekoloji, Sucul böcekler, Çeşitlilik, Türkiye

INTRODUCTION

Most dytiscids are excellent swimmers with compact, flattened, streamlined bodies. The flattened oar-like mid and hind legs often bear fringes of long swimming hairs. Adults and larvae of dytiscids occur together and have adapted to almost all aquatic habitats imaginable. Most dytiscids occur in lentic habitats such as shallow, weedy lakes, ponds, ditches, stock ponds, and springs while

some occur in more specialized habitats ¹. Both adults and larvae are aquatic and predaceous, feeding not only on a wide range of invertebrates such as molluscs, annelids, and insect larvae, but also on vertebrates such as fish fry and small amphibians ². Generally, densely vegetated waters have a more diverse dytiscid fauna than barren ones ³.

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The Dytiscidae includes about 4000 species and 177 genera. About 1000 species have been recorded from the Palaearctic region. The number of Palaearctic genera is currently 66^{4,5}. The presence of about 149 species belonging to 29 genera in 6 subfamilies in Turkey is largely based on recent studies. The type localities of 24 of these 149 species are themselves located in Turkey⁴⁻⁸. According to Fery (2003), 30 species of *Hygrotus* were known to occur in the Palaearctic region, 25 of these belonging to the subgenus *Coelambus*⁹. Eleven *Hygrotus* species have been recorded from Turkey so far⁴⁻⁶. *Hygrotus (Coelambus) ahmeti* Hájek, Fery and Erman, 2005 is endemic for Turkey⁶.

In this study, our objectives were to determine some bio-ecological characteristics of *H. ahmeti*. Besides, we believe that our study will contribute to the determination of aquatic insect species diversity in highland ponds. Determining biological diversity in these habitats is important for natural conservation.

MATERIAL and METHODS

Study Area and Sampling Method

Aquatic insects were collected once a month from eight different type habitats (overflow of stream, stream bank, swamp, snow pool, and mountain meadow pond) around Erzurum (Çat district) during March-September 2009 period. The insects were collected in the habitat by means of a sieve, ladle and net having a mesh diameter of 0.5 mm. The samples were killed with ethyl acetate or in 70% alcohol solution and then the clayey and muddy substance on their surfaces was brushed off with a small paint brush in the laboratory. The samples were examined using a Nikon type SMZ-U stereo microscope. The species of Dytiscidae and Haliplidae were identified using the keys by Zaitzev¹⁰ and Hájek et al.⁶. Hydrophilidae species were identified by Dr. Abdullah MART (Atatürk University, Turkey) and Hemiptera species were identified by Dr. Ali SALUR (Hitit University, Turkey).

Determining the Distribution of *Hygrotus ahmeti*

Distribution was determined as the percent of sampling sites in which a species was noted, according to the formula¹¹:

$$C = n/N \cdot 100\%$$

Where: C- distribution, n- number of sites of the species, N- number of all sites.

- C1- sporadic appearance (constancy 0 - 20%)
- C2- infrequent (20.1 - 40%)

C3- moderate (40.1 - 60%)

C4- frequent (60.1 - 80%)

C5- constant (80.1 - 100%)

Analysis of Water Quality in Habitats

Physico-chemical parameters of the habitats were measured in each month during insect collect procedures. Anions (NO₂, NO₃, SO₄, PO₄, F, Cl) and cations (Na, K, Mg, Ca, NH₄) of the water were analyzed by using Dionex ICS 3000 and Dionex ICS 1000 model devices (Ion Chromatography System), respectively. Physical parameters (water temperature, dissolved oxygen, pH and conductivity) were measured on site using portable devices. The results of water analysis were evaluated according to water quality criteria of Turkey Ministry of Environment¹². Besides, some basic characteristics of aquatic habitats (appearance, water dept etc.) were recorded.

RESULTS

H. ahmeti could be only sampled from one habitat out of eight different type habitats. This habitat was a mountain meadow pond (39°37'N, 40°59'E; altitude of 1960 m). In this paper, the findings in this habitat where *H. ahmeti* could be only sampled are given. On the other hand, *H. ahmeti* had sporadic appearance (C=12.5%) in the study area.

Aquatic Insects Species and Their Population Fluctuations in the Habitat

The population fluctuations of aquatic insects during the study are shown in [Table 1](#). A total of 546 aquatic insects of the 27 species present were collected during the study in the habitat. Aquatic insects could be sampled continuously during March-September period. During October period habitat water was dried up completely. The number of aquatic insects was very low in March (n=10). The number of the insects was peaked in May (n=172) and June (n=165).

H. ahmeti could not be sampled during March-May period in the habitat. The species could be sampled very low during June-August period (one each specimen), and it reached its peak in September (n=10) ([Table 1](#)).

Familia Dytiscidae (11 species), Hydrophilidae (7 species) and Notonectidae (3 species) had high species diversity in the habitat. *H. ahmeti* could be sampled densely with three species from its own family (*Hygrotus inaequalis*, *Hyphydrus ovatus*, *Laccophilus minutus*), *Haliplus* sp., *Notonecta* sp. and *Plea minutissima*.

Table 1. Monthly population fluctuations of 27 aquatic insect species in the study area**Tablo 1.** Çalışma alanındaki 27 sucul böcek türünün aylık populasyon dalgalanması

	Family	Species	March	April	May	June	July	August	September	Total
Coleoptera	Dytiscidae	<i>Hygrotus ahmeti</i>	-	-	-	1	1	1	10	13
		<i>Hygrotus inaequalis</i>	-	-	-	-	7	3	4	14
		<i>Hygrotus parallellogrammus</i>	6	3	5	7	6	-	-	27
		<i>Hydroglyphus geminus</i>	-	-	4	2	13	-	-	19
		<i>Hydroporus marginatus</i>	-	-	1	1	1	-	-	3
		<i>Hydroporus palustris</i>	-	-	-	7	3	-	-	10
		<i>Hydroporus thracicus</i>	-	-	-	2	1	-	-	3
		<i>Hyphydrus ovatus</i>	-	-	23	4	1	1	7	36
		<i>Laccophilus hyalinus</i>	-	-	-	2	10	-	-	12
		<i>Laccophilus minutus</i>	-	-	11	27	-	6	20	64
		<i>Scarodytes halensis</i>	3	2	5	3	-	-	-	13
		Total	-	-	-	-	-	-	-	214
	Gyrinidae	<i>Gyrinus</i> sp.	-	-	1	-	-	-	-	1
		Total	-	-	-	-	-	-	-	1
	Halipilidae	<i>Haliplus</i> sp.	-	1	95	60	2	2	14	174
		Total	-	-	-	-	-	-	-	174
	Hydrophilidae	<i>Berosus luridus</i>	1	9	15	18	-	3	1	47
		<i>Helophorus discrepans</i>	-	-	-	-	3	4	-	7
		<i>Helophorus longitarsis</i>	-	-	-	-	2	-	-	2
		<i>Laccobius sulcatulus</i>	-	-	-	-	7	-	-	7
		<i>Berosus signaticollis</i>	-	-	-	16	-	-	-	16
		<i>Helophorus</i> sp.	-	3	2	-	-	-	-	5
		<i>Helochaeres</i> sp.	-	-	2	-	-	-	-	2
		Total	-	-	-	-	-	-	-	86
Hemiptera	Corixidae	<i>Corixa</i> sp.	-	1	-	-	-	1	1	3
		<i>Sigara nigrolineata</i>	-	1	-	2	-	1	1	5
		Total	-	-	-	-	-	-	-	8
	Naucoridae	<i>Ilyocoris cimicoides</i>	-	1	2	5	9	1	2	20
		Total	-	-	-	-	-	-	-	20
	Notonectidae	<i>Notonecta glauca</i>	-	1	1	-	-	-	-	2
		<i>Notonecta viridis</i>	-	-	-	1	-	-	-	1
		<i>Notonecta</i> sp.	-	-	-	5	2	-	7	14
		Total	-	-	-	-	-	-	-	17
	Pleidae	<i>Plea minutissima</i>	-	-	5	2	-	-	19	26
Total		-	-	-	-	-	-	-	26	
General Total			10	22	172	165	68	23	86	546

The Valeus of Physico-chemical Parameters in the Habitat of *H. ahmeti*

Monthly values of physico-chemical parameters in the breeding habitat of *H. ahmeti* are shown in Table 2. Sodium, Magnesium and Calcium were the highest in September period (31.65, 40.86 and 151.7, respectively). The highest values of ammonium, nitrite and nitrate were 0.42, 0.12 and 2.62, respectively during the study. Phosphate values were too low to be measured, except September period. Values of chlorine, flour and sulphate were very low. The highest water temperature was 24.6°C, and the lowest dissolved oxygen was 2 mg/l in the habitat during the study. pH was between 7 and 9.27, and while conductivity was the lowest in August

(177 µS/cm), it was 442 µS/cm in September.

Habitat waters were determined as class I waters with regard to the parameters, except nitrite in March-April period and dissolved oxygen in September.

Some Basic Characteristics of Aquatic Habitat of *H. ahmeti*

The habitat was mountain meadow type pond which was natural, clear water, without current, generally permanent, exposed to direct sunlight, vegetation with *Potamogeton* sp. It continues to exist through some leakage throughout season. The water depth was between 5 and 60 cm in the area whose size was about 1.000 m².

Table 2. Monthly fluctuations of physico-chemical parameters in the breeding habitat**Tablo 2.** Üreme habitatındaki fiziko-kimyasal parametrelerin aylık dalgalanması

Parameter	March	April	May	June	July	August	September
Sodium (mg Na ⁺ /l)	12.31	5.54	13.4487	6.97	9.75	8.94	31.65
Potassium (mg K ⁺ /l)	12.02	8.1	12.9507	5.84	6.60	5.75	11.63
Magnesium (mg Mg ⁺ /l)	9.89	9.47	24.291	8.81	6.95	7.29	40.86
Calcium (mg Ca ⁺ /l)	37.23	30.04	51.7122	34.44	20.59	18.53	151.7
Ammonium (mg NH ₄ ⁺ /l)	0.31	0.4196	0.3397	<0.01	-	-	<0.001
Nitrite (mg NO ₂ ⁻ /l)	0.12	0.02	-	0.0036	-	-	<0.002
Nitrate (mg NO ₃ ⁻ /l)	2.62	0.85	-	0.52	-	0.097	0.1
Sulphate (mg SO ₄ ⁻ /l)	46.33	37.02	33.4842	32.02	28.14	17.85	8.97
Phosphate (mg PO ₄ ⁻³ /l)	-	-	-	-	-	-	<0.0173
Flour (mg F/l)	0.18	0.33	0.2641	0.41	0.45	0.37	0.40
Chlorine (mg Cl ⁻ /l)	7.56	5.79	4.3931	6.78	7.73	7.86	11.31
Water temperature (°C)	14.8	12.7	22.8	19.5	24.6	21.2	8
Dissolved oxygen (mg O ₂ /l)	9.5	8.8	8.6	6.4	5.73	8.76	2
pH	8.52	8.57	8.68	8.42	8.9	9.27	7
Conductivity (µmhos/cm)	290	273	289	248	190	177	442

DISCUSSION

Low population density (2.38%) in the habitat and limited distribution (12.5%) in the study area of *H. ahmeti* can be caused by its low ecological tolerance. In a previous study, this species could be only sampled from four temporary habitats (Erzincan and Erzurum province of Turkey) and it was given as a new record for the world ⁶. Since water was dried up in October in our study, this habitat can be evaluated as temporary/ generally permanent. While the number of aquatic insects decreased in the habitat in September, the number of *H. ahmeti* increased. This phenomenon might be caused by biology of *H. ahmeti*. According to our findings, *H. ahmeti* needs more time than most aquatic insects to be mature in the same habitat.

Since there were no polluting sources around the habitat and it was away from residential areas, low water parameters are normal. The reason why some parameters, especially nitrite, nitrate and sulphate, were high in March in the habitat could be related to the materials, carried into the habitat by snow melting. Basic reason of an increase of most physico-chemical parameters in September can be a decrease of water in the habitat because of evaporation. Environmental factors such as water chemistry, size of water, or habitat homogeneity are assumed to be imported in determining assemblage structure of diving beetles ¹³⁻¹⁵. On the other hand, Nilsson and Söderberg (1996) studied boreal lakes and did not detect a clear relationship between dytiscid faunas and water chemistry ¹⁶. Since *H. ahmeti* could be only sampled from one habitat in our study, we cannot explain accurately the effect of water parameters on the population density of the species.

In this study, sampling 27 species belonging to two

Ordo (Coleoptera and Hemiptera) in only one habitat shows the importance of ponds in highland areas for biological diversity. Ponds are valuable resource for the conservation of freshwater biodiversity and indicator taxa for the conservation of pond can be different in various geographical areas ¹⁷. The 55% of Turkey's territory is above 1.000 m. This geographical position is important with regard to variety of ponds in plateau and mountains. The Eastern Anatolian Region has high endemism on account of freshwater invertebrates because of its geographical structure ¹⁸. On the other hand, scientific data in Turkey is restricted for the ecology and protection of ponds. At a regional level, ponds can contribute highly to freshwater biodiversity, with recent evidence showing that they often support considerably more species, more unique species and more scarce species than other water-body types ¹⁹. Although temporary ponds have recently been recognized as a wetland type of international importance by the Ramsar Convention, political recognition of ponds as an entity and as an important part of the water environment remains insignificant throughout Europe ²⁰.

With this study both some data about bio-ecology of *H. ahmeti* and the species diversity of the ponds in highland area are obtained. We recommend multi-disciplinary studies of the biological diversity in ponds. Multidisciplinary studies will provide us valuable data related to rare and endemic species, and these data are important for natural conservation.

ACKNOWLEDGMENTS

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A Scanning Electron Microscopic Study on Some Morphological Properties of Three Local Silkworm Breeds (Alaca, Bursa Beyazı and Hatay Sarısı)

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Summary

In this study the cocoon, egg and silk gland of three local silkworm breeds were analysed by the scanning electron microscopy (SEM). The egg and cocoon morphology of Bursa Beyazı was observed as different from other breeds. The cocoon fibers were tightly packed in the cocoons of Bursa Beyazı which have a higher silk quality whereas the fibers were loosely packed in the cocoons of Alaca and Hatay Sarısı. In addition, the motif structure on Alaca's egg surface was irregular. Interestingly, there was a regular motif structure on the Bursa Beyazı's and Hatay Sarısı's egg surface.

Keywords: *Silk, Cocoon, SEM, Silk gland, Bombyx mori*

Üç Yerli İpekböceği Irkının (Alaca, Bursa Beyazı ve Hatay Sarısı) Bazı Morfolojik Özellikleri Üzerine Bir Elektron Mikroskop Çalışması

Özet

Bu çalışmada üç yerli ipekböceği irkının kokon, yumurta ve ipek bezleri taramalı elektron mikroskobu (SEM) ile incelenmiştir. Bursa Beyazı'nın yumurta ve kokon morfolojisinin diğer ırklardan farklı olduğu gözlenmiştir. Yüksek ipek kalitesine sahip Bursa Beyazı'nın kokon fiberleri daha sık paketlenirken Alaca ve Hatay Sarısı'nın kokon fiberleri daha gevşek paketlenmiştir. Ayrıca Alaca'nın yumurta yüzeyindeki motif yapısı oldukça düzensizdi. İlginç olarak Bursa Beyazı ve Hatay Sarısı'nın yumurta yüzeyinde düzenli bir motif yapısı vardı.

Anahtar sözcükler: *İpek, Kokon, SEM, İpek bezi, Bombyx mori*

INTRODUCTION

The silk of lepidopteran insects secrete a solid fiber, the silk ¹. The silk is secreted from labial silk glands. In the domestic silkworm *Bombyx mori* ², the silk fibre core, which is secreted in the posterior section of the silk glands (PSG), consists of heavy-chain fibroin (H-fibroin) ¹, light-chain fibroin (L-fibroin) and protein p25. The middle silk gland sections (MSG) secrete several sericins that sticky coating of the silk ¹. Fibroin is the water insoluble core of the silk fiber whereas sericin is the water soluble proteinaceous glue ³. The structure and properties of the silk filaments of wild silkworms has been studied by a number of workers ⁴.

in Bursa Provincial Directorate of Agriculture. Alaca and Bursa Beyazı are diapausing genotypes but Hatay Sarısı is nondiapausing genotypes. Bursa Beyazı have a higher silk quality than others.

In this study the cocoon, silk gland and egg morphology of three local silkworm breeds studied by the Scanning Electron Microscopy (SEM).

MATERIAL and METHODS

Collection of Samples: Cocoons, larvae and eggs of three Turkish silkworm breeds were obtained from Bursa

There are three local silkworm breeds are inbreded



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Silkworm Breeding Research. The silk glands were obtained from the last instar larvae of three local silkworm breeds.

Preparation of the Silk Gland, Cocoons and Eggs for the SEM: The silk glands and cocoons were fixed in 3% glutaraldehyde solution buffered in 0.2 M phosphate buffer for 1 h; post fixed in 1% osmium tetroxide ⁵ in the same buffer for 1.5 h. For scanning electron microscopic observations dehydrated through 50%, 60%, 70%, 80%, 90%, 99% and 100% series of alcohol. Then the samples were dried in the room for the night and gold coated for the SEM ⁶. The eggs were gold coated and observed under the SEM ⁷.

Preparation of Degummed Silk Fibers: Two different method were used to remove the sericin layer. In the first; the fibers were washed in boiling distilled water for 1 h. Then the samples were dried in the room and observed by the SEM. In the second method; the fibers were processed by extraction of sericin by using an aqueous solution containing 0.02 M Na_2CO_3 and white soap powder for 60 min at 90°C as previously described ⁸. Dehydrated through 50%, 60%, 70%, 80%, 90%, 99% and 100% series of alcohol. Then the samples were dried in the room for the night and gold coated for the SEM ⁶.

RESULTS

The cocoon filaments and degummed fibers of three breeds were examined under scanning electron microscope. The morphological structure of cocoon filaments were different in silkworm breeds. The cocoon fibers were tightly packed in the cocoons of Bursa Beyazı which have a higher silk quality whereas the fibers were loosely packed in the cocoons of Alaca and Hatay Sarısı. Another difference was observed on the surface of the silk fibers. There were more voids on the fibers of Bursa Beyazı than fibers of Alaca and Hatay Sarısı (Fig.1).

The sericin layer wasn't removed by washing with distilled water (Fig. 2). This glue-like layer was removed by washing with Na_2CO_3 and white soap powder as previously described ⁸ (Fig. 3). It's clear that this sericin layer occurs breed-special fiber design.

The egg morphology of three breeds were different. The motif orientation on Alaca's egg surface was irregular. Moreover, shape of all motifs and the distances between motifs were different. Interestingly, there was a regular motif orientation on the Bursa Beyazı's egg surface.

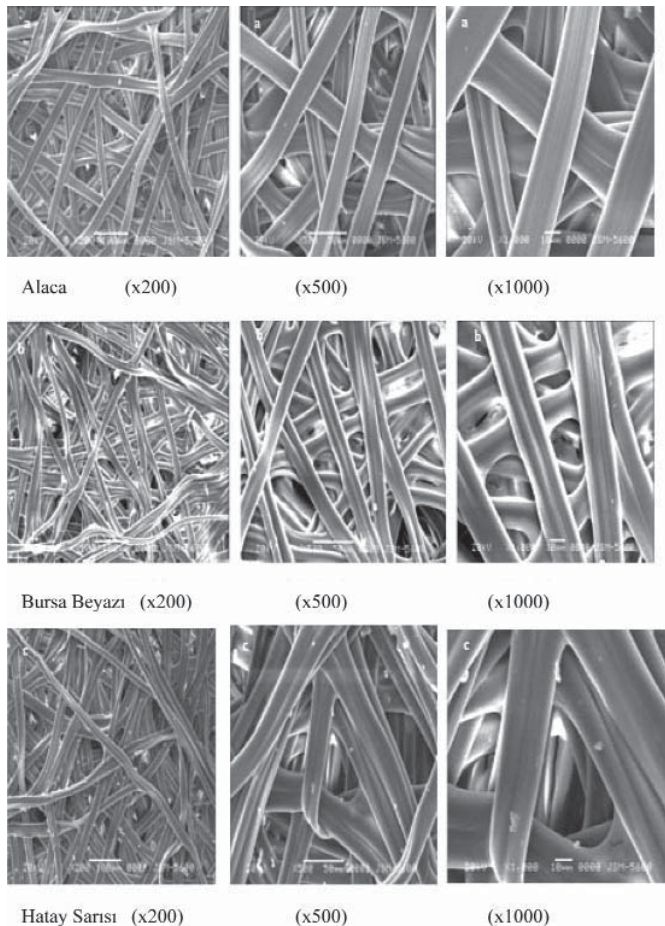


Fig 1. The morphology of cocoon in local silkworm breeds

Şekil 1. Yerli ipekböceği ırklarında kokon morfolojisi

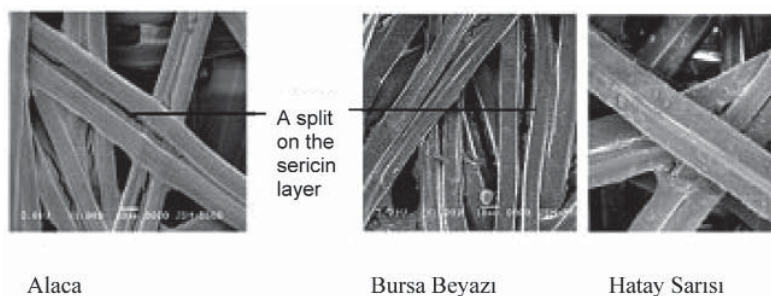
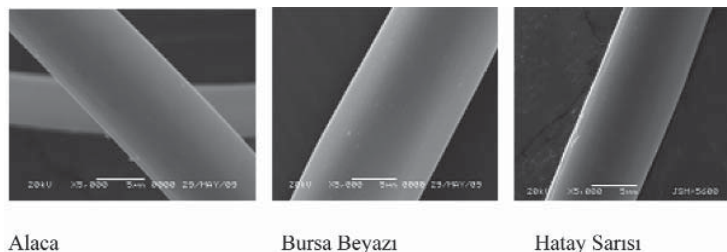


Fig 2. Degummed silk fiber surfaces of local silkworm breeds by washing with distilled water (X1000)

Şekil 2. Yerli ipekböceği ırklarının distile su ile yıkanarak soyulmuş ipek fiber yüzeyleri (X1000)

Fig 3. Degummed silk fiber surfaces of local silkworm breeds by washing with Na_2CO_3 and soap powder (X5000)

Şekil 3. Yerli ipekböceği ırklarının Na_2CO_3 ve sabun tozu ile yıkanarak soyulmuş ipek fiber yüzeyleri (X5000)



Because the dimension of motifs were the same and the distances between motifs were equal. In addition, each motif had more than one honeycomb structures. There was a regular motif orientation on the Hatay Sarısı's egg surface, too. The egg morphology of Hatay Sarısı was resemble to Bursa Beyazı. But the honeycomb structure in motifs wasn't so clear as in Bursa Beyazı (Fig. 4).

There was no difference between local three breeds' silk glands (Fig. 5) There were much secretory tubes on the posterior part. Middle silk gland was very smoothy which is a depot part of the silk gland.

DISCUSSION

According to these observations the cocoon morphology of Hatay Sarısı and Alaca were similar in each other. Similarly, Mondal et al.⁴ had determined that fibers had tightly packed in pure bivoltine breeds (have a higher silk quality) whereas fibers had loosely packed in pure multivoltine breeds. The number of voids in pure multivoltine breeds had been determined much higher than had found in pure bivoltine breeds. Narumi et al.⁹, had studied the cocoon filaments of various wild silkworm species under a SEM and had found voids in the cocoon filaments. Narumi et al.¹⁰, had reported that the number of voids in male filament was larger than that of female silkworms. Lin et al.¹¹, had determined that the presence of voids in the cocoon filament is a characteristic of the fine structure of cocoon filament in wild silkworms belonging to the family Saturniidae. Mondal et al.⁴ had determined that voids in the cocoon filament are important in the selection process of good quality of silk cocoons for silkworm breeding.

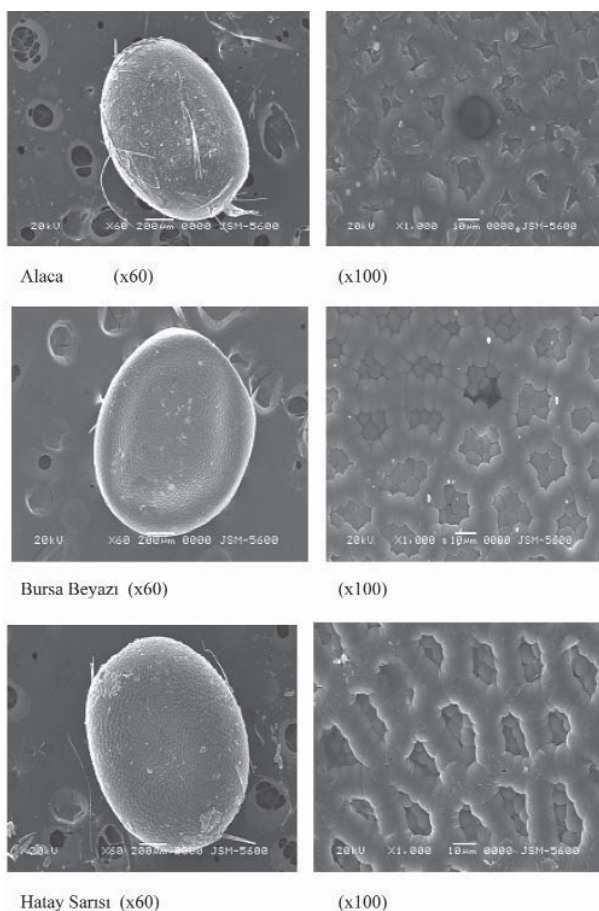


Fig 4. The morphology of egg in local silkworm breeds
Şekil 4. Yerli ipekböceği ırklarında yumurta morfolojisi

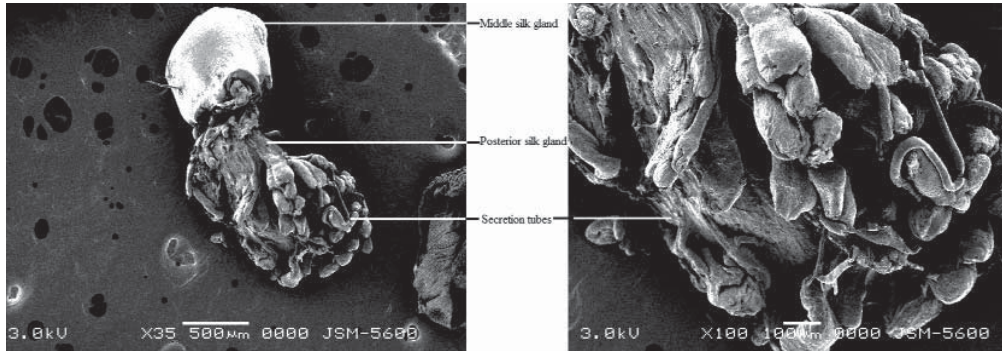


Fig 5. The morphology of silk gland in local silkworm breeds

Şekil 5. Yerli ipek-böceği ırklarında ipek bezi morfolojisi

Interestingly, there was a regular motif orientation on the Hatay Sarısı's and Bursa Beyazı's egg surface. The motif orientation on Alaca's egg surface was irregular. The studies on the silkworm eggs are limited. Keino and Takesue ¹² had studied morphological changes on the surface of eggs of the silkworm *Bombyx mori* L. during the early developmental stages by scanning electron microscopy.

In this study secretory tubes were observed on the posterior part of the silk gland. These tubes secrete the fibroin fibers and is surrounded by a liquid secretion ¹³. In the middle secretory portion, this secretion receives the sericin layers, which envelopes the fibroin fibers ¹⁴. Sasaki and Tashiro ⁵ had showed two microtubule systems in the posterior silk gland cells, a radial and a circular microtubule system.

This is the first morphological study of three Turkish silkworm breeds. The egg and silk morphology of Bursa Beyazı was different from other breeds. So the data which obtained from this study can be important for characterization of them. These preliminary results can be potentially utilized in improvement programmes of Turkish silkworm strains in future.

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Endocrine Cells in the Gastrointestinal Tract Mucosa of Zander (*Stizostedion lucioperca*)

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Summary

The distribution and relative frequency of endocrine cells in the gastrointestinal tract of the zander (*Stizostedion lucioperca*) were studied immunohistochemically using the peroxidase anti-peroxidase complex method. Our aim was to determine the distribution of specific immunoreactivities in some regions of digestive tract of the zander against the antisera Calcitonin gene related peptide (CGRP), Bombesin, Somatostatin-14, Secretin, Trk-A, Trk-B, Trk-C, Histamine, Neurotensin, Neuropeptide Y (NPY). The samples were taken from the stomach, intestine and pyloric caeca. Data were subjected to analysis of variance SPSS 10 statistical software programme and analysis of variance results were significant in the region of average density immunoreactive cell type were compared with ANOVA test. The immunoreactivities against all antisera were observed as intensively in the cells of the gland and less in the cells of Lamina epithelialis. The most intensive region of immunoreactive cells were determined as the fundus. Immunoreactivity was not observed in the nerves belonging to the myenteric plexus and muscle tissue and also ganglion cells.

Keywords: Zander, *Stizostedion lucioperca*, Endocrine cells, Gastrointestinal tract

Sudak (*Stizostedion lucioperca*) Gastrointestinal Kanal Mukozasındaki Endokrin Hücreler

Özet

Sudak balığı (*Stizostedion lucioperca*) gastrointestinal kanal endokrin hücrelerinin dağılım ve yoğunluğu peroksidaz anti peroksidaz immunohistokimyasal yöntemi kullanılarak çalışıldı. Spesifik antiserumların Calcitonin gene related peptide (CGRP), Bombesin, Somatostatin-14, Sekretin, Trk-A, Trk-B, Trk-C, Histamin, Neurotensin, Neuropeptide Y (NPY)'nin sudak balığı sindirim kanalının bazı bölgelerinde dağılımlarının belirlenmesi amaçlandı. Örnekler mide, bağırsak ve pilorik sekalaradan alındı. Veriler SPSS 10 istatistiksel yazılım programında varyans analizine tabi tutuldu ve varyans analizi sonucu önemli bulunan bölgelerdeki immunreaktif hücre tipi ortalama yoğunlukları ANOVA testi ile karşılaştırıldı. Immunoreaktivite gösteren hücrelerin bezlerdeki hücrelerde Lamina epitelyalisdeki hücrelere kıyasla daha yoğun olduğu gözlemlendi. En yoğun immunoreaktif hücrelerin midede yer aldığı belirlendi. Immunoreaktivite miyenterik sinir ağındaki hücrelerde, kas dokusunda ve gangliyon hücrelerinde gözlenmedi.

Anahtar sözcükler: Sudak, *Stizostedion lucioperca*, Endokrin hücre, Gastrointestinal kanal

INTRODUCTION

A large number of endocrine-type secretions are produced in the gastrointestinal tract mucosa of the fish. These secretions, which are called peptide and/or amine, are detected in lamina epithelialis, glands, several connective tissue cells, mucosal nerve ganglions ^{1,2}, and intermuscular nerve plexus ³. These secretions assure the mobility as well as the proper functioning of the

nervous system, regulation of the secretion through the cellular interaction ⁴, cellular proliferation ^{5,6}, regulation of the intestinal epithelium ⁷, and the contraction of the smooth muscles ⁸. Some researchers suggest that a relationship exists between the immune and endocrine systems ⁹ and that some secretions which belong to the endocrine cells may be effective in developing immunity

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against parasital diseases ¹⁰. Also, it is argued that some peptides (GLP-2) may be used in gastrointestinal region for related injuries ⁶.

In the studies which were conducted on mammals and fish ^{3,11,12}, endocrine cells in the digestive tract were observed to interact with each other as well as with the neurons, and thus leading to a complex system. In recent years, endocrine cells, which are identified in fish and which secrete peptide and/or amine hormones like gastrin, secretin, CCK (cholecystokinin), and serotonin containing pancreatic polipeptide, such as enteroglucagone, somatostatin, substance P, vasoactive intestinal peptide (VIP), bombesin, gastric inhibitory polypeptide (GIB), motilin have been reported to be found in a scattered manner in pancreas apart from the gastrointestinal mucosa ^{1,3,7,11,13}. In addition to all these traditional ones, such peptides as Trk-like proteins (TrkA-like, TrkB-like, TrkC-like), neuropeptide Y, calcitonin gene-related peptid (CGRP), neurotensin, metenkephalin are reported to be found in fish, as well. These peptides regulate the autonome nervous system as well as the activities of the digestive system in many respects ^{4,7,14-18}.

In this study, our aim was to determine the distribution and mucosal location of the cells, which contain CGRP, bombesin, somatostatin-14, sekretin, Trk-A, Trk-B, Trk-C, Histamine, NPY, neurotensin peptides, within the different sections of the gastrointestinal tract (cardia, fundus, pylorus, pyloric caeca, anterior and posterior intestine) of zander (*Stizostedion lucioperca*).

In this study, our aim was to determine the distribution and mucosal location of the cells, which contain CGRP, bombesin, somatostatin-14, sekretin, Trk-A, Trk-B, Trk-C, Histamine, NPY, neurotensin peptides, within the different sections of the gastrointestinal tract (cardia, fundus, pylorus, pyloric caeca, anterior and posterior intestine) of zander (*Stizostedion lucioperca*).

MATERIAL and METHODS

Stomach, pyloric caece and intestine of 30 zander (*Stizostedion lucioperca*) were used for this study. The samples which were taken from the esophagus, adjacent parts of the stomach with the intestine (cardia and pylorus), fundus region, pyloric caeca, anterior and posterior intestine were fixed in Bouin's solution for 12 h. The samples which were excised and processed with routine histologic tissue methods were blocked in paraffin. Peroxidase-antiperoxidase (PAP) complex method modified by Sternberger 19 was applied to the 6-7 micron-thick sections. In accordance with this method, the sections exposed to xylol and alcohol were washed and then waited in a hydrogen peroxide solution (0.3 % H₂O₂). The sections which were kept in normal goat serum (1:100) for 1 h were kept in antisera, the name and the dilutions of which are separately given in [Table 1](#), at +4°C' for 12-18 h. After the cleaning process with PBS (Phosphate buffered saline 0.01 M, pH 7.4), the sections were kept in anti-rabbit IgG solution (1:200) under room temperature for 1 h. In subsequent stages, they were washed with PBS and waited in PAP complex (1:400). The sections which were exposed to 3,3'-diaminobenzidine tetrahydrochloride (DAB) stain were treated with alcohols together with xylol, and then the slides were mounted entellan.

RESULTS

CGRP, Secretin, Trk-A immunoreactive (IR) cells in the lamina epithelialis of cardia were found to be distributed at medium density. On the other hand, Som-14, Bombesin Trk-B and Trk-C IR cells were observed to be less in number in proportion to other peptides. The cells which contain Histamine, NPY and Neurotensin were not detected in this region. Besides, Trk-A and CGRP IR cells found in the glands of cardia region were determined to

Table 1. Primary antisera used in this study

Tablo 1. Bu çalışmadaki primer antiserumlar

Antiserum	Item No	Dilution	Source
CGRP	sc-28920	1: 200	Santa Cruz Biotec. INC.
Bombesin	NCL-BOMp	1: 200	Nova Castra Lab. UK
Histamine	H 7403	1: 200	Sigma USA
Somatostatin-14	CL-SOMATOp	1: 200	Nova Castra Lab. UK
Sekretin	sc-20938	1: 200	Santa Cruz Biotec. INC.
Neurotensin	sc-20806	1: 200	Santa Cruz Biotec. INC.
NPY	sc-28943	1: 200	Santa Cruz Biotec. INC.
Trk-A	sc-118	1: 200	Santa Cruz Biotec. INC.
Trk-B	sc-12	1: 200	Santa Cruz Biotec. INC.
Trk-C	sc-117	1: 200	Santa Cruz Biotec. INC.

be densely populated. These were followed by Trk-C and Bombesin immunoreactive cells in number, respectively. However, Som-14, Secretin, Trk-B, Histamine NPY and Neurotensin immunoreactive cells were not detected.

With respect to the relative frequency in the lamina epithelialis of fundus region, NPY IR cells were observed to rank first and followed by Trk-C and Histamine IR cells in number, respectively. Except for the small number of Trk-B immunoreactive cells, the other peptides were determined to be at medium intensity. In fundus glands, we observed that no Trk-B IR cell was found but a small number of Trk-C and Neurotensin IR cells. The cells which proved immunoreactivity against the other antisera were at medium intensity.

Bombesin IR (Fig. 1) cells were observed to be highly dense in the lamina epithelialis of pylorus region, and this was followed by Trk-B IR cells. Apart from Trk-A and NPY IR cells which were found to be small in number in the pylorus region, the other peptides (Fig. 2) were at medium density, and there was no Neurotensin immunoreactive cell. In pylorus glands, however, Som-14 immunoreactive cells were determined to be dense, CGRP IR ones at medium density, while Bombesin, Secretin, Trk-A and Histamine cells were in small number (Fig. 2). We could not detect Trk-B, Trk-C, NPY, and Neurotensin IR cells.

Som-14 and Bombesin IR cells were found to be at medium density in the lamina epithelialis of the pyloric caeca region, while there was no Trk-B, Histamine and Neurotensin IR cell detected. Also, other IR cells were determined to be small in number. As for the density in the glands of pyloric caeca region, CGRP and Som-14 IR cells were determined to rank first (Fig. 3). Bombesin, and Histamine immunoreactive cells were found to be at medium density, while the cells which contain the other peptides couldn't be detected.

In the lamina epithelialis of the anterior intestine,

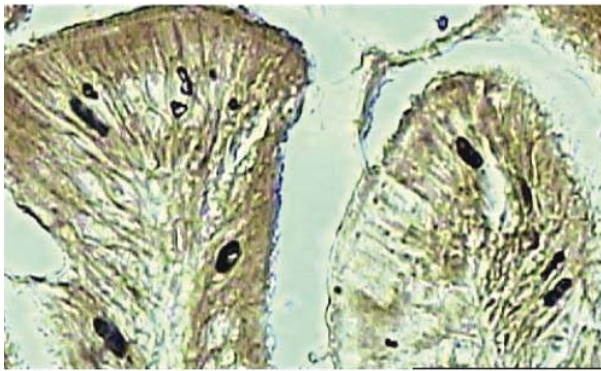


Fig 1. Bombesin IR cell Pylorus x400

Şekil 1. Bombesin IR hücre Pylorus x400

CGRP, Bombesin, Som-14, Sekretin, and Trk-A IR cells were found to be distributed at medium density. No immunoreactivity of the other peptides was detected. As for the case in the glands, the density of CGRP, Bombesin, Som-14, Sekretin, Trk-A, and Trk-C IR cells were found to be low, whereas no Trk-B, Histamine, NPY, and Neurotensin IR cell proved to exist.

In the lamina epithelialis of the posterior intestine, Sekretin IR cells were found at medium intensity, while CGRP, Bombesin, Som-14 (Fig. 3). Trk-A, Trk-C, and NPY immunoreactive cells were comparatively less denser.

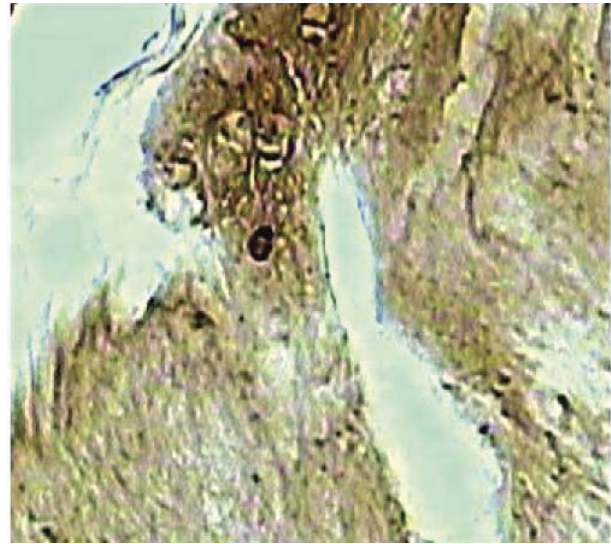


Fig 2. Somatostatin-14 IR cell Pylorus x400

Şekil 2. Somatostatin-14 IR hücre Pylorus. x400

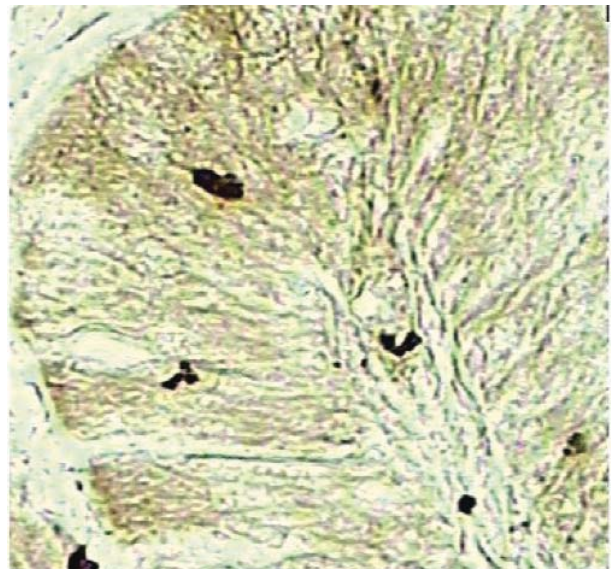


Fig 3. Somatostatin-14 IR cell Post. intes. x400

Şekil 3. Somatostatin-14 IR hücre Post. bağı. x400

Trk-B, Histamine, and Neurotensin immunoreactivities were not detected. With respect to the relative frequency in the glands, the first place was reserved by Sekretin IR cells, and these cells were followed by Som-14, CGRP and Bombesin IR cells in number. Trk-A, Trk-C, and NPY immunoreactive cells, on the other hand, were not observed in the glands.

In this study, a comparison between the cells, which contain 10 different peptides and which are found in 6 regions from which samples were taken, was made using the statistical analysis (ANOVA) (Table 2). It was observed that there was no difference when $P=0.000$ or $P<0.0000$ and that the peptides proved to be different in density when $P>0.000$. Accordingly, significant differences in density were observed to exist between the peptides in the glands and lamina epithelialis of the cardia and fundus regions as well as in the glands and the lamina epithelialis of the posterior intestine. Since $P=0.000$ in other regions, no remarkable difference was noted. As a result of the immunoreactive staining (PAP), the resulting reaction towards all the antisera was determined to prove weak.

DISCUSSION

As it is the case in fish, the endocrine cells in the gastrointestinal tract of the vertebrates were found to have very different types in related studies. At the same time, these cells were observed to vary in density in the different regions of the gastrointestinal tract²⁰⁻²².

In this study, CGRP immunoreactive cells *Anguilla anguilla*¹⁷ and *Salmo trutta*¹⁰ whose regional distribution and localization are to be determined, were reported to be densely distributed in the gastrointestinal tract. Besides, CGRP immunoreactivity was reported to exist in intestinal myenteric plexus nerve fibres and ganglion cells^{10,17,23}. In this study, however, no immunoreactivity has been detected in the mentioned regions. On the other hand, CGRP IR cells were found to have been localized in the epithelialis and glands of the stomach and intestine.

Bombesin is a peptide which is produced by ECs cells and found in mammals, amphibians, and gastrointestinal tract of fish¹¹. Endocrine function of bombesin regulates the secretion of gastric acid and its motility¹⁶. In a study conducted on *Amia calva*¹⁶ Bombesin immunoreactive cells were found to be densely distributed in the fundus region, which is in accordance with the findings of this study. While bombesin immunoreactive cells which are found in the fundus region of this species 16 were reported to have a strong immunostaining characteristic in the former study, these cells were observed, in this

study, to have a weaker immunostaining characteristic as it was the case with the other peptides.

Although Bombesin IR cells in the pyloric caeca and intestine of *Salmo trutta*¹¹ were found to be small in number, these cells proved to have a mean density in both epithel and glands of the mentioned regions..

Somatostatin is a polypeptide of 14 amino acids and has been originally isolated from the sheep hypothalamus²⁴. As it is the case in this study, somatostatin-14 immunoreactive cells were reported to be localized in the gastrointestinal tract of the species *Oncorhynchus mykiss*⁸, *Osteoglossomorpha*²⁵, *Pelteobagrus fulvidraca*, *Monopterus albus*, *Siniperca chuatsi*³, *Zacco platypus*²⁴, *Coreoperca herzi*²². These cells were also reported to be found in endocrine pancreas of some fish species^{3,13,25}. In this study, while these cells were observed to have a mean density in the epithelium and glands of the anterior intestine, they were found high in number in 7 different teleost fish species without stomach³. Pan et al.³, Domeneghini et al.¹⁷, Maake et al.²³ states that they observed somatostatin immunoreactivity in nerve plexus and fibres as well, whereas we could not detect this immunoreactivity in the mentioned regions.

Secretin is a polypeptide of 27 amino acids and a member of VIP/glucagon family²⁶. In this study, secretin IR cells were found in intestines and pyloric caeca in small number, which is in accordance with the findings of Dezfuli et al.¹¹ and Bosi et al.¹². On the other hand, these cells were found to exist neither in the stomach nor within the other mentioned regions of *Zacco platypus*²⁴ and *Coreoperca herzi*²² species.

Trk-like (A-B-C) proteins which are secreted by the cells making up the sub-population of the endocrine cells carry out the neurotrophin synthesis, amine and/or peptide storage⁴ as well as the regulation of the blood circulation of the gastrointestinal tract²³. The fact that Trk-like proteins (TrkA, TrkB, TrkC) are distributed throughout the intestines of the teleost fish was reported by Lucini et al.²⁷. Also, the same researchers stated that these immunoreactivities were found in the nerve fibres of the intestine myenteric plexus and within the ganglion cells. The existence of the cells which show this immunoreactivity in the stomach and intestine of the species *Pagrus major* and *Dentex dentex* has been proved²⁸. In a study conducted on different teleost species²⁷, Trk-A IR cells were found only in the intestines, Trk-B IR cells only in the stomach, and Trk-C IR cells both in the stomach and intestines. In this study, Trk-A IR cells were observed in all the studied regions, whereas Trk-B ones were detected in cardia, pylorus, and pyloric caeca. Trk-C immunoreactivity

Table 2. Anova test and the mean values of the immunoreactive cells in the lamina epithelialis and the glands of the regions studied
Tablo 2. Anova testi ile Lamina epitelyalis ve bezlerdeki immunoreaktif hücre yoğunluğu ortalama değerleri

Region	CGRP	Bombesin	Som-14	Sekretin	Trk-A	Trk-B	Trk-C	Histamine	NPY	Neurotensin	Anova (F)	P
Cardia Lam epit.	1.33±0.577	0.67±0.577	1.00±0.000	1.33±0.577	1.33±0.577	0.67±1.155	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	3.210	0.014
Cardia Gland	2.00±1.000	0.33±0.577	0.00±0.000	0.00±0.000	2.33±0.577	0.00±0.000	0.66±0.577	0.00±0.000	0.00±0.000	0.00±0.000	11.926	0.000
Fundus Lam epit.	1.33±1.528	0.67±0.577	1.33±0.577	1.67±0.577	1.33±1.528	0.67±1.155	2.00±1.000	2.00±1.000	4.00±1.000	1.00±1.000	3.111	0.017
Fundus Gland	2.33±1.527	2.00±1.000	2.66±0.577	2.33±0.577	2.00±1.000	0.00±0.000	1.00±1.000	2.00±1.000	2.00±1.000	1.00±1.000	2.206	0.068
Pylorus Lam epit.	1.33±0.577	4.00±1.000	1.33±0.577	1.33±0.577	0.33±0.577	2.00±1.000	1.00±0.000	1.00±0.000	0.67±0.577	0.00±0.000	9.990	0.000
Pylorus Gland	1.00±1.000	0.66±0.577	5.33±0.577	0.66±0.577	0.33±0.577	0.00±0.000	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000	29.514	0.000
Ant. Int. Lam epit.	1.00±0.000	1.67±0.577	0.67±0.577	1.00±1.000	1.67±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	7.333	0.000
Ant. Int. Gland	1.33±0.577	2.66±1.527	2.66±0.577	1.00±0.000	2.00±1.000	0.00±0.000	1.00±0.00	0.00±0.000	0.00±0.000	0.00±0.000	8.852	0.000
Post. Int. Lam epit.	0.67±0.577	1.00±1.000	1.33±0.577	2.00±1.000	0.33±0.577	0.00±0.000	1.00±1.000	0.00±0.000	1.00±1.000	0.00±0.000	2.637	0.034
Post. Int Gland	1.66±1.154	1.33±1.527	2.33±1.154	2.66±0.577	0.33±0.577	0.00±0.000	0.33±0.577	0.00±0.000	0.33±0.577	0.00±0.000	4.918	0.001
Pyl. Cae. Lam epit.	1.67±1.155	2.67±0.577	3.00±0.000	1.00±0.000	2.00±1.000	0.00±0.000	0.33±0.577	0.00±0.000	1.33±0.577	0.00±0.000	11.378	0.000
Pyl. Cae. Gland	3.00±1.000	2.00±1.000	3.00±1.000	0.00±0.000	2.66±0.577	0.66±0.577	0.00±0.000	1.33±0.577	0.00±0.000	0.00±0.000	12.741	0.000

was detected in the regions except pylorus gland, anterior intestine, lamina epithelialis, and pyloric caeca.

Histamine is a peptide which assures the smooth muscle contraction of the gastrointestinal tract and stimulates the stomach acid secretion²⁹. In accordance with the findings obtained from the studies on the species *Oncorhynchus mykiss*³⁰. Histamine IR was found to exist in the fundus and pylorus regions of the stomach. As far as the species *Oncorhynchus mykiss* that Fairgrieve et al.³⁰ fed with diets containing protein at different doses are concerned, they noted that the diets containing high doses of protein had increased the Histamine immunoreactivity in the samples. However, in this study, Histamine immunoreactive cells which have not been observed in the intestines were found to exist in the intestines of the species *Oncorhynchus mykiss*³¹, *Salmo salar*³², *Dicentrarchus labrax*³³. Histamine IR was determined to be much higher in density in the samples of the species *Oncorhynchus mykiss*³¹, *Salmo salar*³², and *Dicentrarchus labrax*³³, which carry parasites. In the parasite-carrying samples of the species *Salmo salar*, mast cells in the connective tissues of the intestine were noted to secrete a high amount of Histamine³². Once *Oncorhynchus mykiss* was given toxic substances, the Histamine immunoreactivity in the intestine was determined to increase³¹.

Neuropeptide Y (NPY) is a 36 amino acid peptide which is abundant and widespread in the central nervous system of all the vertebrates³⁴. In this study, these cells were observed in the epithel and glands of the fundus and posterior intestine. Similar findings were reported in previous studies, as well^{12,17,25}. Similar to the findings obtained from the studies on *Salmo trutta*¹⁰, a small amount of NPY IR cells were detected in the intestines in this study, as well. Rombout & Reinecke¹⁴ stated that the neurotensin immunoreactivity was found to exist densely in the intestines, myenteric plexus nerve fibres and ganglion cells of the species *Barbus conchoniensis*. In this study, however, these cells were not observed in the intestines but only in the fundus region. This immunoreactivity was not observed in Plexus myenteric nerve fibres and ganglion cells, either.

Certain difficulties were reported to appear in identifying the mucosal peptides of the digestive tract of fish. Rajjo et al.¹⁶ suggest that these peptides are long-structured in proportion to the mammals and that the determination of the peptide is difficult even if specific antiserum is applied. Besides, Larsson et al.³⁵ emphasize that apart from the the dilution, heterogeneity, and specificity of the antiserum used, the species should also be taken into consideration. Elbal and Agulleiro¹⁵ state that the difference induced by the terminal ends of the

antisera might be species-specific, whereas Holmgren and Nilsson³⁶ assert that this can be attributable to immunoreactive material difference in neuron and mucosal cells. This resulting interaction was defined as "Quasi Immunoreactivity" by Holmgren and Jönsson⁵. However, some researchers have argued that the positive results obtained in fish-related studies show a structural relationship with the mammals¹⁵ and that although they vary, amino acid arrangements of peptides may also show similarities³⁵. Similarly, Rajjo et al.¹⁶ reported that the anticipated innervations happened when antiserum-specific peptide was administered to the fish. Moreover, though the antisera used were obtained from the mammals, it was concluded that the peptides in the digestive tract of the mammals and fish have a similar nature due to the immunoreactivities detected.

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Kafkas Arısı (*Apis mellifera caucasica* Pollmann, 1889) (Hymenoptera: Apidae)'nın, Dış Yüzey ve Sindirim Sistemi Mikrofungus Florasının Belirlenmesi

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Özet

Bal arıları, ürünleri ve tozlaşmaya sağladıkları katkılar ile hem ülkemiz hem de dünyada büyük bir ekonomik öneme sahiptir. Bu nedenle arılar üzerinde birçok mikroflora çalışmaları yapıldı. Bu araştırmalar, önceleri sadece patojen izolasyonuna yönelik iken, günümüzde patojen olmayan flora üyelerinin ekolojik rolleri ve bunlardan nasıl yararlanılabileceği araştırılmaktadır. Bu çalışma, 2009 yılında Kars Platosunda, farklı istasyonlardan alınan Kafkas ırkı (*Apis mellifera caucasica* Pollmann, 1889), canlı ve ölü arı örneklerinin, vücut yüzeyleri ile sindirim sistemlerine ait mikrofungus florasının belirlenmesi amacı ile yapıldı. Araştırma sonucunda; *Penicillium*, *Alternaria*, *Mucor*, *Trichoderma*, *Fusarium*, *Aspergillus*, *Ulocladium*, *Verticillium* ve *Zythia* cinslerine ait toplam 13 farklı fungus türü teşhis edildi ve bu fungusların arılarla ilişkileri literatüre dayalı olarak tartışıldı.

Anahtar sözcükler: *Apis mellifera caucasica*, Kafkas arısı, Sindirim sistemi, Mikrofungus

Determining Microfungus Flora of Body Surface and Intestinal System of Caucasian Race Bees (*Apis mellifera caucasica* Pollmann, 1889) (Hymenoptera: Apidae)

Summary

Honey bees are economically important species to Turkey and many other regions of the world due to honey production and also vital pollinators for many species. Therefore, extensive microfloral honey bee research conducted in the literature. Early research was focused on the isolation of pathogen, however contemporary efforts are devoted to unveil ecological role of the non-pathogen members of flora and their potential ecological benefits. In the present study, we evaluated microfungus flora of digestion system and body surface of dead and alive Caucasian (*Apis mellifera caucasica* Pollmann, 1889) race bees collected from Kars Plateau in 2009. We identified 13 different species of fungi belonging *Penicillium*, *Alternaria*, *Mucor*, *Trichoderma*, *Fusarium*, *Aspergillus*, *Ulocladium*, *Verticillium*, and *Zythia* genuses. The relationship between these fungi and honey bees was discussed further in the light of the existing literature.

Keywords: *Apis mellifera caucasica*, Caucasian honey bee, Intestine system, Microfungus

GİRİŞ

Arılar, Hymenoptera takımında APOIDEA üst familyasının Apiformes grubunu oluşturan böceklerdir ¹. Yeryüzünde 25.000 kadar tanımlanmış arı türü bulunmak-

tadır ². Bal arısı (*Apis mellifera*) dışındaki türler yabancı arıları olarak bilinmektedir. Ancak *Apis mellifera* dışında yeryüzünde 10 civarında daha bal arısı türü mevcut olup



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bunlar Uzak Doğu ülkelerinde bulunmaktadır. Ülkemizin iklim koşulları, topoğrafik yapısı ve yeryüzündeki konumu, bitki örtüsünü ve buna bağlı olarak diğer canlıların çeşitliliğini artırdığı gibi, arı faunasının da çok zengin olmasına olanak sağlamıştır. Ülkemizde toplam 2.000 civarında arı türü olacağı tahmin edilmektedir ³. Kuzey Doğu Anadolu'da Apoidea ile ilgili sınırlı sayıda çalışma mevcuttur ⁴.

Bal arısı; bal, balmumu, arı sütü, arı zehiri ve propolis gibi ürünler üreterek insanoğluna sunarken, bunlardan daha da önemlisi yaban arıları ile birlikte allogamiye (karşı tozlaşmaya) gereksinim duyan kültür bitkilerinde tozlaşmayı gerçekleştirerek, ürünün nicelik ve nitelik yönünden üstün olmasını sağlar ⁵⁻⁷. Biyotik tozlaşma çeşitleri arasında en önemlisi ve yaygın entemogami (böceklerle tozlaşma) olup, bunun da büyük bir kısmını arıların gerçekleştirdiği bilinmektedir ⁸. Bal arıları tozlaşmaya olan katkıları yönünden ele alındığında dünya üzerinde yetiştiriciliği yapılan en değerli hayvanlar olup tarımsal üretime sağladıkları katkı, bal ve yan ürünlerinden sağladıkları katkıdan daha fazladır ⁹. Delaplane ve Mayer ¹⁰, bütün dünyada total olarak insan gıdasının %90'ının 82 bitki türünden elde edildiğini ve bunlardan 63 türün, yaklaşık %77'sinin tozlaştırıcısının da arılar olduğunu belirtmişlerdir. Arılar, tozlaştırma fonksiyonları ile biyolojik çeşitliliğin devamını sağlarken, erozyonun önlenmesi gibi, özellikle ülkemiz için hayati önem arz eden bir işlevi de yerine getirmektedirler. Ülkemizin Doğu ve Kuzeydoğu Anadolu Bölgelerinde arazi yapısı çok engebeli ve erozyona açık olup, bu bölgelerde başta *Fabaceae* olmak üzere *Asteraceae*, *Boraginaceae*, *Brassicaceae* ve *Campanulaceae* gibi familyalara ait bitki türlerinin yaygın olması, erozyonun önlenmesinde onları çok önemli kılmaktadır. Bu familyalara ait bitkilerin çoğunun, arıların tozlaştırması ile nesillerini devam ettirip, yayıldıkları bilinmektedir ³.

Kafkas arısı dünyada yaygın olarak yetiştiriciliği yapılan bal arısı ırklarından birisidir ¹¹. Kuzeydoğu Anadolu'nun özgün arısı olan Kafkas arısı, Türkiye bal arısı ırk ve ekotiplerinden biridir ¹²⁻¹³.

Apis mellifera ve onların besinleri; bakterileri, küfleri ve mayaları içeren mikroorganizma grupları ile birlikte bulunur. Arılar ve onların besinlerinden 6.000'in üzerinde mikroorganizma izole edilmiş ve tanımlanmıştır. Arılar üzerinden yapılan izolasyonlarda, bakterilerden daha çok fungus ve mayaların olduğu belirlenmiştir ¹⁴.

Doğan ve ark.¹⁵'nin, Benjamin ve ark.'dan aktardıklarına göre fungusların çoğu akar ve diğer eklembacaklı hayvanlarla değişik şekillerde simbiyotik ilişkiye sahiptirler. Bazen bu ilişki oldukça belirgin, bazen de sadece bir fungusun varlığını ortaya çıkarmak için böceklerin

mikroskopik incelenmesi, dikkatli diseksiyonu ve fungus bulaşmış organizmaların hayat döngüleri boyunca yapılan gözlemlere gereksinim duyulur. Bu ilişkiler yoluyla yayılabilen funguslar nekrotrof ve biyotrof parazittir. Diğer etkileşimlerde ise böcek, fungusu doğrudan besin olarak veya enzim kaynağı olarak kullanır. Bu funguslardan bazıları kendi çevrelerinde eklembacaklılar tarafından taşınır.

Arılardan izole edilen mikroorganizma türleri oldukça değişiklik göstermektedir. Polen ve nektar toplayan işçi arılardan izole edilen mikroorganizma sayısı ve çeşitliliği kovanda kalan bakıcı arılarından daha fazladır. Çünkü polen toplayan arıların vücutlarına, kovan dışındaki aktivitelerinden dolayı, kovan içerisindeki bakıcı arılardan daha fazla bulaşmaktadır. Larvalar bu mikroorganizmaları, kontamine olmuş besinleri yemek suretiyle alırlar. Yetişkin arılar ise; polenlerin tüketilmesiyle ve kolonideki diğer arılar ile besin değişimi sırasında bu florayı birbirlerine bulaştırırlar ¹⁴.

Fungal sporlar, arının depoladığı polenlerde, peteklerde, balda ve kovan içindeki bütün yüzeylerde bulunabilir. Sporlar yıllarca canlı kalabilir ve bu sporlar sürekli enfeksiyon kaynağı olabilirler ¹⁶⁻¹⁸.

Aspergillus P. Micheli ex Link, *Penicillium* Link ve *Mucor* Fresen türlerinin, arı larva ve prepupalarında sekonder istilacı oldukları bilinmektedir ¹⁹. Bu cinslere ait funguslar genellikle işçi arıların sindirim kanalında bulunur. *Penicillium glabrum* (Wehmer) Westling, *Penicillium aurantiogriseum* Dierckx, *Aspergillus flavus* Link, *Aspergillus niger* var. *niger* Tiegh bu cinslere ait sıklıkla izole edilen türlerdir. İşçi arıların bağırsaklarından izole edilen diğer funguslar ise *Cladosporium cladosporioides* (Fresen) G.A. de Vries ve *Alternaria tenuissima* (Kunze) Wiltshire'dır ¹⁴. Ayrıca *Penicillium*, *Aspergillus*, *Mucor* ve *Trichoderma* Pers cinslerine ait funguslar; kolonideki ölü arılar, petek, arı besini, bal ve polenler gibi bir çok yüzeyden izole edilmiştir ²⁰. Arı kovanlarından fungusların sıklıkla izole edilmeleri, kovan içindeki sıcaklık ve nem şartları ile yakından ilgilidir ²¹.

Benoit ve ark.²², bal arılarında zararlı akar olan *Varroa destructor*'un mikrofungus florasını araştırmış; bu floranın *Aspergillus*, *Penicillium*, *Fusarium* Link, *Trichoderma*, *Alternaria* Nees, *Rhizopus* Ehrenb ve *Mucor* gibi kozmopolit toprak saprofitlerinden oluştuğunu belirlemiştir. Muz ⁹'un aktardığına göre bal arılarında zararlı olan *Varroa destructor* akarı, arının hemolenfinden beslenmektedir. Bu esnada açtığı yaralar, arının vektörlüğünü yaptığı mikrobiyal patojenlere geçiş kolaylığı sağlar.

Özakın ve ark.²³ da, eski ve yeni petekleri bakteri-

yolojik ve mikolojik yönden incelemişler ve mikrofungus florası olarak; *Aspergillus fumigatus* Fresen, *Cladosporium corroni* ve *Penicillium spp.* türlerini izole etmişlerdir. Özkırım ve Keskin ²⁴, Ankara ili ve çevresinden aldıkları petek örneklerinden kireç hastalığı etkeni *Ascosphaera apis* (Maasen ex Claussen) ve taş hastalığının etmeni *Aspergillus flavus* türlerini izole etmişlerdir.

Fusarium, *Penicillium*, *Rhizopus* ve *Aspergillus* gibi cinslere ait bazı fungusların, zaman zaman kovandaki besinleri bozduğu ve besin kaynağının azalmasından dolayı doğal populasyonların sönmeye yol açtığı bildirilmektedir ²⁰.

Ülkemizde arı ürünleri; petek vs. üzerinden fungus izolasyon çalışmaları az miktarda da olsa yapılmış olup, arılar üzerinden ve özellikle de bölgesel öneme sahip olan Kafkas ırkı arılar üzerinden yeterli düzeyde mikoflora çalışması bulunmamaktadır. Bu çalışmada, Kars platosundaki farklı istasyonlardan alınan Kafkas ırkı arıların (*Apis mellifera caucasica* Pollmann, 1889), vücut yüzeyi ile sindirim sistemlerine ait mikrofungus florası belirlendi ve arı-fungus ilişkileri; entomopatojen, fitopatojen, parazitik ve antagonistik özellikler bakımından çeşitli yönleriyle literatüre dayalı olarak tartışıldı. Böylece arılar üzerinde yapılan sınırlı sayıda mikroflore çalışmaları, izole edilen fungus türleri ile katkı sağlanmaya çalışıldı.

MATERYAL ve METOT

Kafkas ırkına ait canlı ve kovan ön tahtasından toplanan ölü arılar, steril şartlarda laboratuara getirildi. Dış yüzey florası için arılar, içerisinde 5 ml steril fizyolojik su bulunan cam tüplere bırakılarak karıştırıldı ve bu karışımların her birinden, patates dekstroza agar (PDA) iktiva eden petri kaplarına ekim yapıldı ²⁵. Bağırsak florasının belirlenmesi için de cam tüplerden alınan arıların, yüzey sterilizasyonu sağlandı ve aseptik şartlarda diseksiyon yapılarak çıkarılan bağırsaklar homojenize edilerek, PDA besiyeri üzerine bırakıldı ²⁶. Petri ler 25°C'de aerobik şartlarda bir hafta inkübe edilerek izolasyon yapıldı. İzolatlardan koloni morfolojisi farklı olanlar saflaştırıldı. Arıların tür tanımları Kırpık ¹¹'e göre, gelişimlerini tamamlayan küf kolonileri de makroskopik ve mikroskopik yapıları incelenerek Hasenekoğlu ²⁷'e göre tanımlandı. İzole edilen ve teşhisi yapılan saf kültürler, PDA besiyeri içeren yatık agarlara alınarak 25°C'de bir haftalık inkübasyondan sonra +4°C'de saklandı.

BULGULAR

İzolasyonlar sonucunda arıların yüzeyleri ve bağırsaklarından toplam 167 koloni elde edildi. Bunlardan 39

tanisi maya olarak belirlenmiş ve çalışmaya dahil edilmedi. Mikrofunguslardan %22.6'sı *Penicillium*, %21'i *Alternaria*, %9.3'ü *Mucor*, %8.5'i *Trichoderma*, %5.4'ü *Fusarium*, %3.9'u *Aspergillus*, %1.5'i *Ulocladium* Preuss, %1.5'i *Verticillium* Nees, %1.5'i *Zythia* Fr. cinslerine ait toplam 13 farklı fungus türü izole edildi.

Tablo 1. Arılardan (*Apis mellifera caucasica*) izole edilen mikrofunguslar

Table 1. Microfungus isolated from bees (*Apis mellifera caucasica*)

İzole Edilen Funguslar	Sağlıklı Arı		Ölü Arı	
	Bağırsak Florası	Yüzey Florası	Bağırsak Florası	Yüzey Florası
<i>Alternaria alternata</i>	x	x	x	x
<i>Aspergillus flavus</i>	x	-	-	-
<i>Aspergillus fumigatus</i>	x	-	-	-
<i>Aspergillus niger</i>	-	x	x	-
<i>Fusarium sp.</i>	-	x	-	x
<i>Mucor circinelloides</i>	x	-	-	-
<i>Mucor sp.</i>	-	x	-	x
<i>Penicillium sp.</i>	x	x	x	x
<i>Trichoderma harzianum</i>	x	x	x	x
<i>Trichoderma koningii</i>	-	x	-	-
<i>Ulocladium consortiale</i>	-	x	-	-
<i>Verticillium sp.</i>	-	x	-	-
<i>Zythia sp.</i>	x	x	-	-
Steril fungus	x	x	x	x

Tablo1'de görüldüğü gibi; *Alternaria alternata* (Fr.) Keissl., *Trichoderma harzianum* Rifai ve *Penicillium sp.* hem sağlıklı hemde ölü arıların yüzeylerinden ve bağırsaklarından izole edildi. Diğer taraftan, *Ulocladium consortiale* Simmons, *Verticillium sp.* ve *Trichoderma koningii* Oudem sağlıklı arıların yüzeylerinden; *Mucor circinelloides* Tiegh, *Aspergillus fumigatus* Fresen ve arılarda taş çürüklüğü etmeni olarak bilinen *Aspergillus flavus* sağlıklı arıların bağırsaklarından, *Aspergillus niger* ise sağlıklı arıların yüzeyinden ve ölü arıların bağırsaklarından izole edildi. *Fusarium sp.* ve *Mucor sp.* sağlıklı ve ölü arıların yüzeylerinden; *Zythia sp.* ise sağlıklı arıların yüzeylerinden ve bağırsaklarından izole edildi.

TARTIŞMA ve SONUÇ

Bal arısı kolonilerinde *Aspergillus* ve *Penicillium* cinslerinin yaygın olduğu bilinmektedir ¹⁴. Çalışmada bu cinslere ait *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* ve *Penicillium sp.* türleri izole edilmiştir. *A. flavus* ile *A. fumigatus*, sadece sağlıklı arıların sindirim sistemlerinden izole edilmiştir. *A. niger*, sağlıklı arıların yüzeylerinden ve ölü arıların sindirim sistemlerinden izole edilmiştir. *Penicillium sp.* ise sağlıklı ve ölü arıların hem sindirim sistemlerinden hem de yüzeylerinden izole edilmiştir. Daha önceki çalışmalarda *A. niger*, işçi arıların sindirim sistemlerinden, *A. fumigatus* peteklerden izole

edilmiş; *A. flavus* ise, hem işçi arıların sindirim sistemlerinden hem de peteklerden izole edilmiştir ^{14,23,24}. Dört ayrı izolasyon çalışmasında da rastlanan *Penicillium* türlerinin ölü arı, kovan, polen, bal ve petekler gibi bir-çok yüzeyden izole edildiği bilinmektedir ²⁰. Bu verilere göre değerlendirdiğimizde; diğer çalışmalarda peteklerde ve kovanlarda rastlanılan türlere, bu çalışmada arıların yüzey florasında rastlanması, bal ve polenlerde rastlanan türlere ise arıların sindirim sistemlerinde rastlanması beklenen bir sonuçtur.

Mucor ve *Fusarium* türleri sağlıklı ve ölü arıların yüzey florasından izole edilmiştir. Sindirim sistemlerinde bu funguslara ait sporlara rastlanmamıştır. *Mucor* türleri; kovanlarda birçok yüzeyden, ölü arıların üzerinden, polenlerden ve arı larvalarından izole edilebilmektedir ²⁰. Glifiski ve Buczek ¹⁹, *Mucor* türlerini arılarda sekonder istilacı olarak tanımlamışlardır. Bu cinsin, toprak kaynaklı kozmopolit ve saprofit türleri içerdiği bilinmektedir ²⁷. Bu nedenle *Mucor* türlerinin, arıların yüzey florasında bulunması ve arıların ölümlünden sonra saprofitik gelişme göstermesi muhtemeldir.

Fusarium türlerinin ise kovanlardan izole edildiği bilinmektedir ²⁰. *Fusarium* türleri, tarımsal ürünlerde *Fusarium* solgunluğu olarak bilinen fungal hastalıklara neden olmaktadır ²⁸. Buna göre arıların, polen ve nektar toplamak için ziyaret ettikleri bitkilerden fungus sporlarını aldıkları ve aynı yolla kovana da taşımış olabilecekleri düşünülmektedir.

Trichoderma ve *Alternaria* türlerinin, bal arılarında parazit olan *Varroa destructor* akarından izole edildiği bildirilmiştir ²². Ayrıca *Trichoderma* türleri, *Aspergillus*, *Penicillium* ve *Mucor* türleri gibi, arı kolonilerinde sık rastlanılan türlerdendir ²⁰. Bazı fungusların, bitki zararlısı funguslar için antagonistik özelliklerinin olduğu bilinmektedir. *Trichoderma* türleri bitki gelişimini hızlandırdığı, bitki savunma mekanizmalarını stimüle ederek bitkileri toprak kaynaklı patojenlere karşı dirençli hale getirdiği ve çeşitli antibiyotik bileşikler ürettiği için biyolojik mücadelede tercih edilmekte ve aynı zamanda ticari olarak üretilen çok sayıda preparatı bulunmaktadır ²⁹⁻³². Kapongo ve ark.'nın ³³ aktardığına göre; bal arıları ve *Bombus* arıları, bitki patojenlerini baskılayan fungusların vektörü olarak etkili bir şekilde kullanılmıştır. Bu çalışmada izole edilen *Trichoderma koningii* ve *T. harzianum* türlerinden, *T. harzianum*, biyolojik kontrol ajanı olarak çilek, üzüm ve domates gibi kültür bitkilerinde kurşuni küf hastalığına sebep olan *Botrytis cinerea* Pers.'e karşı biyolojik mücadelede kullanılan antagonistik bir fungus olup, bal arıları ve bombuslar vektör olarak kullanılarak *T. harzianum*, hedef bitkiye ulaştırılmış ve önemli ölçüde verim artışı sağlanmıştır ³⁴⁻³⁶.

Toprakta ve çürümüş bitki materyali üzerinde bol miktarda bulunan fitopatojen fungus *Alternaria alternata* birçok bitkide "*alternaria kahverengi çürüklüğü*"ne sebep olmaktadır ³⁷. Çalışmamızda canlı ve ölü arıların hem yüzeylerinden hem de sindirim sistemlerinden *Alternaria alternata* izole edilmiştir. Bu durum *Alternaria alternata*'nın taşıyıcıları arasında bal arılarının da bulunduğunu düşündürmektedir.

Verticillium, çeşitli bitki ve böcek patojenlerini barındıran bir cinstir ^{38,39}. *Ulocladium consortiale*, domates meyvesinde *Alternaria*'nın yaptığına benzer siyah lekeler oluşturmaktadır ⁴⁰. *Zythia* ise, 40 kadar türü olan ve bu türler arasında bitki patojenleri olduğu bilinen bir cinstir. Örneğin *Gnomonia comari* Karst. çilek yapraklarında gri lezyonlar oluşturan bir fitopatojendir ⁴¹. *Zythia versoniana* (Sacc), nar bitkisinde meyve, dal ve gövde üzerinde enfeksiyonlara sebep olmaktadır ⁴². *Verticillium* sp. ve *Ulocladium consortiale*, sağlıklı arıların yüzeyinden; *Zythia* sp. ise, sağlıklı arıların hem yüzeyinden hem de sindirim sistemlerinden izole edilmiştir. Arılar üzerinde yapılan mikoflora çalışmaları arasında *Ulocladium*, *Verticillium* ve *Zythia* cinslerine ait herhangi bir izolasyona rastlanmamıştır. Fakat bu cinsler içerisinde bitki patojeni ve böcek patojeni olan türlerin bulunması, arılar üzerinde yapılacak çeşitli izolasyonlarda, bu cinslere ait funguslara rastlanabileceğini düşündürmektedir.

Dünyada, arılarla birlikte bulunan mikroorganizmaların ortaya çıkarılmasına ve bu mikroorganizmaların görevlerine yönelik birçok çalışma bulunmaktadır. Ancak bu araştırmalarda daha çok patojen ve parazitleri saf dışı ederek, arı ürünlerini arttırmak ve ayrıca arılar üzerinde patojen olmayıp, zararlıların biyolojik kontrolünde kullanılabilme potansiyeli olanların arılar aracılığıyla dağıtım amaçlanmıştır.

Bu çalışmada izole edilen funguslar arasında; arılarda taş hastalığına neden olan *Aspergillus flavus* ve *Aspergillus fumigatus* türleri ⁴³ hariç, arı patojeni olan fungus bulunmamaktadır. Dolayısıyla arı - fungus ilişkisi patojenite kavramı dışında diğer yönleriyle değerlendirilmeye çalışılmıştır. Arılar üzerinde herhangi bir zarar teşkil etmeyen ancak henüz tespit edilmediğini düşündüğümüz daha birçok mikroorganizmanın ortaya çıkarılmasına yönelik çalışmalara ihtiyaç vardır. Bu nedenle ülkemiz için önemli bir bölgesel değere sahip olan Kafkas ırkı arılarının, mikoflorasının tespitine ilişkin bir katkıda bulunulmaya çalışıldı.

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Salih Adası Civarındaki Kültür ve Doğal Deniz Balıklarındaki Monogenean Trematodlar ve Crustacean Parazitlerin Araştırılması ^[1]

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Özet

Bu araştırma Salih adası (Bodrum) civarında Şubat 2008 - Aralık 2008 tarihleri arasında gerçekleştirilmiştir. Çalışmada kültürü yapılan levrek, çipura, mırmır, fangri, sivriburun karagöz, minekop, sinağrit (*Dicentrarchus labrax*, *Sparus aurata*, *Lithognathus mormyrus*, *Pagrus pagrus*, *Diplodus puntazzo*, *Umbrina ciroa*, *Dentex dentex*) olmak üzere toplam 7 tür balık ile doğal ortamdan avlanan levrek, çipura, isparoz, karagöz, sargos, mırmır, izmarit, kupes, sarpa, mercan, tiryaki, güneş balığı, iskorpit, kaya balığı, dikenli çütre, tekir, istavrit, sarı kuyruk balığı (*Dicentrarchus labrax*, *Sparus aurata*, *Diplodus annularis*, *Diplodus vulgaris*, *Diplodus sargus*, *Lithognathus mormyrus*, *Spicara flexuosa*, *Boops boops*, *Sarpa salpa*, *Pagellus erythrinus*, *Uranoscopus scaber*, *Coris julis*, *Scorpaena scrofa*, *Gobius sp.*, *Stephanolepis diaspros*, *Mullus surmuletus*, *Trachurus mediterraneus*, *Seriola dumerili*) toplam 18 tür balık parazitolojik açıdan incelenmiştir. Kültür balıklarında *Microcotyle chrysophrii*, *Diplectanum aequans*, *Furnestinia echeneis*, *Lernanthropus kroyeri*, *Caligus minumus*, *Ceratothoa oestroides*, *Praniza larvası* parazitlerine rastlanılmıştır. Doğal balıklarda ise *Microcotyle chrysophrii*, *Microcotyle erythrini*, *Diplectanum aequans*, *Furnestinia echeneis*, *Cyclocotyle bellones*, *Choricotyle chrysophrii*, *Lernanthropus kroyeri*, *Hatschekia sargi*, *Hatschekia pygmaea*, *Caligus minumus*, *Ceratothoa oestroides*, *Praniza larvası* parazitleri bulunmuştur. Ayrıca çalışmada kafes sistemlerinde yetiştirilen 7 tür balıktan çipura, levrek ve mercana doğal ortamda da rastlanırken, 4 türe fangri, sivriburun karagöz, minekop ve sinağrit'e bu bölgenin doğal ortamında rastlanmamıştır. Hem doğada hem kültürel ortamda ortak bulunan 3 balık türüne ait parazitolojik sonuçlar istatistiksel olarak karşılaştırılmıştır. Doğal ve yetiştiricilik ortamındaki değerler istatistiki açıdan karşılaştırıldığında *Microcotyle chrysophrii* için Şubat ayında istatistiki fark önemli bulunurken diğer aylarda önemli bulunmamıştır. *Lernanthropus kroyeri* için Şubat, Ekim, Aralık aylarında fark anlamlı olurken Nisan, Haziran, Ağustos aylarında önemsiz bulunmuştur.

Anahtar sözcükler: Salih Adası, kültür balığı, Deniz balığı, Monogenean trematod, Crustacean parazit

Investigation of the Monogenean Trematods and Crustacean Parasites of the Cultered and Wild Marine Fishes near Salih Island

Summary

This investigation was realized between February and December 2008 near Salih Island (Bodrum). In this study, 7 species of culture fishes european seabass, gilt-head seabream, striped seabream, common seabream, shi drum, common dentex (*Dicentrarchus labrax*, *Sparus aurata*, *Lithognathus mormyrus*, *Pagrus pagrus*, *Diplodus puntazzo*, *Umbrina ciroa*, *Dentex dentex*) and 18 fish species caught from natural surroundings european seabass, gilt-head seabream, annular seabream, common two-banded seabream, white seabream, striped seabream, picarel, bogue, salema, common pandora, stargazer, rainbow wrasse, red scorpionfish, goby, lazenge filefish, striped red mullet, mediterranean horse-mackerel, great amberjack (*Dicentrarchus labrax*, *Sparus aurata*, *Diplodus annularis*, *Diplodus vulgaris*, *Diplodus sargus*, *Lithognathus mormyrus*, *Spicara flexuosa*, *Boops boops*, *Sarpa salpa*, *Pagellus erythrinus*, *Uranoscopus scaber*, *Coris julis*, *Scorpaena scrofa*, *Gobius sp.*, *Stephanolepis diaspros*, *Mullus surmuletus*, *Trachurus mediterraneus*, *Seriola dumerili*) were investigated in terms of parasite. *Microcotyle chrysophrii*, *Diplectanum aequans*, *Furnestinia echeneis*, *Lernanthropus kroyeri*, *Caligus minumus*, *Ceratothoa oestroides*, *Praniza* in culture fishes and *Microcotyle chrysophrii*, *Microcotyle erythrini*, *Diplectanum aequans*, *Furnestinia echeneis*, *Cyclocotyle bellones*, *Choricotyle chrysophrii*, *Lernanthropus kroyeri*, *Hatschekia sargi*, *Hatschekia pygmaea*, *Caligus minumus*, *Ceratothoa oestroides*, *Praniza* in natural fishes were found in wild fishes. The parasitological results belongs to three fish species found in both culture and naturel environment were compared statistically. No statistically signeficant differences were observed for *Microcotyle chrysophrii* for any month ($P>0.05$) between culture and wild fishes except in February ($P<0.05$). The statistically differences were determined for *Lernanthropus kroyeri* in February, October, December ($P<0.05$), while differences were not found in April, June, August ($P>0.05$).

Keywords: Salih Island, Culture fish, Wild fish, Monogenean trematod, Crustacean parasite



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GİRİŞ

Deniz kafes sistemlerinin olduğu ortamda doğal balıklarda yaşamakta bazen sürüler halinde bulunmaktadır. Yapılan çalışmalar Akdeniz Bölgesindeki çiftliklerin çevresinde doğal balıkların toplandığını, bu doğal balık türlerinin büyük bir çoğunluğunun clupeidae, sparidae, mugilidae ve carangidae familyalarına dahil olduğunu ortaya koymuştur. Araştırmacılar ayrıca ortamdaki bu balık türlerinin çiftliklere ve mevsimlere göre de farklılıklar gösterdiğini bildirmiştir ¹.

Deniz kafes sistemindeki balıklar ile çevresindeki doğal balıklar birbirleri ile etkileşim içindedirler. Bu etkileşimin en önemli sonuçlarından biri de patojen transferidir. Kültür stokları ile doğal balık stokları arasında hastalık etkenlerinin bulaşma şekli şimdiye kadar yeterli düzeyde araştırılmamış olup bu konuda birçok eksiklikler bulunmaktadır ². Deniz kafes sistemlerinde balıklar mükün olan en yüksek yoğunlukta yetiştirilmekte olup bundan dolayı büyük bir stres altındadırlar. Böyle durumlarda hastalık riski artar, hastalanan balıklardaki patojenler çevreye yayılır. Bazı durumlarda ise çevredeki balıklarda görülen patojenler kafes kültür sistemini etkiler. Doğal ve kültür balıkları arasındaki hastalık ilişkisi farklı coğrafik bölgelerde değişiklik göstermektedir ^{3,4}.

Ülkemizde deniz balığı yetiştiriciliği son 20 yılda çok büyük bir gelişme göstermiştir. FAO tarafından yapılan bir çalışma ile Türkiye'nin dünyada yetiştiricilik üretimi en hızlı büyüyen 3. ülke olduğu belirlenmiştir (FAO, 2007). Türkiye İstatistik Kurumunun 2008 yılı verilerine göre ülkemiz deniz balığı yetiştiriciliğinde yıllık 83 bin tonluk üretimi ile Akdeniz Ülkeleri arasında Yunanistan'dan sonra 2. ülke konumundadır. Sektörün bu hızlı gelişmesi sürecinde resmi otoriteler tarafından belli bölgeler potansiyel üretim alanı olarak belirlenmiştir. Bu bölgelerden biride balık çiftliklerinin yoğun olarak yer aldığı Muğla ilindeki Salih Adası'dır.

Dünyada olduğu gibi Akdeniz ülkelerinde de gerek doğal gerek kültür balıklarının parazitleri üzerinde çok çeşitli çalışmalar bulunmaktadır ⁵⁻⁸. Ülkemizde de son yıllarda doğal ve kültür balıklarının parazitleri üzerine çeşitli akademik tezler ⁹⁻¹¹ ve yayınlara ¹²⁻¹³ rastlanılmaktadır. Bu çalışmalar baz alınarak Öktemer ¹⁴ Türkiye'nin doğal deniz balıklarındaki, Kayış ve ark. ¹⁵ ise kültür balıklarındaki parazitleri bir checklist halinde bildirmişlerdir.

Bu çalışmada Türkiye'de deniz balığı yetiştiriciliğinde önemli bir bölge olan Salih Adası civarındaki yetiştiriciliği yapılan balık türleri ile bölgenin doğal balık türlerinin metazoon parazitlerinin (Monogenean Trematod, Crustacean parazit) araştırılması amaçlanmıştır. Ayrıca tespit edilen parazit türlerinin enfeksiyon yoğunluğu,

yüzdesi gibi parazitolojik parametrelerin belirlenmesi ve bu parametrelerin mevsimsel değişiminin incelenmesi ile doğada ve kültürel ortamda ortak bulunan balık türlerine ait parazitolojik sonuçların istatistiksel olarak karşılaştırılması da planlanmıştır.

MATERYAL ve METOT

Balık örnekleri ağırlıklı olarak Kılıç Deniz Ürünleri'nin kafes üniteleri olmak üzere Ada Su Ürünleri ve Fjord Marine Deniz Ürünleri'ne ait kafes sistemlerinden temin edildi. Doğal balık örnekleri ise Salih adası civarında galsama ağları, olta, sepet gibi çeşitli av araçları kullanılarak yakalandı. Bütün örnekler canlı olarak Kılıç Deniz Ürünleri'nin Balıklar Ar-Ge birimine getirildi ve inceleninceye kadar canlı olarak tutuldu. Örnekler Şubat 2008 - Aralık 2008 tarihleri arasında iki ayda bir olmak üzere (Şubat, Nisan, Haziran, Ağustos, Ekim, Aralık) bir yıl boyunca basit rastgele örnekleme yöntemiyle alındı. Getirilen bu balıklar klasik parazitolojik metodlar ^{16,17} kullanılarak incelendi. İncelenen balıkların tür teşhisinde ^{18,19}, parazitlerin teşhisinde ise ^{7,20-23} den yararlanıldı. Her parazit türü ile ilgili enfeksiyon değerleri ²⁴'e göre belirlenirken istatistiki analizler ANOVA'ya göre yapıldı.

BULGULAR

Bu çalışmada kafes ünitelerinde yetiştirilen 7 türe ait [çipura (*Sparus aurata*), levrek (*Dicentrarchus labrax*), mırmır (*Lithognathus mormyrus*), mercan (*Pagrus pagrus*), sivriburun karagöz (*Diplodus puntazzo*), minakop (*Umbrina ciroso*), sinarit (*Dentex dentex*)] toplam 864, doğal ortamdan avlanan 18 türe ait [levrek (*Dicentrarchus labrax*) çipura (*Sparus aurata*), isparoz (*Diplodus annularis*), karagöz (*Diplodus vulgaris*), sargos (*Diplodus sargus*), mırmır (*Lithognathus mormyrus*), izmarit (*Spicara flexuosa*), kupes (*Boops boops*), sarpa (*Sarpa salpa*), mercan (*Pagellus erythrinus*), tiryaki (*Uranoscopus scaber*), güneş balığı (*Coris julis*), iskorpit (*Scorpaena scrofa*), kaya balığı (*Gobius sp.*), dikenli çütre (*Stephanolepis diaspros*), tekir (*Mullus surmuletus*), istavrit (*Trachurus mediterraneus*), sarı kuyruk (*Seriola dumerili*)] 562 adet balıkta metazoon parazitler arandı. Bulunan parazit türleri, konakçıları ve enfeksiyon yüzdeleri Tablo 1'de görülmektedir. Şubat (19), Nisan (35), Haziran (22), Ağustos (18), Ekim (21), Aralık (30) olmak üzere incelenen toplam 145 adet mercan, Şubat (6), Nisan (5), Haziran (5), Ekim (4) olmak üzere incelenen toplam 20 adet sinaritte ve Şubat (5), Haziran (9), Ağustos (16), Ekim (15), Aralık (15) olmak üzere incelenen 61 adet minakopta herhangi bir metazoon parazite rastlanılmazken Şubat (4), Nisan (4), Haziran (5), Ağustos (3) olmak üzere toplam 16 adet sivriburun karagöz balığının yalnız Ağustos örnekle-

Tablo 1. Bulunan parazit türleri, konakçıları ve enfeksiyon değerleri**Table 1.** Parasite species, their host and infection values

Enfeksiyon Değerleri Parazit Türleri		Konakçı	Parazit Türü	İncelenen Balık Sayısı	Parazitli Balık Sayısı	Enfeksiyon Yüzdesi
DOĞAL	Monogenea	Microcotyle chrysophrii	Sparus aurata	287	136	47.39
		Diplectanum aequans	Dicentrarchus labrax	201	48	23.88
		Furnestinia echeneis	Sparus aurata	287	4	9.46
	Crustacea	Lernanthropus kroyeri	Dicentrarchus labrax	201	194	96.52
		Caligus minumus	Dicentrarchus labrax	201	3	1.49
		Ceratothoa oestroides	Sparus aurata	287	3	1.05
			Dicentrarchus labrax	201	1	0.50
			Lithognathus mormyrus	114	17	14.91
Diplodus puntazzo,	16		1	6.25		
KÜLTÜR	Monogenea	Microcotyle chrysophrii	Diplodus annularis	152	12	7.89
		Microcotyle erythrini	Lithognathus mormyrus	31	2	6.45
			Diplodus vulgaris	26	3	11.54
			Diplodus sargus	9	1	11.11
			Spicara flexuosa	27	1	3.70
			Boops boops	52	6	11.54
			Sarpa salpa	19	3	15.79
			Pagellus erythrinus,	47	2	4.26
			Dicentrarchus labrax	65	14	21.54
		Diplectanum aequans	Sparus aurataCoris julis	74	7	9.46
		Furnestinia echeneis	Boops boops	52	1	1.92
		Cyclocotyle bellones	Pagellus erythrinus	47	1	2.13
		Choricotyle chrysophrii	Dicentrarchus labrax	65	62	96.34
	Crustacea	Lernanthropus kroyeri	Diplodus annularis	152	2	1.32
		Hatschekia sargi	Diplodus sargus	9	2	22.22
			Coris julis	16	4	25
		Hatschekia pygmaea	Dicentrarchus labrax	65	3	4.62
		Caligus minumus	Sparus aurata	74	5	6.76
		Ceratothoa oestroides	Diplodus annularis	152	27	17.26
			Diplodus vulgaris	26	4	15.38
			Lithognathus mormyrus	31	1	3.23
			Pagellus erythrinus	47	1	2.13
			Dicentrarchus labrax	65	3	4.62
			Boops boops	52	2	3.85
			Sarpa salpa	19	2	10.53
			Coris julis	16	1	6.25
			Spicara flexuosa	27	3	11.11
			Sparus aurata	74	5	6.76
		Praniza larvası	Diplodus annularis	152	8	5.26
			Diplodus vulgaris,	26	7	26.92
			Lithognathus mormyrus	31	1	3.23
			Spicara flexuosa	27	4	14.81
			Pagellus erythrinus	47	2	4.26
			Coris julis	16	3	18.75
			Scorpaena scrofa	7	1	14.29
			Stephanolepis diaspros	8	1	12.50

mesinin 1 adedinde ağız içinde isopoda grubundan 2 adet anaç *Ceratothoa oestroides*'e rastlanıldı. Çipura, levrek ve mırmır balıklarında ise her örnekleme periyo-

dunda değişik tür parazitlere rastlanıldı. Bu balık türlerine ait parazit türlerinin enfeksiyonla ilgili bulgular ve bu bulguların mevsimsel değişimleri [Tablo 2, 3, 4](#)'te verildi.

Ay	Microcotyle chrysophrii		Furnestinia echeneis		Ceratothoa oestroides (yavru)		Praniza Larvası		Poliparazitizm	
	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes
Şubat	22 (7) 20 (1-5) %31.82 2.86	55 (37) 284 (1-35) %67.27 7.68			22 (2) 39 (2-37) %9.09 19.50	55 (1) 2 (2) %1.82 2	22 (1) 2 (2) %4.55 2.00		22 (10) %45.45 1 türle 9 2 türle 1	55 (37) %67.27 1 türle 36 2 türle 2
Nisan	8 (2) 7 (3-4) %25 3.50	57 (27) 107 (1-25) %47.37 3.96	8 (2) - %25 -	57 (4) - %7.02 -	8 (1) 5 (5) %12.50 5.00	57 (2) 3 (1-2) %3.51 1.50	8 (2) 4 (1-3) %25 2.00		8 (5) %62.50 1 türle 3 2 türle 2	57 (27) %47.37 1 türle 23 2 türle 2 3 türle 2
Haziran	17 (5) 14 (2-5) %29.41 2.80	59 (22) 65 (1-8) %37.29 2.95	17 (4) - %23.53 -		17 (2) 17 (3-14) %11.76 8.50				17 (7) %41.18 1 türle 3 2 türle 4	59 (22) %37.29 1 türle 22 -
Ağustos	9 (1) 3 (3) %11.11 3.00	33 (9) 20 (1-5) %27.27 2.22	9 (1) - %11.11 -				9 (2) 5 (1-4) %22.22 2.50		9 (3) %33.33 1 türle 2 2 türle 1	33 (9) %27.27 1 türle 9 -
Ekim	7 (2) 19 (2-17) %28.57 9.50	31 (16) 52 (1-6) %51.61 3.25							7 (2) %28.57 1 türle 1 2 türle 1	31 (16) %51.61 1 türle 16 -
Aralık	11 (3) 7 (1-4) %27.27 2.33	52 (30) 86 (1-7) %57.69 2.87							11(3) %27.27 1 türle 3 -	52(30) %57.69 1 türle 30 -
Toplam	74 (20) 70 (1-17) %27.03 3.50	287 (136) 614 (1-35) %47.39 4.51	74 (7) - %9.46 -	287 (4) - %1.39 -	74 (5) 61 (2-37) %6.76 12.20	287 (3) 5 (1-2) %1.05 1.67	74 (5) 11 (1-4) %6.76 2.20		74 (30) %40.54 1 türle 21 2 türle 9	287 (141) %47.39 1 türle 136 2 türle 3 3 türle 2

İ.B.S.: İncelenen Balık Sayısı

P.B.S.: Parazitli Balık Sayısı

T.P.S.: Toplam Parazit Sayısı

Çipura ve levrek balıklarında bazı parazit türlerin belli periyotlarda rastlanırken çipurada monogenetik trematodlardan *Microcotyle chrysophrii*'ye, levrekte ise copepodlardan *Lernanthopus kroyeri*'ye her periyotta rastlanıldı. Bu parazitlerin mevsimlere göre dağılımı istatistiki açıdan irdelendiğinde $P>0.05$ hesaplanarak istatistiki fark önemli bulunmadı.

Çipura balığında *Microcotyle chrysophrii*, levrek balığında ise *Lernanthopus kroyeri*'ye gerek doğal gerekse yetiştiricilik ortamında her mevsimde rastlanılmıştır. Bundan dolayı istatistik analizler bu iki tür üzerinde gerçekleştirilmiştir. Şekil 1'de çipura balığında görülen *Microcotyle chrysophrii*'nin ile levrek balığında görülen *Lernanthopus kroyeri*'nin doğal ve yetiştiricilik ortamında enfeksiyon yüzdesi, yoğunluğu ve abundans değerlerinin karşılaştırılması ve mevsimlere göre değişimleri gösterilmiştir.

Gerçekleştirilen istatistiki analizlerde gerek yetiştiricilik gerek doğal ortamdaki çipura balığında görülen *Microcotyle chrysophrii*'in mevsimler arasındaki değerlerinde (yetiştiricilikte $P=3.94$ $P>0.05$, doğal ortamda $P=0.43$ $P>0.05$) anlamlı bir fark bulunmamıştır.

Doğal ve yetiştiricilik ortamındaki değerler istatistiki açıdan karşılaştırılınca Şubat ($P=0.01$ $P<0.05$) ayında fark önemli bulunurken diğer aylarda Nisan ($P=0.51$ $P>0.05$), Haziran ($P=0.115$ $P>0.05$), Ağustos ($P=0.55$ $P>0.05$), Ekim ($P=0.43$ $P>0.05$), Aralık ($P=0.112$ $P>0.05$) önemli bir fark bulunmamıştır.

Gerek yetiştiricilik gerek doğal ortamdaki levrek balığında görülen *Lernanthopus kroyeri*'nin mevsimler arasındaki değerlerinde (yetiştiricilikte $P=0.42$ $P>0.05$, doğal ortamda $P=0.23$ $P>0.05$) anlamlı bir fark bulunmamıştır.

İ.B.S. (P.B.S.)
T.P.S. (Min-max)
Enfeksiyon yüzdesi
Enfeksiyon yoğunluğu

Tablo 3. Doğal ve kafes ortamındaki Levrekte (*Dicentrarchus labrax*) rastlanan parazitlerin enfeksiyon değerlerinin mevsimsel değişimi
Table 3. Seasonal variation in infection values of parasites species in cultured and wild sea bass (*Dicentrarchus labrax*)

İ.B.S. (P.B.S.)
Enfeksiyon yoğunluğu
1 türle
2 türle

Ay	<i>Lernanthropus kroyeri</i>		<i>Diplectanum aequans</i>		<i>Ceratothoa oestroides</i> (yavru)		Praniza Larvası		<i>Caligus minumus</i>		Poliparazitizm	
	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes
Şubat	9 (8) 142 (6-44) %88.89 17.75	33 (30) 293 (1-46) %90.91 9.77				33 (1) 2 (2) %3.03 2					9 (8) %88.89 1 türle 8	33 (30) %90.91 1 türle 29 2 türle 1
Nisan	17 (17) 294 (4-51) %100 17.29	42 (41) 384 (1-35) %97.62 9.37	17 (7) - %41.18 -	42 (19) - %45.24 -							17 (17) %100 1 türle 10 2 türle 7	42 (41) %97.62 1 türle 22 2 türle 19
Haziran	11 (11) 141 (2-22) %100 12.82	22 (22) 166 (2-16) %100 7.55	11 (4) - %36.36 -	22 (20) - %90.91 -	11 (1) 3 (3) %9.09 3						11 (11) %100 1 türle 6 2 türle 5	22 (22) %100 1 türle 2 2 türle 20
Ağustos	6 (6) 67 (3-22) %100 11.17	29 (26) 173 (1-18) %89.66 6.65		29 (9) - %31.04 -							6 (6) %100 1 türle 6	29 (27) %93.10 1 türle 19 2 türle 8
Ekim	14 (13) 149 (4-25) %92.86 11.46	47 (47) 378 (2-22) %100 8.04	14 (3) - %21.43 -		6 (2) 9 (2-7) %33.33 4.50					47 (3) 10 (1-5) %6.38 3.33	14 (13) %92.86 1 türle 9 2 türle 3 3 türle 1	47 (47) %100 1 türle 44 2 türle 3
Aralık	8 (8) 83 (3-17) %100 10.38	28 (28) 228 (2-16) %100 8.14					14 (3) 11 (2-6) %21.43 3.67				8 (8) %100 1 türle 5 2 türle 3	28 (28) %100 1 türle 28
Toplam	65 (63) 876 (2-51) %96.92 13.90	201 (194) 1622 (1-46) %96.52 8.36	65 (14) - %21.54	201 (48) - %23.88 -	65 (3) 12 (2-7) %4.62 4.00	201 (1) - %0.50 -	65 (3) 11 (2-6) %4.62 3.67			201 (3) 10 (1-5) %1.49 3.33	65 (63) %96.92 1 türle 44 2 türle 18 3 türle 1	(195) %97.01 1 türle 144 2 türle 51

İ.B.S.: İncelenen Balık Sayısı **P.B.S.:** Parazitli Balık Sayısı **T.P.S.:** Toplam Parazit Sayısı

Doğal ve yetiştiricilik ortamındaki değerler istatistiki açıdan karşılaştırılınca Şubat ($P=0.09$ $P>0.05$), Ekim ($P=0.086$ $P>0.05$), Aralık ($P=0.24$ $P>0.05$) aylarında anlamlı bir fark bulunmazken Nisan ($P=0.0017$ $P<0.05$), Haziran ($P=0.005$ $P<0.05$), Ağustos ($P=0.047$ $P<0.05$) aylarındaki fark önemli bulunmuştur.

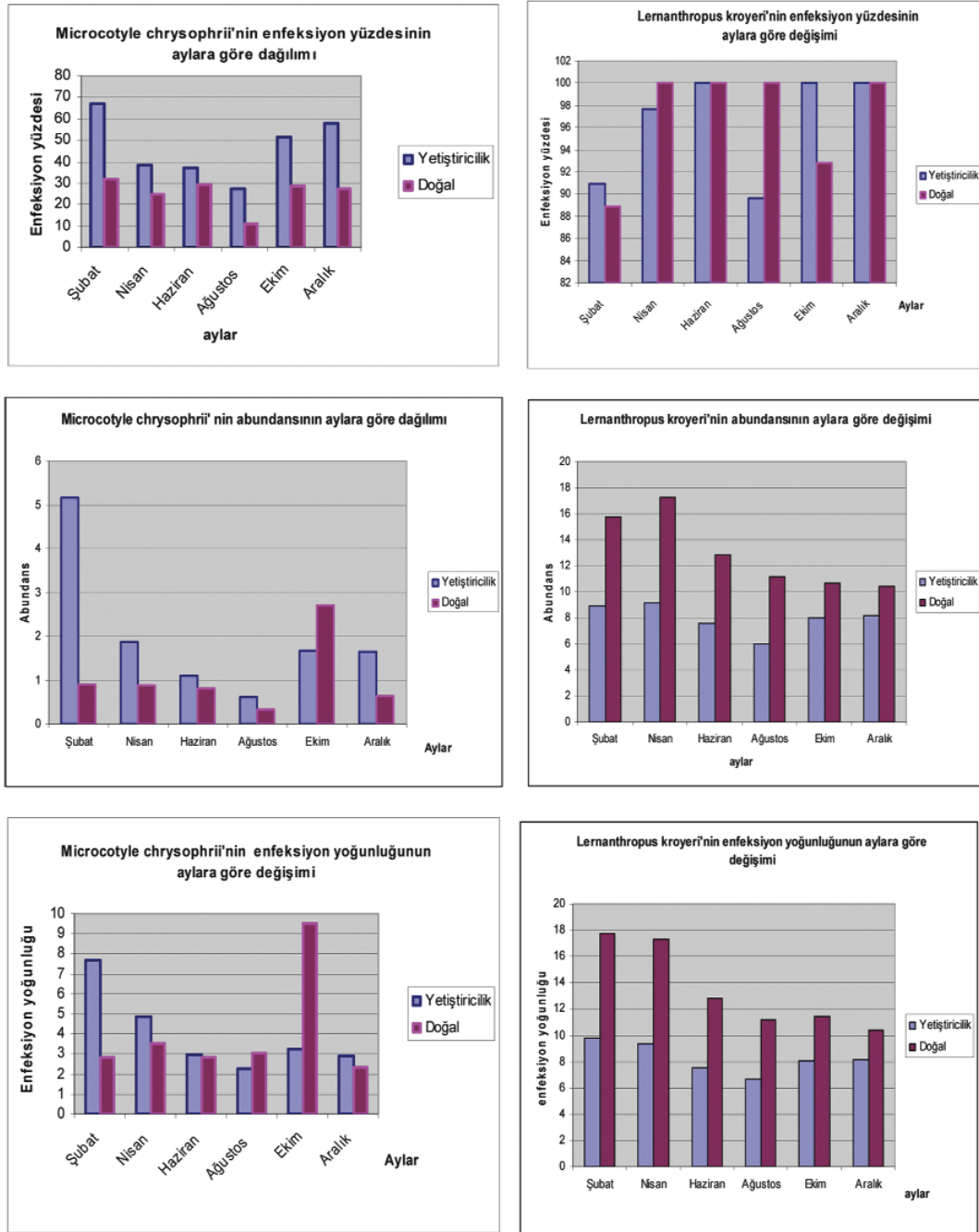
TARTIŞMA ve SONUÇ

Monogenetik trematodlar ve crustacean parazitler balıklarda ektoparazit olarak yaşamakta ve birbirlerine yakın olan coğrafik bölgelerde bile farklı enfeksiyon yoğunluk ve yüzdelerinde görülmektedir. Yunanistan'ın Ege Denizi kıyılarında çipura ve levrek balıkları ile yapılan bir çalışmada tür ayrımı yapmaksızın Monogenetik trematodların bir bölgede %70-90 enfeksiyon yüzdesinde

görülürken diğer bir bölgede aynı mevsimlerde %15-20 düzeylerinde olabildiğini göstermiştir²⁵.

Zararlı bir monogenean türü olan *Microcotyle chrysophryii* hem doğal hemde kültür çipuralarında sıklıkla rastlanılmaktadır. *Microcotyle chrysophryii*'nin enfeksiyon yoğunluğu 10 parazit/balık olduğu zaman balıklarda kondüsyon kaybına yol açmakta, bu enfeksiyon yoğunluğu arttıkça ölümler meydana gelmekte ve ölüm oranları %75-80'lere kadar çıkmaktadır²⁶. Bodrum Bölgesi kültür çipuralarında %50 enfeksiyon yüzdesi ve 5 enfeksiyon yoğunluğunda görülen bu parazit 35 enfeksiyon yoğunluğuna kadar çıkmaktadır. Doğal ortamda ise gerek enfeksiyon yüzdesi gerek enfeksiyon yoğunluğu daha düşük oranda görülmektedir.

Akdeniz Bölgesinde görülen isopod enfestasyon-



Şekil 1. Doğal ve yetiştiricilik ortamında *Microcotyle chrysophrii* ve *Lernanthropus kroyeri*'nin enfeksiyon değerlerinin mevsimsel değişimi

Fig 1. Seasonal variation in infection values of *Microcotyle chrysophrii* and *Lernanthropus kroyeri* in cultured and wild environment

larından *Ceratomyxa parallelus* ve *Ceratomyxa oestroides* türlerinin sorumlu olduğu belirtilmiştir^{27,28}. *C. parallelus* ve *C. oestroides* deri, solungaç ve ağızda bulunarak mekanik yaralanmalara ve strese sebep olur. *Ceratomyxa oestroides* ile parazitlenen levreklerin büyümesinin %7 civarında daha yavaş olduğu bildirilmiştir²⁹. Doğal or-

tamda genellikle kupes, mırmır gibi balıklar bu paraziti taşır ve pullu II safhasında kültür balıklarını enfekte eder. Bölgemizde bu parazite gerek kültür (levrek, mırmır) gerekse doğal balıklarda (kupes, levrek, mırmır, mercan, karagöz vs.) anaç ve larval safhada rastlanılmaktadır.

Ay	Microcotyle erythrini		Ceratothoa oestroides (anaç)		Ceratothoa oestroides (yavru)		Praniza Larvası		Poliparazitizm	
	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes	Doğal	Kafes
Şubat		19 (1) 3 (3) %5.26 3	4 (1) 2 (2) %25 2	19 (2) 2 (1) %10.53 2					4 (1) %25 1 türle 1	19 (3) %15.79 1 türle 3
Nisan	4 (1) 2 (2) %25 2	21 (1) 2 (2) %4.76 2		21 (6) 9 (1-2) %28.57 1.5	4 (1) 3 (3) %25 3	21 (3) 4 (1-2) %14.29 1.33			4 (2) %50 1 türle 2	21(9) %42.86 1 türle 8 2 türle 1
Haziran			7 (1) 1 (1) %14.29 1	25 (2) 4 (2) %8 2		25 (3) 8 (2-4) %12 2.67			7 (1) %14.29 1 türle 1	25 (4) %16 1 türle 3 2 türle 1
Ağustos				12 (2) 2 (1) %16.67 1		12 (1) 1 (1) %8.33 1				12 (3) %25 1 türle 3
Ekim	9 (1) 2 (2) %11.11 2			14 (3) 4 (1-2) %21.43 1.33		14 (1) 1 (1) % 7.14 1			9 (1) %11.11 1 türle 1	14 (4) %28.57 1 türle 4
Aralık		23 (1) 1 (1) %4.35 1	7 (1) 1 (1) %14.29 1	23 (2) 3 (1-2) %8.70 1.5		23 (1) 3 (3) %4.35 3	7 (1) 2 (2) %14.29 2		7 (2) %28.57 1 türle 2	23 (4) %17.39 1 türle 4
Toplam	31 (2) 4 (2) %6.45 2	114 (3) 6 (1-3) %2.63 2	31 (3) 4 (1-2) %9.68 1.33	114 (17) 25 (1-2) %14.91 1.47	31 (1) 3 (3) %3.23 3	114 (9) 17 (1-4) %7.89 1.89	31 (1) 2 (2) %3.23 2		31 (7) %22.58 1 türle 7	114 (27) %23.68 1 türle 25 2 türle 2

İ.B.S.: İncelenen Balık Sayısı **P.B.S.:** Parazitli Balık Sayısı **T.P.S.:** Toplam Parazit Sayısı

Levrek balıkları için spesifik bir parazit türü olan *Lernanthropus kroyeri* bölgede kültür (%96.52) ve doğal (%96.92) ortamda yüksek enfeksiyon yüzdesinde her mevsimde bulunmuştur. Bu parazit balıkların büyümesinin yavaşlamasına sebep olur. İso pod ve Monogenetik trematodlar ile miks bir enfestasyonda ölümler görülebilir ²⁵.

Çalışmamızda doğal balıklarda (levrek, çipura, mırmır, karagöz vs.) bulunmasına rağmen kültür sisteminde rastlayamadığımız praniza larvaları solungaç ve ağız boşluğunda çok sayıda görülebilir ve bu deniz balıkları için bir risk olabilir ³⁰.

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Hormonal Cinsiyet Dönüşüm Metodu Kullanarak Monoseks Gökkuşığı Alabalığı (*Oncorhynchus mykiss*) Üretimi

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Özet

Bu çalışmada, dolaylı hormonal cinsiyet dönüşüm metoduyla tamamı dişi gökkuşığı alabalığı (*Oncorhynchus mykiss*) yetiştirmekte kullanılan erkekleştirilmiş dişi anaçların oluşturulması için uygun hormon protokolü araştırılmıştır. Bu amaçla anaç olarak kullanılacak larvalar ilk yem alma aşamasından itibaren 600 gün-derece süresince kilogramında 1, 2 ve 3 mg 17α -metiltestosteron (MT) içeren yemlerle beslenmişlerdir. Ya da, değişik yaşlardaki keseli larvalar tek ve 48 saat aralıklarla 2 ile 6 adet ikişer saatlik 0.5 mg l⁻¹ MT banyosuna tabi tutulmuşlardır. Kontrol grubuna ise hormon verilmemiştir. Oral MT uygulamalarının her üç dozu da tamamı erkek veya tamamına yakını erkek gökkuşığı alabalığı üretmekte etkili bulunurken, yapılan banyo uygulamalarının tamamı benzer başarıyı yakalayamamış ve çoğu sadece az sayıda interseks bireyin oluşmasına sebep olmuştur. Deneme balıkları 16 aylıkken yapılan kontroller oral yolla yüksek dozda MT verilen balıkların düşük dozda MT verilen balıklara göre daha düşük oranda kanallı fonksiyonel erkeklerden oluştuğunu göstermiştir. Ayrıca yüksek MT dozunun gonad büyüklüğünü olumsuz etkilediği tespit edilmiştir. Sonuç olarak çalışma, gökkuşığı alabalığında erkekleştirilmiş dişi anaçların elde edilmesinde oral MT uygulamalarının daha başarılı ve istikrarlı sonuçlar ürettiğini göstermiştir. Yüksek MT dozlarının hem sperm kanalı gelişimini hem de gonad gelişimini olumsuz yönde etkileyebileceğini ortaya koymuştur. Ayrıca farklı su sıcaklıklarında larvaların maruz kaldığı gerçek MT dozunun değişen metabolik hız dolayısıyla farklı olabileceğini işaret etmiştir.

Anahtar sözcükler: Gökkuşığı alabalığı, *Oncorhynchus mykiss*, Hormonal cinsiyet dönüşüm metodu, 17α -metiltestosteron

Monosex Rainbow Trout (*Oncorhynchus mykiss*) Production by Means of Hormonal Sex Reversal Method

Summary

In this study, efficient hormone protocol for establishment of masculinized female brood stock, which was used in monosex female rainbow trout (*Oncorhynchus mykiss*) production, was investigated. Starting from first feeding, larva that was going to be used as brooders fed formula diets containing 1, 2 and 3 mg 17α -methyltestosterone (MT) for 600 degree-days. Otherwise, sac fry at various ages were immersed once and 2 to 6 times into 0.5 mg l⁻¹ MT baths for 2 h with 48 h intervals. While oral MT treatments were effective to produce all or almost all male populations at all 3 dosages, none of the bath treatments achieved a similar success and most yielded only a few intersex individuals. Control of oral administration fish at 16 months of age showed that fish masculinized by giving the highest MT dosage contained lower ratio of functional males with complete sperm ducts than fish masculinized by giving the lowest MT dosage. Additionally, high MT dosage was determined to have adverse effect on gonad size. In conclusion, the results of the study showed that oral MT administrations produce more successful and consistent results for the production of masculinized female brooders. They demonstrated that high MT dosages can adversely affect both sperm duct development and gonad size in sex reversed fish. Furthermore, it was discussed that the actual dosages of MT that larva were exposed could vary at different water temperatures depending on variable metabolic rates.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, Hormonal sex reversal, 17α -methyltestosterone



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GİRİŞ

Hızla artan dünya nüfusunun besin ihtiyacını karşılamak, var olan sınırlı kaynakların sürdürülebilir kullanımını gerektirmektedir. Bu sebeple dünyanın pek çok gelişmiş ülkesi tarımda, hayvancılıkta ve su ürünleri yetiştiriciliğinde kaynak kullanımını artırmadan üretim verimini artırmak amacıyla biyoteknolojik metotlar kullanılmaktadır. Örneğin ülkemizde entegre üretimi yapılan tek tatlısu balığı olan gökkuşağı alabalığı, *Oncorhynchus mykiss* Walbaum 1792, yetiştiriciliğinde çoğu gelişmiş ülke üretimin tamamını monoseks dişi popülasyonlar kullanarak yapmaktadır ^{1,2}. Çünkü gökkuşağı alabalığı erkekleri birinci yaş içerisinde seksüel olgunluğa erişmeye başlarlar. Erginleşmeyle birlikte besinlerle alınan enerjinin büyük bir kısmı büyüme yerine gonad gelişimine yönlendirildiğinden, erkeklerde büyüme yavaşlar, kaslarda depolanan glikojen miktarının azalmasına paralel olarak et kalitesi düşer ¹. Monoseks dişi alabalık yumurtasını yurt dışından getirebilen büyük üreticiler, Kayseri, Sivas ve Fethiye-Muğla civarındaki bazı işletmeler dışında, ülkemizde çoğu üretici monoseks kültür yapmaktadır ³. Zira ülkemizde gökkuşağı alabalığı ortalama 250 gr ağırlıkta pazarlanmakta ve bu ağırlığa 8-12 aylık sürede ulaşabilmektedir. Fakat özellikle üreme sezonuna yakın dönem olan Sonbahar başlarında yapılan satışlarda, gonadların gelişmesine paralel olarak ikincil cinsiyet karakterleri belirginleşen (renkleri koyulaşmaya ve çeneleri uzamaya başlayan) erkekler kolaylıkla ayırt edilebilmekte ve bu durum üreticilerin bu dönemde pazarlama sorunları yaşamasına sebep olmaktadır. Bunun yanında, son yıllarda Karadeniz’de ve içsularda ağ kafeslerde yetiştirilen ve Salmon olarak pazarlanan kiloluk ve üzeri gökkuşağı alabalığı yetiştiriciliği giderek artmaktadır. Kiloluk veya daha büyük gökkuşağı alabalığı üretiminin ekonomik olabilmesi ise biraz önce belirtilen sebepten dolayı ancak monoseks kültür teknolojisinin uygulanması ile mümkündür.

Androgenez, ginogenez, hibritleştirme, elle seçim gibi çeşitli monoseks üretim tekniklerine göre ekonomik olması, yüksek teknoloji ve uzmanlık gerektirmemesi veya kendileşme gibi istenmeyen genetik yan etkilerinin olmaması nedeniyle balık yetiştiriciliğinde en çok tercih edilen monoseks popülasyon üretim tekniği hormonal cinsiyet dönüşüm (hormonal sex reversal) metodu olmuştur ^{2,4-7}. Balıkların esnek (çevresel ve hormonal faktörlerin etkisine açık) cinsiyet değişim mekanizmalarından ⁸ faydalanan bu teknik, direkt ve dolaylı olmak üzere iki farklı şekilde uygulanır. Direkt uygulamada, embriyonik veya larval gelişimin erken evrelerinde, gonadların somatik büyümesi sırasında, sitolojik değişiminden (primordial üreme hücrelerinin oogonia veya spermatogonia’ya dönüşümünden) önce, banyo veya ağız

yoluyla verilen cinsiyet hormonları vasıtasıyla gonadal cinsiyetin yönü, genetik cinsiyet belirleme mekanizmasının aksi yönünde değiştirilir. Ekonomik balık türlerinin yetiştiriciliğinde ise hormonal cinsiyet dönüşüm metodu genelde dolaylı olarak uygulanır ^{2,7}. Çünkü dolaylı uygulamada her yeni jenerasyonun üretimi için hormon kullanılması gerektirmedikinden direkt uygulamaya göre daha az çevresel etki oluşmakta ve pazara sunulan ürün üzerinde hiç hormon kullanılmamış olması dolayısıyla olası tüketici tepkilerinden kaçınılabilmektedir. Dolaylı hormonal cinsiyet dönüşüm uygulamasında, hormonlarla cinsiyeti değiştirilmiş bireyler (genotipik ve fenotipik cinsiyeti farklı bireyler) anaç olarak kullanılarak monoseks popülasyonlar üretilir ^{2,7}. Örneğin insandaki gibi dişi homogametik (XX dişi/XY erkek) cinsiyet belirleme mekanizmasına sahip olan gökkuşağı alabalığında tamamı dişi popülasyonlar üretmek için keseli larvalara banyo yoluyla ⁹ veya keseyi tüketip serbest yüzmeye başlayan larvalara ağız yoluyla ¹ arzu edilen cinsiyete değil, karşı cinsiyete ait hormonlar verilerek önce gonadal (fenotipik) cinsiyeti değiştirilmiş bireyler (XX erkekleri ya da erkekleştirilmiş dişiler) oluşturulur. Normal dişilerden (XX dişileri) elde edilen yumurtaların, bu erkekleştirilmiş dişilerden elde edilen ve yalnızca dişilik kromozomu (X) taşıyan spermle döllenmesi yoluyla da tamamı dişi popülasyonlar üretilir ².

Ülkemiz alabalık üreticilerinin tamamı dişi üretim teknolojisini uygulaması, sonbahar aylarında porsiyonluk satışlarında yaşanan pazarlama sıkıntılarının önlenmesi yanında kiloluk veya daha büyük alabalık üretiminde verimin artırılmasına yardımcı olacaktır. Ayrıca, yetiştiricilerimizin tamamı dişi alabalık yumurtası için yurt dışı kaynaklara bağımlılığının, bu yolla yurt dışına döviz akışının ve yurt dışından getirilen yumurtalardan kaynaklanabilecek hastalık tehlikesinin ortadan kaldırılmasına da olanak sağlayacaktır. İşte bu öngörüler doğrultusunda, tamamı dişi gökkuşağı alabalığı üretim teknolojisini ülkemiz üreticilerinin yaygın bir şekilde kullanımlarına yardımcı olmak amacıyla İstanbul Üniversitesi, Su Ürünleri Fakültesi, Sapanca İçsu Ürünleri Üretimi Araştırma ve Uygulama Birimi’nde, Muğla Üniversitesi, Su Ürünleri Fakültesi işbirliğiyle 2007-2008 üretim döneminden itibaren çalışmalara başlanılmıştır. Bu çalışmalarda dolaylı hormonal cinsiyet dönüşüm metoduyla tamamı dişi gökkuşağı alabalığı üretmek için gerekli olan erkekleştirilmiş dişi anaçların oluşturulması için uygun hormon protokolü araştırılmıştır. Bu raporla, birimizde gerçekleştirilen bu ön çalışmaların sonuçları paylaşılmaktadır.

MATERYAL ve METOT

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gerçekleştirilen bu çalışmada, birimde üretilen yumurtadan yeni çıkmış, besin kesesini tüketmek üzere veya tüketmiş gökkuşağı alabalığı larvaları kullanılmıştır. Larvalara sentetik erkeklik hormonu olan 17 α -metil-testosteron (MT, Sigma M 7252) oral yolla ve banyo yolu ile verilmiştir (Tablo 1). Oral uygulamalarda, larvalar ilk yem alma aşamasından itibaren 600 gün-derece süresince kilogramında 1, 2 ve 3 mg MT içeren yemlerle beslenilmiştir. Banyo uygulamalarında, değişik yaşlardaki keseli larvalara tek ve 48 saat aralıklarla 2 ile 6 adet ikişer saatlik 0.5 mg l⁻¹ MT banyosu verilmiştir. Kontrol grubuna banyo veya oral yol ile hormon verilmemiştir.

daki hormonlar uzaklaştırılmıştır. Hormon uygulamaları sonrası yavrular gonad gelişiminin tespit edilebileceği büyüklüklere (100 g üzeri) ulaşınca kadar 16 m²'lik açık alan beton havuzlarda bakılmışlardır.

Hormon uygulamalarından bir sonraki üreme sezonunda, gonad gelişiminin tamamlandığı bilinen 10. ay sonrasında yeterli büyüklüklere ulaşan deneme balıkları, hormon uygulamalarının gonad gelişimi üzerindeki etkisini değerlendirmek üzere örneklenmiştir. Örneklemelerde, uygulamaların her tekrarından rasgele olarak 50-120 adet balık alınmıştır. Örneklenen balıkların önce

Tablo 1. 2007 ve 2008 yıllarında gökkuşağı alabalığında fenotipik cinsiyeti yönlendirmede kullanılan 17 α -metiltestosteron (MT) uygulamaları (N=1000)

Table 1. 17 α -methyltestosterone (MT) treatments given in 2007 and 2008 in order to change the direction of sex differentiation in rainbow trout (N=1000)

Uygulama Yılı ve Şekli	Uygulama	Uygulama Başlangıcındaki Larval Gelişim Aşaması
2007 Kontrol	-	-
2007 Oral	600 gün-derece boyunca 1 mg MT kg ⁻¹ yem 600 gün-derece boyunca 2 mg MT kg ⁻¹ yem	Yeme yeni kalkan Yeme yeni kalkan
2007 Banyo	48 saat aralı, 2 saatlik 3 periyodik 0.5 mg l ⁻¹ MT banyosu 48 saat aralı, 2 saatlik 6 periyodik 0.5 mg l ⁻¹ MT banyosu	410 gün-derece 410 gün-derece
2008 Kontrol	-	-
2008 Oral	600 gün-derece boyunca 3 mg MT kg ⁻¹ yem	Yeme yeni kalkan
2008 Banyo	2 saatlik tek 0.5 mg l ⁻¹ MT banyosu 2 saatlik tek 0.5 mg l ⁻¹ MT banyosu 2 saatlik tek 0.5 mg l ⁻¹ MT banyosu 48 saat aralı, 2 saatlik 2 periyodik 0.5 mg l ⁻¹ MT banyosu 48 saat aralı, 2 saatlik 2 periyodik 0.5 mg l ⁻¹ MT banyosu	480 gün-derece 550 gün-derece 620 gün-derece 550 gün-derece 620 gün-derece

Çalışmada kullanılan MT hassas teraziyle tartıldıktan sonra, %96'lık etil alkol içerisinde çözündürülerek stok solüsyonları (1 mg/ml) hazırlanmıştır. Stok solüsyonlar uygulamalar boyunca koyu renkli örnek şişelerinde +4°C de saklanmıştır. Oral uygulamalarda, stok solüsyondan alınan hormonun yeme homojen bir şekilde karışmasını sağlamak için her kilogram yeme katılması gereken miktar, 250 ml, %96'lık etil alkol içerisinde seyreltildikten sonra sürekli karıştırarak küçük hacimlerde yeme eklenmiştir. Bu işlem sonrasında nemlenen 300, 500, 700, 1000, 1200 μ m alabalık yavru yemleri (sırasıyla %57-50 ham protein, Trouw Nutrition TR) havadar ve direkt güneş ışığı almayan, loş bir yerde 24 saat havalandırılarak içerisindeki alkolden ve nemden arındırılmıştır. İçerisindeki alkol uçurulan ve iyice kuruyan yemler kapaklı yem kovaları içerisinde serin ve loş bir alanda korunmuştur.

Banyo uygulamaları larvalara Kaliforniya tipi kuluçka kanallarında verilmiştir. Kanallardaki su hacmi litrede 20 larva olacak şekilde ayarlanmış ve banyo süresince su giriş vanaları kapatılmıştır. İki saatlik banyo süresi sonunda ise, vanalar açılarak su değişimi yoluyla kanal suyun-

abdominal masaj (sağım) yoluyla sperm verip vermediği kontrol edildikten sonra, balıklar yüksek dozda anestetik (2-phenoxyethanol) ile öldürülerek, ağırlıkları 0.1 g ve toplam boyları 1 mm hassasiyetle ölçülmüştür. Daha sonra, bir bistüri yardımıyla abdomenleri açılarak, gonadları bir bütün halde çıkarılmış ve %10'luk nötralize edilmiş formalin içerisinde fikse edilmiştir.

Fikse edilen gonad örneklerinden fast green boyası ve gonadal-ezme metodu kullanılarak yaş preparatlar hazırlanmıştır¹⁰. Yaş preparatların mikroskopik inceleme-siyle gonadlar gözlemlenen hücre tiplerine ve iç morfolojik özelliklerine göre dişi, erkek veya interseks olarak sınıflandırılmıştır.

İki bin yedi yılı oral MT ve kontrol uygulamalarında, örneklemelerden arta kalan balıklar sperm kanalı gelişimi ve cinsiyet dönüşümünün işlevselliğinin değerlendirilebilmesi için büyütülmeye devam edilmişlerdir. Bu balıklardan birinci yaşlarındaki üreme sezonu sonunda (16. ayda), sağım yoluyla sperm elde edilmeye çalışılarak, kanallı (fonksiyonel) erkek bireylerin oranları tes-

pit edilmiştir. Sağımla süt veren ve vermeyen erkeklerden onar adedi yüksek dozda anestetik (2-phenoxyethanol) ile öldürülerek, ağırlıkları 0.1 g ve toplam boyları 1 mm hassasiyetle ölçülmüştür. Daha sonra, bir bistüri yardımıyla abdomenleri açılarak, gonadları bir bütün halinde çıkarılıp, 0.1 g hassasiyetle tartılmış ve gonadosomatik indeksleri ($GSİ = (Gonad\ ağırlığı \cdot 100 / Toplam\ vücut\ ağırlığı)$) hesaplanmıştır.

Veriler SAS (Statistical Analysis System, Carry, NC, USA) yazılımının 8.2 versiyonu kullanılarak analiz edilmiştir. Deneme gruplarının boy, ağırlık ve GSİ ortalamaları arasında fark olup olmadığı çift yönlü ANOVA testi kullanılarak belirlenmiştir. ANOVA testinin deneme gruplarının ortalamaları arasında istatistiki belirgin ($P < 0.05$) farklar işaret ettiği durumlarda, ortalamalar Duncan çoğul aralık testi kullanılarak gruplara ayrılmıştır. Deneme gruplarının cinsiyet oranlarının dengeli %50 dışı: %50 erkek oranına uyum gösterip göstermediği veya kontrol gruplarının cinsiyet oranlarına benzerliği G testi (log likelihood ratio test) kullanılarak belirlenmiştir ¹¹.

BULGULAR

Kullanılan hormonlar, uygulanan dozda gökkuşağı alabalığında toksisiteye sebep olmamıştır. Hormon uygulanan tüm deneme gruplarının yaşam oranları kontrol grubununkine benzer değerler (%70) göstermiştir. On ikinci ayda yapılan tetkikler, 2007 yılı deneme grupla-

rının boy ve ağırlık artışlarında istatistiki olarak belirgin ($P < 0.05$) farklar olduğunu göstermiştir (Tablo 2). Oral yolla düşük dozda ($1\ mg\ kg^{-1}$ yem) MT verilen grubun büyümesi kontrol grubuna benzer kalırken, oral yolla yüksek dozda ($3\ mg\ kg^{-1}$ yem) MT verilen grubun kontrol grubundan daha yavaş büyüdüğü tespit edilmiştir. Periyodik MT banyolarının ise büyümeyi olumsuz etkilediği ve bu iki gruptaki balıkların hem kontrol hem de oral MT gruplarındaki balıklardan küçük olduğu görülmüştür (Tablo 2).

On beşinci ayda yapılan tetkikler, 2008 yılı deneme gruplarının da boy ve ağırlık artışlarında istatistiki olarak belirgin ($P < 0.05$) farklar olduğunu göstermiştir (Tablo 3). Bir grup hariç, yine tüm tek veya çift MT banyosu verilen balıkların ağırlıklarının, kontrol ve oral MT gruplarındaki balıklardan daha düşük olduğu görülmüştür. Kontrol, oral MT ve 620 gün-dereceden itibaren çift MT banyosu gruplarındaki balıkların ağırlıkları benzer bulunurken, 620 gün-dereceden itibaren tek veya çift MT banyosu verilen gruplardaki balıkların kontrol ve oral MT grubundan daha uzun olduğu tespit edilmiştir (Tablo 3).

Oral MT uygulamalarının her üç dozu ($1, 2$ ve $3\ mg\ kg^{-1}$ yem) tamamı erkek veya tamamına yakını erkek gökkuşağı alabalığı üretmekte etkili olmuştur (Tablo 4). MT banyo uygulamaları ise %2.4-6.8 oranında interseks bireyin oluşmasına sebep olmuş, ama erkek oranlarında %50'yi aşan belirgin bir artış yaratmamıştır (Tablo 4). Diğer yandan 2008 yılı kontrol grubunun cinsiyet oranı

Tablo 2. 2007 yılında gerçekleştirilen 17α -metiltestosteron (MT) uygulamalarının 12 aylık gökkuşağı alabalığının büyümesi üzerine etkileri. Aynı sütunda farklı küçük harflerle işaretlenmiş olan ortalamalar istatistiki belirgin farklar göstermektedir ($P < 0.05$)

Table 2. Effects of 17α -methyltestosterone (MT) treatments administered in 2007 on the growth of 12 month old rainbow trout. Means in the same column followed by different lower case letters are significantly different ($P < 0.05$)

Uygulama	N	Ort. Ağırlık (g)	Ort. Boy (cm)
Kontrol	117	193.2±32.1 ^a	25.2±1.6 ^a
1 mg MT kg^{-1} yem	102	197.0±44.6 ^a	25.3±2.4 ^a
3 mg MT kg^{-1} yem	98	166.0±38.9 ^b	24.5±2.3 ^b
410 gün-dereceden itibaren 48 saat aralı, 3 periyodik MT banyosu	106	149.5±29.9 ^c	24.0±1.9 ^b
410 gün-dereceden itibaren 48 saat aralı, 6 periyodik MT banyosu	107	145.2±22.7 ^c	22.7±2.2 ^c

Tablo 3. 2008 yılında gerçekleştirilen 17α -metiltestosteron (MT) uygulamalarının 15 aylık gökkuşağı alabalığının gonad gelişimi ve büyümesi üzerine etkileri ($N=100$). Aynı sütunda farklı küçük harflerle işaretlenmiş olan ortalamalar istatistiki belirgin farklar göstermektedir ($P < 0.05$)

Table 3. Effects of 17α -methyltestosterone (MT) treatments administered in 2008 on the growth of 15 month old rainbow trout. Means in the same column followed by different lower case letters are significantly different ($P < 0.05$)

Uygulama	Ort. Ağırlık (g)	Ort. Boy (cm)
Kontrol	306.1±45.9 ^a	27.8±1.4 ^{cd}
2 mg MT kg^{-1} yem	300.9±60.0 ^a	27.9±1.6 ^{cd}
480 gün-derecede tek MT banyosu	263.5±38.9 ^{bc}	27.7±1.4 ^d
550 gün-derecede tek MT banyosu	260.8±33.8 ^{bc}	28.1±1.3 ^{bcd}
620 gün-derecede tek MT banyosu	270.7±66.9 ^b	28.4±1.9 ^{ab}
550 gün-dereceden itibaren 48 saat aralı, 2 periyodik MT banyosu	253.1±38.1 ^c	28.1±1.4 ^{bc}
620 gün-dereceden itibaren 48 saat aralı, 2 periyodik MT banyosu	307.7±50.4 ^a	28.6±1.4 ^a

dengeli %50 dişi: %50 erkek cinsiyet oranından hafifçe sapma göstermiştir (%39.8 erkek, *Tablo 4*). Kontrol grubunun dişi ağırlıklı cinsiyet oranı ile karşılaştırıldığında, 550 gün-derecede verilen tek ve 550 gün-dereceden itibaren verilen 48 saat aralı çift MT banyolarının dişi birey oranında istatistiki olarak belirgin ($P<0.05$) düşüşe (%60.2'den sırasıyla %45.9 ($G_p/G_T = 8.202/8.584$) ve %36.6'ya ($G_p/G_T = 10.396/10.554$)) sebep olduğu tespit edilmiştir.

Hormon uygulamalarından sonraki üreme sezonunun başında (12. ayda) yapılan kontrollerde, kontrol grubunun %45.7'si abdomene yapılan yumuşak masajla sperm verirken, oral veya banyo yoluyla hormon verilen balıkların sadece %3-7.5'inden sağımla sperm elde edilebilmiştir. Üreme sezonu sonunda (16. ayda) yapılan kontrollerde ise, 600 gün-derece boyunca 1 mg MT kg^{-1} yemle beslenen tamamı erkek grubun %54.7'sinin sağımla süt verdiği gözlenmiştir. Aynı süre yüksek dozda (3 mg kg^{-1}) MT içeren yemle beslenen grubun ise, sadece %26.4'ünden sağımla süt elde edilebilmiştir (*Tablo 5*). Ayrıca sağımla süt vermeyen bireylerin büyümesinin

sağımla süt verenlere göre daha iyi olduğu ve kullanılan MT dozu arttıkça hem büyümenin yavaşladığı hem de GSİ'nin düştüğü tespit edilmiştir (*Tablo 5*).

TARTIŞMA ve SONUÇ

Erkeklik hormonları anabolik etkiye sahip olduğundan yasal izin verilen ülkelerin hayvan yetiştiricileri tarafından büyümeyi destekleyici olarak kullanılabilir. Balık yetiştiriciliğinde bu amaçla kullanılmamakla birlikte, hormonal cinsiyet dönüşüm çalışmaları androjenlerin düşük dozlarının balıklarda da büyümeyi destekleyici etkiye sahip olduğunu, ama yüksek dozlarının aksine büyümeyi yavaşlattığını göstermiştir ^{4,6}. Bizim çalışmamızda sentetik erkeklik hormonu MT oral yolla verildiğinde 1 mg kg^{-1} yem dozunda büyüme üzerinde herhangi bir etki oluşturmazken, 3 mg kg^{-1} yem dozunda büyümeyi yavaşlatmış görünmektedir. Daha ilginç banyo yoluyla verildiğinde, düşük dozda (0.5 mg l^{-1}) bile büyüme üzerinde olumsuz etki göstermiş görünmesidir. Kuzey Amerika kıtasına özgü güneş balıklarıyla yapılan

Tablo 4. 17 α -metiltestosteron (MT) uygulamalarının gökkuşağı alabalığının cinsiyet oranları üzerine etkileri. Yüzde oranlarını takip eden değerler 2 tekrara ait $\pm SS$ 'yi göstermektedir, * $P<0.05$ ve ** $P<0.001$

Table 4. Effects of 17 α -methyltestosterone (MT) treatments on sex ratios of rainbow trout. Values following the percentages show $\pm SD$ belonging to 2 replicates, * $P<0.05$ and ** $P<0.001$

Uygulama Yılı	Uygulama	N	% Erkek	% İnterseks	% Dişi	G
2007	Kontrol	102	45.1	0.0	54.9	0.982
	1 mg MT kg^{-1} yem	85	100.0	0.0	0.0	113.4 **
	3 mg MT kg^{-1} yem	98	98.0	2.0	0.0	116.3 **
	410 gün-dereceden itibaren 3 periyodik MT banyosu	93	51.6	4.0	44.4	0.097
	410 gün-dereceden itibaren 6 periyodik MT banyosu	98	53.1	4.0	42.9	0.368
G_p/G_T						
2008	Kontrol	47 $\pm$ 5	39.8 $\pm$ 0.3	0.0	60.2 $\pm$ 0.5	3.909 */3.911
	2 mg MT kg^{-1} yem	32 $\pm$ 1	98.4 $\pm$ 2.3	1.6 $\pm$ 2.3	0.0	78.42 **/78.42 **
	480 gün-derecede tek MT banyosu	50 $\pm$ 0	44.0 $\pm$ 11.3	0.0	56.0 $\pm$ 11.3	1.443/4.053
	550 gün-derecede tek MT banyosu	50 $\pm$ 1	51.1 $\pm$ 4.4	3.0 $\pm$ 4.2	45.9 $\pm$ 4.4	0.641/1.023
	620 gün-derecede tek MT banyosu	43 $\pm$ 6	49.5 $\pm$ 0.8	0.0	50.5 $\pm$ 0.8	0.012/0.021
	550 gün-dereceden itibaren 2 periyodik MT banyosu	45 $\pm$ 1	56.6 $\pm$ 3.6	6.8 $\pm$ 6.5	36.6 $\pm$ 3.6	1.605/1.763
	620 gün-dereceden itibaren 2 periyodik MT banyosu	42 $\pm$ 4	45.3 $\pm$ 1.5	2.4 $\pm$ 0.2	52.3 $\pm$ 1.5	0.191/0.226

Tablo 5. 2007 yılında gerçekleştirilen oral 17 α -metiltestosteron (MT) uygulamalarının 16 aylık gökkuşağı alabalığında gonad gelişimi ve büyüme üzerine etkileri. Aynı sütunda farklı küçük harflerle işaretlenmiş olan ortalamalar istatistiki belirgin farklar göstermektedir ($P<0.05$)

Table 5. Effects of oral 17 α -methyltestosterone (MT) treatments administered in 2007 on the gonad development and growth of 16 month old rainbow trout. Means in the same column followed by different lower case letters are significantly different ($P<0.05$)

Uygulama	N	Grup	Grup % Oranı	GSİ (N=10)	Ort. Ağırlık (g)	Ort. Boy (cm)
1 mg MT kg^{-1} yem	190	Sağımla sperm veren erkek	54.7	1.9 $\pm$ 1.5 ^a	274.2 $\pm$ 58.5 ^{bc}	27.4 $\pm$ 2.5 ^b
		Sağımla sperm vermeyen erkek	45.3	2.1 $\pm$ 1.5 ^a	375.2 $\pm$ 58.7 ^a	30.9 $\pm$ 1.6 ^a
3 mg MT kg^{-1} yem	144	Sağımla sperm veren erkek	26.4	2.8 $\pm$ 1.9 ^a	210.0 $\pm$ 32.8 ^d	24.7 $\pm$ 1.6 ^c
		Sağımla sperm vermeyen erkek	73.6	0.3 $\pm$ 0.1 ^b	292.0 $\pm$ 26.1 ^b	29.4 $\pm$ 1.0 ^{ab}

hormonal cinsiyet dönüşüm çalışmalarında da, uygulamalar sonrasında yapılan değerlendirmelerde periyodik banyolar şeklinde düşük dozda MT verilen balıkların oral yolla yüksek dozda MT verilen balıklardan ve kontrol grubundan daha küçük kaldığı görülmüştür^{13,14}. Ama uygulamaların bitiminden birkaç ay sonra yapılan değerlendirmeler, telafi büyümesinin gerçekleştiğini ve bu durumun MT dozuyla değil banyo uygulamaları sırasında balıkların yoğun stoklanması ve hareket ettirilmesi sebebiyle oluşan stres kaynaklı olabileceğini göstermiştir. Bu çalışmada deneme balıklarının büyümesi uygulamaların bitiminden çok sonra (12 ve 15. aylarda) değerlendirilmiştir. Fakat ne banyo yoluyla nede oral yolla yüksek dozda MT verilen balıkların daha küçük kalması direkt olarak MT dozu veya uygulama şekliyle ilgili olmayabilir. Çünkü MT uygulamaları sonrası, deneme gruplarının stok yoğunlukları ve besleme oranları sıkı bir şekilde denetim altında tutulmamıştır.

Hormonlarla fenotipik cinsiyetin başarılı bir şekilde yönlendirilmesi ve fonksiyonel olarak fenotipik cinsiyeti değiştirilmiş bireylerin elde edilmesi hormon uygulamasının gonadal gelişime bağlı zamanlamasına, uygulamanın süresine, kullanılan hormonun türüne ve dozuna bağlıdır⁴. Gonadların sitolojik değişimi larvaların serbest yüzme aşamasına denk gelen¹⁵ Pasifik salmonlarında (*O. kisutch* ve *O. tshawytscha*), larvalara ilk yemlemeden itibaren oral yolla verilen hormonlar çok çeşitli sonuçlar üretmiş ve genelde fenotipik cinsiyeti yönlendirmede başarısız olmuştur⁴. Gonadların somatik büyüme (mitotik bölünmeler) aşamasına denk gelen yumurtaların medyan açılması (aynı zamanda döllenmiş yumurtaların yarısının açılmasını tamamladığı evre) sırasında verilen kısa (2 saatlik) ve düşük dozlardaki androjen veya östrojen banyoları ise fonksiyonel olarak tamamı erkek veya tamamı dişi populasyonlar üretmekte başarılı olmuştur¹⁵⁻¹⁷. Bu düşük dozda, kısa süreli hormon uygulamalarından elde edilen yüksek başarı, uygulamaların gonadların fizyolojik olarak hormonların etkisine en duyarlı olduğu dönemde verilmiş olmasına ve verilen steroidlerin salmon larvalarının nispeten büyük besin kesesi tarafından absorbe edilerek, banyo uygulamalarından çok sonra bile larvalara aktarılabilmesine bağlanmıştır¹⁸.

Buna karşılık gonadların sitolojik değişimi (mayoz bölünmeler) larvaların serbest yüzmeye başladığı ay içerisinde gerçekleşen Atlantik salmonu, *Salmo salar*^{4,19}, ve gökkuşağı alabalığında^{19,20}, ilk yemlemeden itibaren oral yolla verilen erkeklik ve dişilik hormonları fenotipik cinsiyeti yönlendirmede başarılı sonuçlar üretmiştir^{4,21}. Fakat bu yolla erkekleştirilen bireylerde sperm kanalı oluşmadığı rapor edilmiştir^{1,21}. Bu durum uygulamaların zamanlamasıyla da ilişkilendirildiğinden, gonadal değişimin hem somatik hem de sitolojik elementlerini etkile-

yebilmek için her iki türde de keseli larval dönemde banyo yoluyla steroid uygulamaları yapılmıştır. Atlantik salmonu ile yapılan çalışmada²², yumurtaların medyan açılmasından sonraki 2 ve 3. veya 3 ve 4. haftalarda (döllenmeden 570-690 gün-derece sonra) verilen üçer saatlik 2 adet 0.4 mg l⁻¹ 17 α -metildihidrotestosteron banyolarının %100 erkek populasyonlar üretmekte etkili olduğu tespit edilmiştir. Ayrıca bu yolla üretilen erkekleştirilmiş dişilerin %84-92 oranında fonksiyonel, kanallı bireylerden oluştuğu rapor edilmiştir²². Gökkuşağı alabalığı yumurtalarına (açılmadan 1 hafta önce) ve larvalarına (medyan açılmada, açılmadan 1 veya 2 hafta sonra) verilen tek veya birer hafta aralı 2 ila 4 adet, 2 saatlik 0.4 mg l⁻¹ MT banyoları ise %16-76 oranında erkekleşmeye sebep olmuştur ve bu yolla erkekleştirilen dişilerin %60-100 oranında kanallı fonksiyonel bireylerden oluştuğu rapor edilmiştir⁹. Ayrıca banyo uygulamaları için en uygun zamanın medyan açılmadan 1 hafta (döllenmeden yaklaşık 425 gün-derece) sonra olduğu bildirilmiştir⁹. Aynı hormonun 410 ile 620 gün-derece arasında biraz (%25) yüksek dozda verildiği bu çalışmada ise, tek veya periyodik MT banyolarının hiçbirisi erkek oranlarında %50'yi aşan istatistiki olarak belirgin bir artış yaratamamıştır. Çalışmada keseli larval dönemde verilen MT banyolarının etkisiz kalması, işletmenin nispeten yüksek su sıcaklıkları (12-14°C) dolayısıyla deneme balıklarının artan gelişim hızına bağlı olabilir. Çünkü balıklar soğukkanlı canlılar olduklarından, balıklarda gelişim hızı, dolayısıyla gelişimin zamanlaması ve süresi su sıcaklıklarına bağlı farklılıklar gösterebilir⁴. Banyo uygulamalarının daha başarılı sonuçlar verdiği önceki çalışmada⁹, yumurta ve larvalar 11.5°C'de bakılmışlardır. Oysa yapılan çalışmada su sıcaklıkları 12-14°C arasında değişim göstermiştir. Yüksek gelişim hızlarında, primordial gonadların fizyolojik olarak hormonların etkisine en duyarlı olduğu kısa dönem banyo uygulamalarına başladığımız 410 gün-derece öncesinde gerçekleşmiş olabilir. Bununla birlikte, yüksek gelişim hızı nedeniyle besin kesesinin hızla tüketilmesi, ayrıca periyodik banyolarında daha sık aralıklarla (1 hafta yerine 48 saat) verilmesi çalışmamızda larvaların maruz kaldığı gerçek MT dozunun daha önceki çalışmada⁹ kullanılan dozdan sadece %25 değil, çok daha yüksek olmasına da sebep olabilir. MT östrojene aromatize olabilen bir androjen olduğundan²³, yüksek dozlarda MT'nin kısa süreli uygulamalarda erkekleşme oranlarında düşüş ve hatta dişi oranlarında artışa (paradoksal dişileşme) sebep olduğu görülmüştür^{17,24}. Nitekim daha önceki çalışmada⁹ banyo sayısı arttıkça erkekleşme oranlarında azalma olduğunu gözlenmiş ve bu durumu banyo uygulamalarının etkisinin artan banyo sayısı ile azalması şeklinde yorumlanmıştır. Fakat çalışmamızda hem tek hem de periyodik banyoların başarısız olması, bu durumun ban-

yo sayısından çok larvaların maruz kaldığı gerçek MT dozu ile bağlantılı olduğunu işaret etmektedir.

Öte yandan, kesenin tüketilmesine doğru (550 günden itibaren) verilen tek veya çift MT banyoları dişi oranını %60.2'den sırasıyla %45.9 ve %36.6'ya düşürmüştür. Fakat tamamı erkek popülasyonlar oluşturamamıştır. Kesenin tüketiminden itibaren oral yolla verilen uzun süreli (600 gün-derece boyunca) MT uygulamaları ise tamamı veya tamamına yakını erkek popülasyonlar üretmekte etkili olmuştur. Bu sonuçlar, gelişmekte olan gonadların keseli larval dönem sonrasında hormonların etkisine açık kaldığını, ama başarı sonuçlar için uygulamaların daha uzun süreli olması gerektiğini işaret etmektedir.

Bununla beraber, keseli larval dönem sonrası başlatılan uzun süreli uygulamalarda da kullanılan MT dozunun iyi ayarlanması büyük önem taşımaktadır. Daha önce gökkuşağı alabalığı veya diğer türlerde yapılan çalışmalar yüksek dozda uzun süreli androjen uygulamalarının kısırılığa varan sonuçlar doğurabileceğini ortaya koymuştur^{4,6}. Yapılan çalışma sonuçları da, sperm kanalı gelişiminin kullanılan MT dozuyla ilişkili olabileceğini ve yüksek MT dozlarının sağımla sperm veren, kanallı erkek oranını azaltabileceğini göstermektedir. Ayrıca yüksek MT dozu kullanılarak erkekleştirilmiş dişilerde GSI'nin de düştüğü görülmüştür. Bütün bunlar göz önüne alındığında, oral uygulamalar için uygun MT dozunun 1 mg kg⁻¹ yem veya altında olduğu düşünülebilir. Fakat banyo uygulamalarında olduğu gibi oral uygulamalarda da metabolik hızı, dolayısıyla tüketilen yem miktarını ve larvaların maruz kaldığı gerçek androjen dozunu su sıcaklıklarının belirleyeceği ve uygun dozun çalışmadakinden farklı su sıcaklıklarında farklı olabileceği unutulmamalıdır.

Sonuç olarak, çalışmamız gökkuşağı alabalığında erkekleştirilmiş dişi anaçların üretilmesinde oral MT uygulamalarının daha istikrarlı sonuçlar verdiğini göstermiştir. Ayrıca yüksek MT dozlarının sperm kanalı gelişimi ve GSI'yi olumsuz yönde etkilediğini ortaya koymuştur. Dahası çalışma sonuçları farklı su sıcaklıklarında larvaların maruz kaldığı gerçek MT dozunun değişen metabolik hız sebebiyle farklı olabileceğini işaret etmektedir. Bu sebeple, çalışmada en iyi sonucun alındığı protokol olan besin kesesini tüketen larvaları ilk yemlemeden itibaren 600 gün-derece süresince kilogramında 1 mg MT bulunan yemle besleme, çalışmanın yapıldığı su sıcaklıklarına (12-14°C) benzer su sıcaklıklarına sahip işletmelere önerilir. Farklı su sıcaklıklarına sahip işletmelerin kullandıkları MT dozunu kendi koşullarına göre optimize etmeleri gerekebilir.

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C-Banded Karyotype and Nucleolar Organizer Regions (NORs) of Marsh Frog, *Rana ridibunda* (Ranidae: Anura) in Central Anatolia

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Summary

Karyotype, C-banding and nucleolar organizer regions (NORs) characteristics of nine *Rana ridibunda* samples collected from Konya (Meram, Beyşehir, Hadim) province were examined. The diploid number of chromosomes (2n) and the fundamental number of chromosome arms (FN) were determined as 26 and 52, respectively. All of the chromosomes of this species have centromeric constitutive heterochromatin (C-band). Nucleolar organizer regions (secondary constriction) were determined on the long arm of the no. 8 submetacentric chromosome by using silver-nitrate staining technique.

Keywords: *Rana ridibunda*, Karyotype, C-band, NORs, Central Anatolia

Orta Anadolu'daki Bataklık Kurbağası, *Rana ridibunda* (Ranidae: Anura)'nın C-Bantlı Karyotipi ve Nükleolar Organizatör Bölgeleri (NORs)

Özet

Konya ilinden (Meram, Beyşehir, Hadim) toplanan dokuz *Rana ridibunda* örneğinin karyotip, C-bantlama ve nükleolar organizatör bölgelerinin (NORs) özellikleri incelendi. Diploid kromozom sayısı (2n) ve temel kromozom kol sayısı (FN) sırasıyla 26 ve 52 olduğu belirlendi. Bu türün kromozomlarının tamamı sentromerik konstitütif heterokromatin (C-band)'e sahiptir. Gümüş-nitrat boyama tekniği ile 8 nolu submetasentrik kromozomun uzun kolu üzerinde nükleolar organizatör bölge (ikincil boğum) tespit edildi.

Anahtar sözcükler: *Rana ridibunda*, Karyotip, C-bandı, NORs, Orta Anadolu

INTRODUCTION

Rana ridibunda Pallas, 1771, a common frog species of Central Europe and Western Asia, was described in Turkey ¹. Karyological techniques provided a reliable tool for cytotoxic analysis, and were effectively used to characterize the *R. ridibunda* karyotypes throughout the world ^{2,3}. Most *Rana* species are characterized by 26 chromosomes with a variable morphology among populations ⁴. C-banding, used to establish heterochromatin regions on the chromosomes is frequently used in animals and thus is useful for examining intra and interspecific chromosomal differences between closely related species ^{5,6}. The first karyotype of this

species was described by Alpagut and Falakalı ⁷ from Turkey. However, there is not any information about C- and Ag-NOR banding of karyotypes. Therefore, this study aims to present conventional, C- and NORs banded karyotypical data on *R. ridibunda* from Central Anatolia.

MATERIAL and METHODS

Nine animals (three male and six female) studied were collected from Konya province (Meram, Beyşehir

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and Hadim). Karyotype preparations were obtained from the bone marrow of the colchicined animal ⁸. In order to identify each autosomal pair and both sex chromosomes, constitutive heterochromatin and nucleolus organizer regions (NORs) were detected with C-banding ⁹ and Ag-NOR staining ¹⁰, respectively.

RESULTS

The karyotype contains 26 chromosomes and the number of fundamental arms (FN) is 52. All the autosomal pairs are bi-armed. Six autosomal pairs are metacentric (nos. 1, 4, 6, 8, 10, 11) and chromosome no. 1 is larger than others and two pairs (nos. 2, 3) are large and four pairs are small submetacentric (nos. 7, 10, 12, 13). All the chromosomes in our samples have centromeric constitutive heterochromatin (C-band). Telomeric bands were not observed in the long and short arms of the chromosomes (Fig. 1).

Active nucleolar organizer regions (NORs) were determined on the long arm of the number 8 submetacentric chromosome by using silver- nitrate staining technique (Fig. 2).

pairs of metacentric, 4 pairs of submetacentric and 2 pairs of subtelocentric at Beyşehir population. These differences may result from the methodology. In the same study polymorphism in a pair of chromosomes at İzmir and Beyşehir populations were observed. However Al-Shehri and Al-Saleh ³, in a study they carried out in Saudi Arabia, reported that sex chromosomes of *R. ridibunda* had different sizes, and X chromosome is a little larger than Y chromosome. Alpagut and Falakalı ⁷ could not differentiate between the sex chromosomes in the karyotype, but they did for this species. However, these researchers determined that chromosome pair 4 of Beyşehir specimens was heteromorph. Schempp and Schmid ¹¹ described chromosome pair 4 as sex chromosomes in *Rana esculenta*, which is the only *Rana* species for which the sex chromosomes have been identified so far.

In most of ranid species C-band analyses were carried out. As a result of these analyses ranid species were discovered to have centromeric bands ¹²⁻¹⁴. In the cytogenetic research done in India on *Rana malabarica*, *Rana temporalis* and *Rana curtipes* by Joshy et al. ¹⁵ noted that all the chromosomes of each three species

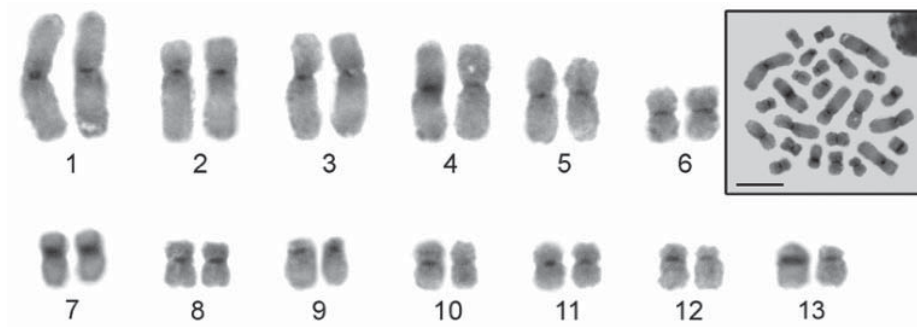
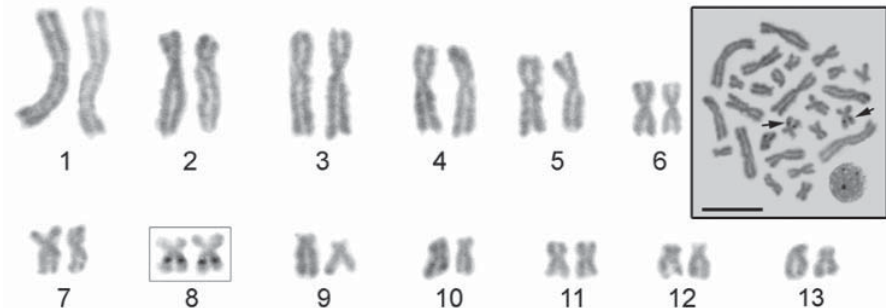


Fig 1. Metaphase spread and C-banded karyotype of *Rana ridibunda* (Scale bar = 10 µm)

Şekil 1. *Rana ridibunda*'nın C-bantlı karyotipi ve metafaz plağı (Scale bar = 10 µm)

Fig 2. Silver-stained metaphase spread and karyotype of *Rana ridibunda*. Arrows indicate the Ag-NORs (Scale bar = 10 µm)

Şekil 2. *Rana ridibunda*'nın Ag-NOR bantlı karyotipi ve metafaz plağı. Oklar Ag-NOR'ları göstermektedir (Scale bar = 10 µm)



DISCUSSION

Alpagut and Falakalı ⁷ studied two populations of this species in Turkey. Unlike our samples, they detected 7

had centromeric C-bands. But these researchers determined that the telomeric C-bands which did not exist in other ranid species existed in two chromosomes of *R. temporalis* and *R. curtipes* and in three pairs of

chromosomes of *R. malabarica*. Joshy et al.¹⁵ argued that the chromosomes which have telomeric bands differed according to the species and this case was a distinctive feature for these three species. It is inferred from these results that all *Rana* species all over the world have centromeric bands while some of them have also telomeric bands.

Alpagut and Falakalı⁷ detected no. 9 secondary constriction in Beyşehir population and no. 10 in İzmir population. Koref-Santibanez¹⁶ found that the *R. ridibunda* in Europe had no. 10 secondary constriction. Al-Shehri and Al-Saleh³ detected secondary node in a pair of chromosomes (no. 10) in the Arabia population of this species as in Europe and Turkey samples. Joshy et al.¹⁵ determined satellite in two pairs of chromosomes of *R. curtipes* via giemsa staining technique in a study they did in India. But these NORs exist in terminal parts of short arms not long arms unlike *R. ridibunda*. Howell and Black¹⁰ reported that active NORs were exactly detected by silver-nitrate stain. The NORs outside the telomers of chromosomes can be detected by normal Giemsa stain. However Silver-nitrate staining must be implemented in order to exactly detect not only the secondary constructions in this region and also the satellites in telomers. According to the results of our silver-nitrate staining, active NOR existence was detected as a secondary constriction on the long arms of the no. 8 chromosome pair in Konya population.

As a result the chromosome morphology in our samples is similar to the studies on *R. ridibunda* in Turkey and around the world as well. Moreover, the existence NOR, which was detected on no. 8 chromosome of this species by routine giemsa staining several cytogenetic studies, was proved by silver-nitrate staining technique. The centromeric C-bands of *R. ridibunda* samples in Turkey was first detected with this study, as it is the first study to detect the centromeric C-bands of *R. ridibunda*, our samples do not have telomeric bands that are in some species related with *Rana* genus.

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First Report of *Tristomella laevis* (Monogenea, Capsalidae) from Aegean Sea in Turkey

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Summary

Tristomella laevis (Monogenea, Capsalidae) was recorded on gills of sword-fish (*Xiphias gladius*), collected from Ayvacık, Çanakkale, for the first time in Turkey. Infested fish weight ranged between 8.1 and 10.5 kg. Parasites were observed generally on small fish rather than big fish. Main prevalence of parasites was determined as 70% and serious gill damage was observed on the host fish.

Keywords: *Metazoa, Parasite, Sword-fish*

Türkiye'nin Ege Denizi'nde *Tristomella laevis* (Monogenea, Capsalidae) İçin İlk Rapor

Özet

Türkiye'nin Çanakkale ili Ayvacık sahillerinde avlanan kılıç balıklarının (*Xiphias gladius*) solungaçlarından *Tristomella laevis* (Monogenea, Capsalidae) ilk kez izole edilmiştir. Enfeste olmuş balıkların ağırlık aralığı 8.1 ve 10.5 kg olarak belirlenmiştir. Parazitler daha çok küçük balıklarda gözlemlenmiştir. Parazitlerin ortalama prevalansı %70 olarak belirlenirken balıklarda ciddi solungaç hasarları tespit edilmiştir.

Anahtar sözcükler: *Metazoa, Parazit, Kılıç balığı*

INTRODUCTION

Monogeneans are lives in freshwater, brackish and marine environments and they all have a direct life cycle. The majority of the monogeneans are ectoparasitic and generally attached on external surfaces of their hosts ¹.

Many reports have presented high pathogenesis of monogeneans in aquatic systems. The parasites can cause different effects of host such as, feeds of the host tissue, mechanic effect, gland secretions and vectors for viruses and bacteria. Therefore monogeneans infestations cause for serious mortality in fish farms ^{1,2}.

Tristomella laevis (synonyms: *Tristoma leave*, *Tristomum histiophori*, *Tristomum laeve* var. *armata* and *Tristomella leave*), is a capsalid monogenean parasites. It was already reported from different fish species (*Xiphias*

gladius, *Makaira indica*, *Makaira nigricans*, *Tetrapterus albidus*). And also *Tristomella laevis* was isolated on cultured Atlantic bluefin tuna (*Thunnus thynnus*) ³.

Broadbill sword-fish (*Xiphias gladius*) is a highly migratory fish species. It shows a wide geographical spread including Atlantic, Indian and Pacific, Mediterranean Sea, the Sea of Marmara, the the Sea of Azov and Black Sea ^{4,5}. *X. gladius* is found in all Turkish sea and annual sword-fish production of Turkey is 386 tones in 2008 ⁶.

Monogeneans are very common parasites of fish ⁷, but there is no record related to *Tristomella* infestation in Turkey. *Tristomella laevis* infestation on sword-fish was exhibited for the first time in Turkish sea in the present study.



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MATERIAL and METHODS

Twenty sword-fish (*Xiphias gladius*) were caught by drift-net in the coast of Ayvacık (39°36' N; 26°24' E) between May and August 2008. The gills of host fish were extracted, and then examined in the laboratory for metazoan parasites⁸. Parasites were observed on gills of fish and then fixed in 10% formalin. Body weight of host fish, length and width of parasites were recorded and the parasites were identified⁹.

RESULTS

Tristomella laevis was found on gills of sword-fish (Fig. 1) and mean prevalence of the parasites was determined 70%. Average density of parasites on the both gills was 10. Infested fish weight ranged between 8.1 and 10.5 kg. Body size of parasites were measured as follows; mean length 14.3 ± 0.2 mm, mean width 13.1 ± 0.5 mm and serious erosions were realized in gills of the fish. And also parasites were observed frequently on small fish rather than big fish.

DISCUSSION

Parasites can cause many different damages on their host fish. *Tristomella laevis* previously was reported on *Thunnus thynnus*³, but any pathological symptoms were

not mentioned in the study. Unlike the previous study, this study presents serious gill damages due to *T. laevis* on *X. gladius*.

Sword-fish are fairly migratory fish. Therefore, they may carry their pathogens from anywhere to another aquatic location. The production of marine fish is very intense (*Sparus aurata* and *Dicentrarchus labrax*) in the coast of Aegean and Mediterranean Sea of Turkey^{10,11}. Although many parasitological studies were realized on fish species^{7,12,13,14}, *Tristomella laevis* was not observed so far on cultured and other fish in Turkish sea. However, location of the parasites shows that the parasite maybe infest economical fish species in the area.

Monogenean fish parasites were commonly reported in *Cyprinus carpio* and *Esox lucius* in Turkey. But sea bass and sea bream are the most common hosts cultivated fish species for the monogenetic parasites⁷. The only parasite report from *X. gladius* in Turkish sea is *Pennella instructa* (Copepoda)¹⁵. In this study is the first monogenean parasite report from *X. gladius* in Turkey. Therefore present study is very important in terms of parasitic studies in the country.

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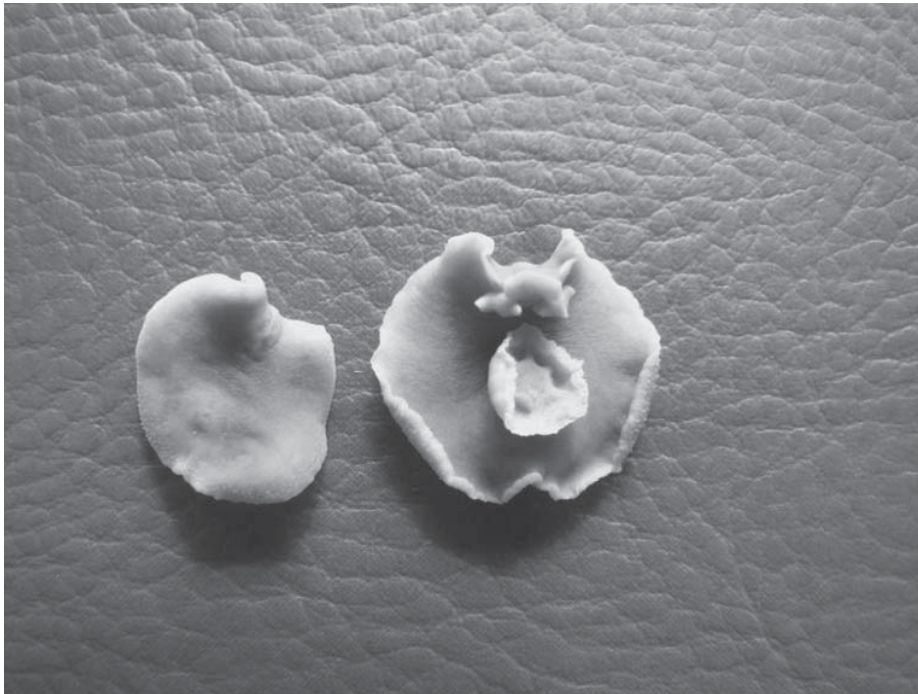


Fig 1. Dorsal and ventral view of *Tristomella laevis*

Şekil 1. *Tristomella laevis*'in dorsal ve ventral görünüşü

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Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 2 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.

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Yazarla ilgili kişisel ve kuruma ait bilgiler ana metin dosyasına değil, on-line başvuru sırasında sistemdeki ilgili yerlere unvan belirtilmeksizin eklenmelidir.

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Example: McIlwraith CW: Disease of joints, tendons, ligaments, and related structures. **In,** Stashak TS (Ed): Adam's Lameness in Horses. 4th ed. 339-447, Lea and Febiger, Philadelphia, 1988.

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