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## CONTENTS

### Research Articles

**Determination of heavy metal pollution in honey samples collected from Ardahan and counties**  
Hüsamettin EKİÇI Kemal YAZICI  
1-7

**A comparison of activating solutions with hatchery water in artificial insemination of rainbow trout (Oncorhynchus mykiss)**  
Burak Evren İNANAN, Ümit ACAR, Hüseyin URÇUK, Ersin ÇELİK  
8-15

**Klinoptilolit kuzularda giardiazis sağıltına yönelik alternatif ve doğal bir çözüm olabilir mi?**  
Adnan AYAN, Deniz ALİÇ URAL, Serdar PAŞA, Songül ERDOĞAN, Hasan ERDOĞAN  
16-20

**Marmara ve Karadeniz Bölgesi istavrit karaciğerinde GPx, SOD, CAT enzim aktiviteleri ve vitamin A değerleri**  
Güzin ÇAMKERTEN ve Hilal KARAGÜL  
21-29

**Karadeniz barbunya balığının (Mullus barbatus ponticus) ilk üreme boyunun tahmini**  
Yakup ERDEM  
30-37

### Letter to Editor

**Pnömonili danalarda L-laktat konsantrasyonları**  
Hasan ERDOĞAN, Songül ERDOĞAN, Tahir ÖZALP, İsmail GÜNAL, Kerem URAL  
38-42
Determination of heavy metal pollution in honey samples collected from Ardahan and counties

Abstract

In this study the concentrations of some elements in 180 honey samples were investigated. Samples were obtained from beekeepers of all the counties of Ardahan province (Center, Hanak, Çıldır, Göle, Damal) in 2015. The levels of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn elements in honey samples were determined by ICP-OES instrument. The samples were digested in microwave oven using nitric acid and hydrogen peroxide. The mean concentration of elements and the lowest and highest values were determined. Of the samples analysed, Cd, Co, Cr and Pb values were found below the detection limit. As a result of analysis, the concentration of elements in honey samples were detected as 124,863 ± 313,44 ppb, 1227,56 ± 892,22 ppb, 67,352 ± 34,636 ppb, 6484,904 ± 2078,892 ppb, 302,551 ± 323,329 ppb, 4,636 ± 3,943 ppb, 3118,69 ± 835,149 ppb and 10,535 ± 14,73 ppb, for Al, Ca, Fe, K, Mg, Mn, Na and Sr respectively. To conclude, the concentrations of detected heavy metals in collected honey samples were below the maximum residue limits of some international residue limits. Therefore it would not pose a risk to human health.

Key Words: ICP-OES, honey, element, pollution, Ardahan

Introduction
Beekeeping is one of the most ancient and widespread production activities in Turkey. Turkey has rich vegetation and different climatic zones and an important potential for bee colony existence, so beekeeping is an important activity for Turkey (Boluktepe and Yılmaz, 2008; Parlakay et al., 2008).

Honey is among the most ancient nutrients. It has been used as an important source of food supplying energy and used instead of drugs in many diseases for thousands of years (Samarghandian et al., 2017). The mineral content of honey is stated to be low (Mendes et al., 2006). The average mineral content of nectar originated honey have been determined as % 0,169, on the other hand the secretion honey kinds are rich in mineral content (Samarghandian et al., 2017). It is possible to detect elements like calcium, phosphor, chlorine, sulphur, magnesium, manganese, silica, sodium and most particularly potassium in honey. Besides, trace elements such as copper, zinc, iron, iodine, which are important for living beings, can be found in small amounts in honey (Mendes et al., 2006; Samarghandian et al., 2017).

The concentration of metals in honey are affected from some factors. Primarily, the mineral rates vary in flower honey kinds just according to the herbal source (Herrero et al., 2017). The mineral rate in any plant shows dependency on the soil that plant grows up, material inside the soil, kind of the plant and to the environmental factors during the manuring and development period (Kaya and Pirincci, 2002). In addition, the acidity of the soil affect the mineral rate in plants. Infact the plants growing in acidic soils cause Pb, Mn, Fe, and Zn poisoning, and the calcic and the high pH rates of the soil reduce solubility of these elements (Vicil et al., 2012).

Techniques using bioindicators have gained importance in recent years in the identification and observation of environmental pollution. Up to the acquired scientific datas, choosing a relevant organism a bioindicator constitutes an important step of the observation activity. Because bioindicator living beings are beings responding differently against various polluting agents, they can hold the polluting agents for a long time by storing them in their bodies (Conti and Botre, 2001). Honey bees are good biological indicators. Bees show the chemical deterioration in environment by the high death rates in their population or storing the polluters in their bodies. With a proper laboratory analysis the polluters which cause pollution on bees and bee products can be identified (Porrini et al., 2003).

It has been able to produce pretty good quality of honey in different parts of Turkey. Furthermore, it has been stated that %75 percent of the required plant flora for honey is available in Turkey (Sancak et al., 2013). Beekeeping has been stated to be among the occupational sectors which are important and have economical dimension for Ardahan Province. The number of the people dealing with beekeeping is %0,2 percent of Turkey in Ardahan province (Aygun and Akbulak, 2017).

The aim of this study is to detect and make comparison of some element concentrations in honey samples collected in Ardahan province which is known to let beekeeping carried out intensively in Turkey. The data that obtained from the study will help to show the level of enviromental pollution, and evaluate the importance of this subject in terms of public health.

Material and Method
The honey samples were collected in 2015 from Ardahan Province and its counties (Centre, Hanak, Posof, Cildır, Gole and Damal). A total of 180 samples, 30 from each locations, were obtained. 100 grams of honey samples were taken into sample containers, the contact between air and honey were prevented by securing the covers of
the sample containers tightly. The samples were stored in dark, under room temperature until the analysis were carried out.

The process of extraction was carried out according to the method advised by Yucel and Sultanoglu (2013), and measurements were actualized in inductively coupled plasma optical emission spectrometry (ICP-OES, Spectroblue, Germany). According to this, 20 honey samples were taken from each sampling field and transferred into the sterilized tubes and in order to prevent crystallization. The samples were taken into the water tank (NÜVE ST 30, Turkey) which is 70 °C average for a while to homogenize the honey samples. 0.5 g was taken from these samples, then 9 ml (HNO3) %65 nitric acid and 1 ml %30 hydrogen peroxide were added. The samples were burned in a microwave device (CEM MARS 6 System 240/50, USA). The working conditions of the microwave device were given in Table 1. The burned samples were subtilized with 5 ml of pure water. The prepared blind (Blank) samples were applied with the same process.

| Table 1. The working conditions of Microwave Device |
|---|---|
| Temperature and power | Time |
| At 70°C 400W | 5 min. |
| At 100°C 800W | 5 min. |
| At 150°C 800W | 10 min. |
| At 200°C 800W | 10 min. |
| Ventilation | 10 min. |

The main stock solution (1000 mg/L) was prepared from ICP Multi-Element standard Solution IV (Merck Millipore, 111355, Darmstadt, Germany) including aluminum (Al), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb) and Strontium (Sr) and Zinc (Zn) which were diluted with citric acid. Calibration standards have been prepared from this main stock solution as 0, 125, 250, 500, 1000, 2500, 5000 µg/L for Ba, Fe, Mg, Na, Ni and for the others as 0, 10, 20, 50, 100, 200, 500, 1000 µg/L. The metal levels in honey were carried out by ICP-OES device in Kirikkale University Centre for Scientific and Technological Researches and Applications. The device was calibrated with prepared calibration standards. The working conditions of the ICP-OES Device were given on Table 2.

According to the analyses carried out, the limits of determination for the elements Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn were detected as 1.77 ppb; 0.112 ppb; 4.44 ppb; 0.0366 ppb; 0.289 ppb; 0.148 ppb; 0.51 ppb; 0.65 ppb; 63.5 ppb; 20.7 ppb; 0.375 ppb; 94 ppb; 0.649 ppb; 1.44 ppb; 1.27 ppb; 0.107 ppb, respectively.

“SPSS 15.0 for Windows” statistic packet programme was used for the statistical calculations. The data were explained by arithmetic mean ± standard deviation. The data were statistically evaluated by one-way analysis of variance (ANOVA). If the F values were significant, Duncan’s Multiple Range Test was
performed to identify the specific differences between the metal accumulation means at a probability level of $P<0.05$.

**Results**

The means and standard deviation of the ash contents of 180 honey samples for Al, Ca, Fe, K, Mg, Mn, Na and Sr were found as 124.86±313.44 ppb, 1227.56 ± 892.22 ppb, 67.35 ± 34.63 ppb, 6484.90 ± 2078.89 ppb, 302.55 ± 323.32 ppb, 4.63± 3.94 ppb, 3118.69 ± 835.14 ppb, 10.53 ± 14.73 ppm, respectively. The Cd, Co, Cr and Pb levels of the analyzed samples were all below the detection limit. Ba were found only in samples taken from Çıldır County, Ni were found under determination limit in counties of Çıldır and Göle.

The mean concentrations of minerals in Centre, Çıldır and Damal were as follows: Mn<Sr<Fe<Al<Mg<Ca<Na<K; the mean concentrations of minerals in Göle and Horat and Posof were as follows: Mn<Sr<Al<Fe<Mg<Ca<Na<K. There were statistically significant differences ($p <0.05$) in Al, Ca, Fe, K, Mg, Mn and Na concentrations in terms of mean element levels; but no statistical difference ($p <0.05$) was found for Sr. Statistical comparisons were calculated for regional differences (Table 3).

**Discussion**

The increase in the industrial activities and the human population cause environmental pollution. The problems due to environmental and food pollution pose a threat to the public health. There can be elemental residue problem at a potentially toxic level in nutrients which are essential for human and living beings. Just because of the mining and industrialization, poisonous materials can join into the environment thereby into the food chain of human and animal. Toxic elements are intensively used in various industrial sectors and used as an agricultural fertilizer (Leita et al., 1996; Vicil et al., 2012). Just like the animals, the plants need some minerals throughout their progress. Taking these elements in high levels may cause poisonings both in plants and living beings. Plants may store the elements in their structures which are not necessary for their progress and for their growth. The animals feeding with these plants may accumulate these elements in their tissues. This may cause residue problem in animal products. (Kaya and Pirincici, 2002). Throughout their life, the bees are constantly in relation with environment. Thereby bees can be affected from these harmful materials. Consequently the pollutants can be found in honey and other bee products (Carmen and Cristina, 2001).

As the results obtained from this study were surpassed maximum Pb (1 mg/kg) and Cd (0.1 mg/kg) residue limits accepted by European Union and as any kind of residue limit was not determined for other metals, such a comparison was not done.

Different kinds of honey are produced in different regions of Turkey just peculiar to their plant flora. The element content of honey depends on the soil which shelters the plant with the nectar, the period the plant was grown, the climate, season and environmental pollution (Tuzen et al., 2007; Sultanoglu, 2011). The importance of determining the element content of honey have been gaining importance. Thereby, it has been thought that bees and bee products can be important indicators for observation of environmental pollution (Przybylowski and Wilczyńska, 2001; Yucel and Sultanoglu, 2013). In a study carried out to determine the metal concentrations of honey samples taken from different parts of Italy, it was determined that the regional differences did not cause any problem in terms of Pb, Ni and Cr (Porrini et al., 2003).

The results of our study showed that the concentrations of Al was found pretty lower than the results obtained from a study carried out by Van Der Steen et al. (2012). When considered in terms of Cu, the
concentration of Cu was lower than the results obtained from a study carried out by Tuzen (2002), Demirezen and Aksoy (2005), Erbilir and Erdogrul (2005), Tuzen and Soylak (2005), Silici et al. (2008) and Van Der Steen et al. (2012). In terms of Fe, the concentrations were lower than the results obtained from a study carried out by Tuzen (2002), Erbilir and Erdogrul (2005), Tuzen and Soylak (2005), Silici et al. (2008) and Saghaei et al. (2012). The concentrations of Mn found in our study was lower than the results obtained from a study carried out by Tuzen (2002), Erbilir and Erdogrul (2005), Tuzen and Soylak (2005), Silici et al. (2008) and Van Der Steen et al. (2012); higher than a study carried out by Saghaei et al. (2012). Ni concentrations was found to be lower than the results of a study carried out by Porrini et al. (2003) and Van Der Steen et al. (2012).

The element concentrations, which are possible to determine in honey, can be affected from climate and seasonal conditions and flora and the physical conditions of the soil. In addition to this it is necessary to take the polluting factors, which can be exposed to in storage conditions and in production conditions, into consideration. The existence of elements in studies carried out upon honey samples in different levels can be attributed to these reasons above.

Conclusions
In this study, the honey samples were evaluated in terms of Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn. The results show that Cd, Co, Cr and Pb were under the determination limits of ICP-OES device. The results obtained from this study were under the maximum residue limits when compared with some other international limits. The samples analyzed does not pose any kind of danger against human health. Although the levels detected were below the permissible residue limits, beekeepers dealing with beekeeping will be able to have safer and more qualified products by practicing beekeeping activities in fields that can be affected from pollution factors in minimum levels.

In addition to the studies being carried out across the country and the other studies which will be carried out intended to measure metal levels in bees and bee products, this study is qualified enough to help in determination of polluting sources and their distribution into the environment. This study can contribute other studies, which will be carried out in other regions and provinces, in the determination of possible element concentrations in honey.

Acknowledgments
This study was financially supported by the Scientific Research Project Coordination Unit of Kırıkkale University (KUBAP No: 2015/049). A part of this study was presented at the The 2nd International Conference on Advances in Veterinary Sciences and Technics on 4-8 October 2017, in Skopje, Macedonia.

References


Table 3: The concentrations (ppb) of analyzed elements up the counties (Ardahan Merkez, Cıldır, Damal, Göle, Horat, Posof).*

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Ca</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>Sr</th>
</tr>
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<tr>
<td>Centre</td>
<td>81.18±58.55</td>
<td>1327.81±979.31</td>
<td>67.42±19.17</td>
<td>5948.46±1574.51</td>
<td>248.93±92.28</td>
<td>3.47±2.61</td>
<td>3135.09±450.47</td>
<td>8.84±5.14</td>
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<tr>
<td></td>
<td>34.65-366.71</td>
<td>688.15-5271.15</td>
<td>30.86-105.47</td>
<td>3701.31-10874.1</td>
<td>162.46-699.72</td>
<td>1.22-13.19</td>
<td>2099.3-4433.79</td>
<td>5.04-30.42</td>
</tr>
<tr>
<td>Cildır</td>
<td>60.23±22.21</td>
<td>798.55±216.27</td>
<td>45.73±17.84</td>
<td>5081.74±1439.31</td>
<td>206.30±37.83</td>
<td>2.80±2.59</td>
<td>2644.11±140.30</td>
<td>6.98±4.44</td>
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<td></td>
<td>35.36-132.94</td>
<td>80.29-1302.32</td>
<td>22.78-78.17</td>
<td>3219.93-8908.79</td>
<td>132.63-261.11</td>
<td>0.98-11.80</td>
<td>2332.12-2909.52</td>
<td>4.28-30.05</td>
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<td>Damal</td>
<td>137.69±253.79</td>
<td>1185.28±1199.99</td>
<td>60.52±18.15</td>
<td>8097.80±2340.05</td>
<td>410.11±665.63</td>
<td>7.35±4.75</td>
<td>3263.5±1226.24</td>
<td>14.99±30.87</td>
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<td>43.63-1290.61</td>
<td>218.41-7454.01</td>
<td>31.21-93.04</td>
<td>5191.69-11749</td>
<td>182.03-3915.36</td>
<td>2.44-14.15</td>
<td>2722.4-9675.72</td>
<td>4.57-172.71</td>
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<tr>
<td>Göle</td>
<td>63.20±12.41</td>
<td>1148.13±617.49</td>
<td>72.83±21.62</td>
<td>5493.47±653.98</td>
<td>260.24±132.96</td>
<td>3.13±2.35</td>
<td>3182.29±819.86</td>
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<td>47.28-97.86</td>
<td>740.82-4042.06</td>
<td>37.56-127.64</td>
<td>3631.02-6971.72</td>
<td>153.02-916.34</td>
<td>1.93-15.40</td>
<td>2349.38-7085.13</td>
<td>4.68-36.09</td>
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<td>Horat</td>
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<td>1204.12±815.59</td>
<td>62.49±21.40</td>
<td>6748.23±2402.79</td>
<td>311.49±229.77</td>
<td>5.27±4.63</td>
<td>3122.34±686.69</td>
<td>10.60±10.10</td>
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<td>46.83-88.81</td>
<td>695.28-4261.2</td>
<td>25.58-122.23</td>
<td>3441.21-11596.3</td>
<td>157.82-1217.02</td>
<td>1.07-15.34</td>
<td>2667.41-5442.31</td>
<td>5.22-47.69</td>
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<td>Posof</td>
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<td>1699.10±983.92</td>
<td>95.10±63.87</td>
<td>7506.64±1775.8</td>
<td>378.22±299.8</td>
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<td>32.71-384.94</td>
<td>4520.94-12414.8</td>
<td>193.11-1875.51</td>
<td>1.63-19.35</td>
<td>2676.24-8684.44</td>
<td>6.29-76.60</td>
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*mean±Standard deviation and min-max
A comparison of activating solutions with hatchery water in artificial insemination of rainbow trout (*Oncorhynchus mykiss*)

Abstract

In the propagation of rainbow trout, the available water supply used for incubation of fertilized egg is generally used for also spermatozoa being activated to reach eggs. The aim of the present study was to assess comparisons of the effects of two lab-made activating solutions and hatchery water on progressive sperm motility percentage (%), duration of progressive sperm motility (s), and fertilization success in artificial insemination of rainbow trout. For this purpose, an activating solution (A1) containing 60 mM NaHCO$_3$, 50 mM Tris (pH=9.0) and another activating solution (A2) containing 20 mM Tris, 30 mM glycine, 125 mM NaCl, (pH=9.0), and also hatchery water (HW) were used for activation of spermatozoa and fertilization. The average motility percentages of samples activated by HW, A1 and A2 were observed >90% with no significant differences, while the durations of progressive motility were found to be significantly different as 22.5±0.7 s, 30.0±1.4 s and 30.5±0.7 s respectively. The lowest average fertilization rate (64.6±1.4 %) was obtained using HW, while those values were 89.4±5.1 and 91.3±0.6 % using A1 and A2 respectively. Consequently, both motility durations and fertilization rates obtained by using A1 and A2 were significantly higher than those values of obtained by HW.

Key Words: Activating solutions, hatchery water, artificial insemination, rainbow trout

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Introduction

The reproductive function in teleosts fish is unique in several ways such as wide variation in the amount of spermatozoa produced, the periods during which spermatogenesis occurs differ widely (Billard, 1986). Moreover, in most of them, the sperm cells which are immotile in the testis are activated only after release into their living environment for a short period (from 30 s to several minutes) of forward motility (Scott and Baynes, 1980).

Motility of spermatozoa is the main reason affecting the fertilization success. This motility is generally initiated by osmolality in marine fish and many freshwater fish while spermatozoa of Salmonids and Acipenserids is activated by the low K⁺ concentration (5 mM) in combination with osmolality. When spermatozoa released and meet with the natural medium (fresh water, sea or brackish water) their motility are limited by some factor such as temperature and pH, ions (Cosson, 2004; Alavi and Cosson, 2005; Alavi and Cosson, 2006). Spermatozoa directly released in the natural medium are subjected to also other factors such as thermic shock, micropolluants, and toxic residues. This stage of reproduction is very susceptible to being damaged (Billard, 1988).

Spermatozoa and eggs are simply put together and available water supply (fresh or salt water) as an external medium is generally added in artificial insemination. Especially in the traditional propagation of freshwater species as a rainbow trout (Oncorhynchus mykiss), hatchery water is used for spermatozoa being activated to reach eggs (Billard and Jensen, 1996).

The present investigation was conducted with the purpose of comparing the effects of two lab-made activating solutions and hatchery water on progressive sperm motility percentage (%), duration of progressive sperm motility (s), and fertilization success in artificial insemination of rainbow trout.

Materials and Methods

Fish and collection of the gametes

Female and male rainbow trout (Oncorhynchus mykiss) individuals were obtained from a commercial fish hatchery located in Muğla, Turkey. All fish were fed with the same diet. Gametes were collected during the spawning in December since it is accepted as middle of the spawning season for the region. Six males (two years of age) and three females’ (three years of age) gametes were obtained by gentle abdominal massage, avoiding any contamination. Gamete collection does not harm the fish as a routine application in the fish farm. December is accepted as middle of the spawning season for the region. Both sperm and eggs samples were mixed and pooled samples obtained for each before the experiments.
Activating solutions, evaluation of the post-thaw motility of the spermatozoa and fertilization

An activating solution (A1, Billard, 1992) containing 60 mM sodium bicarbonate (NaHCO₃), 50 mM Tris pH=9.0 and another activating solution (A2, Lahnsteiner, 2000) containing 20 mM Tris, 30 mM glycine, 125 mM NaCl, pH=9.0, and also hatchery water (HW) were used for activation of spermatozoa and fertilization. These two solutions are commonly used in previous studies. Temperature (11.0±0.1 °C) of activating solutions was adjusted to the same of HW. Percentages of progressive motility (%) and durations of progressive motility (s) were determine under a phase-contrast microscope at 200× magnification immediately while fertilization rates were calculated using a stereomicroscope at 20× magnification eighth days after the insemination. The sperm motility percentages were estimated as the percentage of cells that exhibited progressive forward movement (Horváth, et al., 2003). The durations of motility were determined as the times until forward movement stopped and circular movement began. The percentages of sperm motility were assessed using an arbitrary scale with 10% interval increments in which non-motility was recorded as 0% (modified from Borges, et al., 2005). The evaluation of motility characteristics was performed subjectively by 3 different researchers examining 5 different microscopic fields. Three aliquots of each sample were determined by each researcher, and the average motility characteristics were then calculated and sperm samples were diluted 1:400 with A1, A2 and HW during motility measurements. Fertilization was performed at approximately 300,000:1 sperm-to-egg ratios. To adjust sperm volume, an immobilizing solution (110 mM NaCl, 28.18 mM KCl, 1.22 mM MgSO₄·7H₂O, 1.77 mM CaCl₂·2H₂O, 10.05 mM Bicine, and 9.99 mM Hepes, pH=8.2) was used (Robles, et al., 2003). A1, A2 and HW were added to the eggs in plastic cups (350 ± 20 eggs). Next, the sperm sample was immediately added and the gametes were gently mixed for 60 s. After 5 min to allow fertilization to occur, the eggs were rinsed with HW and incubated for 15 min to water-harden the eggs, and then transferred to hatchery trays supplied with constant water flowing continuously through the system. Eight days after fertilization, at least 100 eggs were taken from each replicate placed in petri dishes containing a clearing solution of acetic acid:methanol:distilled water (1:1:1 v/v/v). After a 10 min, fertilized eggs were distinguished from unfertilized eggs by the presence of a clearly back bone. The fertilization success of a sample obtained from an activating solution was estimated by calculating the percentage of fertilized eggs in relevant replicates (Geffen and Evans, 2000). Fertilization tests were carried out in triplicate for each for each activating solutions.
**Statistical analysis**

All values are represented mean±standard deviation. Statistical differences in the durations of sperm progressive and fertilization rates were tested using one-way ANOVA, following Tukey’s HSD post-hoc, at the 0.05 probability level. Statistics were performed using SPSS software version 20.0.

**Results and Discussion**

Many teleost fish spermatozoa vary from the spermatozoa of mammalian species in terms of some specific properties such as they are immotile in testis, their motility is activated by releasing into the water, and their progressive motility occurs within minutes (Kime, et al., 2001). The improvement and maintenance of this short duration of motility are critical for determining fertilization success. In general, the percentages of progressive motility (%) and the durations of progressive motility (s) which determined also in this study are the two clearest parameters for assessing sperm quality.

The average sperm density of the pooled samples was calculated as $10.46 \pm 0.50 \times 10^9$ spermatozoa/ml. The effects of different activating solutions (HW, A1 and A2) on the percentage and duration of progressive sperm motility in rainbow trout are shown in Fig. 1. No significant differences ($P > 0.05$) were found in the average motility percentages of samples activated by HW, A1 and A2 ($P > 0.05$) even though a slight increase was noted in motility percentages of A1 and A2 was noted. In contrast, the average motility durations have shown significant differences ($P < 0.05$). The highest motility durations were achieved using A1 and A2 ($30.0\pm1.4$ s and $30.5\pm0.7$ s, respectively). Those values were significantly higher than the average motility duration obtained by HW ($22.5\pm0.7$ s).

Cejko, et al., (2013) compared sperm motility parameters determined by 4 different activating solutions with those values achieved by distilled water in *Cyprinus carpio* sperm. They found some differences among motility parameters, particularly the percentage of motile sperm (82.7%) and percentage of progressively motile sperm (approximately 40%). The highest values were found sperm samples activated by a activating solution containing 100 mM NaCl and 10mM Tris, (pH=9) while other activating solutions were lower pH values and had different contents while they did not report any fertilization rates. In our study, similar to motility durations, significant difference ($P < 0.05$) in the fertilization rates was found between HW and the others (Fig. 2). The lowest average fertilization rate ($64.6\pm1.4$ %) was obtained using HW, while those values were $89.4\pm5.1$ and $91.3\pm0.6$ % using A1 and A2 respectively.
Figure 1. The percentage (A) and duration (B) of progressive motility levels in rainbow trout (*Oncorhynchus mykiss*) as a function of different activating solutions. In the box plots, different superscripts indicate the differences (P < 0.05).

Figure 2. Fertilization rates obtained from sperm samples of rainbow trout (*Oncorhynchus mykiss*) activated with different activating solutions. In the box plots, different superscripts indicate the differences (P < 0.05).

Sperm activating solutions are beneficial not just for aquaculturist, also are useful also for researchers. Obviously, motility parameters are more precisely determined using with them. The activating solutions used for spermatozoa of fish species living in marine and fresh water have different contents. Even though fish are
living the same environment, the activation solutions could be species depending. Compositions of activating solutions using commonly in activation of spermatozoa of some freshwater fish species were listed in Table 1. As seen in Table 1, osmolality of activating solutions used for freshwater fish are provided by mainly NaCl. Having regard to these studies listed Table 1, the advantages found in our study seem to arise from two eventual reasons; pH and osmolality. Optimum pH and osmolality values are key elements to trigger and improve motility (Cosson, 2004; Alavi and Cosson, 2005; Alavi and Cosson, 2006). Activating solutions has broader buffering capacity than water. Thus, these solutions are very likely to prevent pH alterations emerge from during spermatozoa activation.

Conclusions
Choosing a proper activating solution can play an extremely useful role in artificial insemination of fish species. Fertilization rates could be increased when the proper activating solutions are used instead of the hatchery water. Besides, preparation of these solutions is usually simple, rapid, and cheap as opposed to common belief. In this study, the effects of two lab-made activating solutions on sperm motility characteristics and fertilization rates were compare with those values obtained by the hatchery water during the artificial insemination of rainbow trout. As with all activating solutions designed for use with relevant fish species, they are in need of much more improvement and study. Moreover, a proper activating solution should be designated for less-studied or new species

Acknowledgement
The study was presented in 1st International Symposium on Limnology and Freshwater Fisheries, 4-6 October 2017 Eğirdir, Isparta, Turkey by poster presentation.

References


### Table 2. Compositions and pH levels of activating solutions using commonly in activation of spermatozoa of potential aquaculture freshwater fish species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Compositions of Activating Solutions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Freshwater fish</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alburnus alburnus</em></td>
<td>100mM NaCl, 10mM Tris pH 9.0</td>
<td>Lahnsteiner, et al., 1996</td>
</tr>
<tr>
<td><em>Chalcalburnus chalcoides</em></td>
<td>50 mM NaCl, 20 mM Tris pH 9.0</td>
<td>Lahnsteiner, et al., 1999</td>
</tr>
<tr>
<td><em>Clarias macrocephalus</em></td>
<td>~70 mM NaCl</td>
<td>Vuthiphandchai, et al., 2009</td>
</tr>
<tr>
<td><em>Cyprinus carpio</em></td>
<td>68 mM NaCl, 50 mM urea pH 7.7</td>
<td>Billard, et al., 1995</td>
</tr>
<tr>
<td></td>
<td>86 mM NaCl</td>
<td>Kucharczyk, et al., 2008</td>
</tr>
<tr>
<td></td>
<td>5mM KCl, 45mM NaCl, 30mM Tris pH 8.0</td>
<td>Perchec, et al., 1996</td>
</tr>
<tr>
<td></td>
<td>45 mM NaCl, 5 mM KCl, 30 mM Tris pH 8.2</td>
<td>Li, 2013</td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td>40 mM NaCl, 20 mM HEPES pH 8.5</td>
<td>Ingermann, et al., 2011</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>125 mM NaCl, 0.1 CaCl₂, 30 Tris pH 8.5</td>
<td>Cosson, et al., 1995</td>
</tr>
<tr>
<td></td>
<td>125 mM NaCl, 1 mM CaCl₂, 20 mM Tris, 30 mM glycine pH 9.0</td>
<td>Billard, 1977</td>
</tr>
<tr>
<td></td>
<td>140 mM NaCl, 90 pH 9.0</td>
<td>Ubilla and Valdebenito, 2011</td>
</tr>
<tr>
<td><em>Sander lucioperca</em></td>
<td>120 mM NaCl, 119 mM NaHCO₃, 0.5% BSA</td>
<td>Jarmolowicz, et al., 2010</td>
</tr>
<tr>
<td><em>Silurus glanis</em></td>
<td>17 mM NaCl, 5 mM Tris–HCl pH 8.0</td>
<td>Linhart, et al., 2005</td>
</tr>
</tbody>
</table>

*BSA: bovine serum albumin*
Klinoptilolit kuzularda giardiazis sağaltımına yönelik alternatif ve doğal bir çözüm olabilir mi?

May clinoptilolite be an alternative and natural solution for the treatment of giardiasis in lambs?

Özet
Amaç: Bu saha çalışması giardiasisli kuzularda oral yolla uygulanan klinoptilolitin etkinliğinin belirlenmesi amacıyla gerçekleştirilmiştir.


Bulgular: Kontrol ve sağaltım grupları arasında karşılaştırmada 10. günde klinoptilolit ile sağaltılan grupta kist atılımında % 97,2'lik azalma belirgindi (p 0˂0,05).

Sonuç: Giardiazisli kuzularda oral yolla 10 gün uygulanan klinoptilolitin kist atılımını etkileşimi gözlemlendi ve antiparaziter etkinliği sağladığı görüldü.

Anahtar kelimeler: Kuzu, giardiazis, klinoptilolit

Abstract
Objective The aim of this field study was to detect the therapeutic efficacy of oral clinoptilolite in lambs with giardiasis.

Methods: A total of fourteen lambs naturally infected with G. duodenalis at the age of 23 to 56 days, of both sexes were enrolled into the study from a lamb farm located in Aydin province. Diagnosis was based on rapid diagnostic test kits with ELISA principle. Lambs with giardiasis were randomly assigned into two groups (n=7), group I [1 g/kg clinoptilolite for 10 days or group II [were left as control without receiving any drug]. Therapeutic efficacy of clinoptilolite was determined by microscopical examination of faecal samples collected rectally on days 0 and 10.

Results: Comparison of control and treatment intragroups revealed 97.2% reduction (p 0˂0,05) for cyst excretion in clinoptilolite treated group.

Conclusion: In conclusion, it may be safely suggested that oral clinoptilolite administration for 10 days effectively reduce cyst excretion with antiparasitic efficacy among lambs with giardiasis.

Key Words: Lamb, giardiasis, clinoptilolite
Giriş


Bu saha çalışması giardiasisli kuzularda oral yolla uygulanan klinoptilolitin etkinliğinin belirlenmesi amacıyla gerçekleştirildi.

**Materyal ve Metot**


**Gruplandırma**

Giardiazisli kuzular her grupta n=7 olarak rastgele I. gruba [1 g/kg dozda 10 gün oral yolla kl (Natmin-9000) uygulanırken] ya da II. gruba [ilaç uygulaması yapılmaksızın kontrol gruba] ayrıldı. Klinoptilolit etken maddesini içeren 40 mikronize toz halindeki preparat 1’e 10 oranında sulandırılarak orogastrik sonda aracılığıyla verildi. Uygulama esnasında çalışmanın yer alan araştırmacıların tamamı ilgili analiz günlerin de işletmeye bizzat iştirak etmiş, aktif rol dağılımı teşkil etmişlerdir.

**Dışkı analizleri**

 Çalışmanın ilk aşamasında ve gruplara rastgele dağılım öncesi dışkıda *G. duodenalis*’e ait kist ve trofozoitlerinin saptanması için rektumdan direkt alınan dışkı numuneleri iki ince yama fotro hakraran flotasyon ile *Gi. boyama* ile incelendi (15). Kuzularda kl sağaltımının etkinliği 0. ve 10. günlüklerde elde edilen dışkı numuneleri mikroskobik muayenesiyle tespit edildi. Kuzulardan elde edilen dışkı numuneleri potasyum dikromat çözeltisi aracılığı ile mikroskobik muayene aşamasına kadar +4°C’de tutuldu (15). Müteakip 1.18 dansite çinko sülfat flotasyon ile muamele edilerek ×40 veya ×100 büyümekte gram dışkı kist sayısına bakıldı. Kist atılımındaki değişikliklerin yüzdesi ilgili ve onceden gerçekleştirilmiş yayınlar eşliğinde (16, 17) geometrik ortalamannın Henderson Tilton formülüne (18) işlemlmesiyle hesaplandı.
İstatistiksel analizler
Gram dışkı başına kist sayları göz önünde bulundurularak, analizi gerçekleştirilen değerlerin geometrik ortalama tanımlayıcı istatistiksel analizler ile yorumlandı. Klinoptilolit sağaltım ve kontrol gruplarının 0. ve 10. günlerdeki kist sayıları arası farklılıkların belirlenmesinde Friedman testi, her bir gün için gruplar arası farklılıkların değerlendirilmesinde ise Mann Whitney U testi kullanıldı. İstatistiksel analizlerin gerçekleştirilmesinde SPSS 15.0 paket programından yararlanıldı.

Bulgular
Analizler başlamadan hemen öncesinde her 2 grupta ishal mevcuttu. Hızlı test kitleri ile dışkı analizlerinde giardiazis dışında farklı bir patojen rastlanmadı. Sağaltım grubundaki kuzuların 2. günden itibaren dışkı kıvamlarının normal olduğu, kontrol grubunda dışkı karakterinde yumuşamanın 7.-8. günler kadar devam ettiği gözlandı. Sağaltım grubundaki hayvanlarda kl uygulamasına ilişkin herhangi bir yan etki (özellikle konstipasyon ya da sıvı elektrolit kayıbı) görülmedi. Klinik ve hem de parazitolojik kür (dışı kafa tekrarlayan mikroskopik muayene) kl uygulanan hayvanlarda belirgin. Kontrol grubundaki hayvanlar çalışma bitikten sonra etik kurallar çerçevesinde standart anti-giardial sağaltıma (seknidazol 30 mg/kg oral tek dozda) tabi tutuldu. Gerek kontrol gerekse sağaltı gruplarından kizilorda 0. ve 10. günlerde belirlenen kist atılımının geometrik ortalama ve sağaltı sonrası belirlenen kist atılmındaki azalma yüzdesini Tablo 1’de sunuldu. Kontrol ve sağaltı gruptarında 0. gruba grupta kist atılmında %97,2’lik azalma belirgin (p 0<0,05).

Tartışma

Klinoptilolitin doğal yolla oluşan giardiazisli 16 oğlakta karşı sağaltı etkinliğinin belirlendiği yakından zamandaki bir çalışmada I. gruba 1 g/kg dozda 10 gün oral yolla kl uygulanırken, II. gruptaki hayvanlar plasebo kontrol grubu olarak bırakılmıştır. Uygulama yapılan grupta giardiazise karşı 10. günde kist atılımında %95 etkinlik ile azalma sağlanmasına karşın, kontrol grubunda 0. gün ile (165832) 10. günlerde (162096) kist atılmının geometrik ortalamanın düşüşlerin belirgin olmadığı saptanmıştır (15). Yine bir başka araştırma ishalli ve G. duodenalis ile doğal enfekte buzağılarda [I. grupta 1 g/kg dozda 10 gün kl uygulanırken, II. grupta herhangi ilaç uygulaması yapılmadı] kl 7. günde %74, 14. günde %84 etkinlik sağlanmıştır (22). Her 2 çalışmada da gerek oğlak (15), gerekse buzağılarda (22) giardiazise karşı oral yolla 10 gün uygulanan klinoptilolitin kist atılmında azalmaya sebep olabileceğini belirtilmiştir.

Sonuç
Giardiazisli kizilorda oral yolla 10 gün uygulanan klinoptilolitin kist atılmını etkin şekilde azalttiği ve antiparaziter etkinlik sağladığı görüldü
Tablo 1: Kontrol ve sağaltılm gruplarındaki kuzularda 0. ve 10. günlerde belirlenen kist atılımlarının geometrik ortalaması ve sağaltım sonrasında belirlenen kist atılımındaki azalma yüzdesi.

<table>
<thead>
<tr>
<th>Kontrol</th>
<th>0. gün</th>
<th>10. gün</th>
<th>Sağaltılm</th>
<th>0. gün</th>
<th>10. gün</th>
</tr>
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<tr>
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<td>244840</td>
<td>25258</td>
<td>Örnek 1</td>
<td>170480</td>
<td>6</td>
</tr>
<tr>
<td>Örnek 2</td>
<td>160230</td>
<td>15256</td>
<td>Örnek 2</td>
<td>160083</td>
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<tr>
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<td>220060</td>
<td>23060</td>
<td>Örnek 3</td>
<td>224048</td>
<td>10</td>
</tr>
<tr>
<td>Örnek 4</td>
<td>192056</td>
<td>22042</td>
<td>Örnek 4</td>
<td>216075</td>
<td>8</td>
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<tr>
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<td>26341</td>
<td>Örnek 5</td>
<td>182540</td>
<td>6</td>
</tr>
<tr>
<td>Örnek 6</td>
<td>180802</td>
<td>18420</td>
<td>Örnek 6</td>
<td>201040</td>
<td>8</td>
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<tr>
<td>Örnek 7</td>
<td>272407</td>
<td>29048</td>
<td>Örnek 7</td>
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<td>6</td>
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<tr>
<td>Ortalama</td>
<td>218061</td>
<td>22775</td>
<td></td>
<td>191808</td>
<td>7</td>
</tr>
</tbody>
</table>

Kist saçılımındaki azalma (%): 99,97%

Kaynaklar


Ayan ve ark. / (VetBio), 2018, 3(2), 16-20


Marmara ve Karadeniz Bölgesi istavrit karaciğerinde GPx, SOD, CAT enzim aktiviteleri ve vitamin A değerleri*

GPx, SOD, CAT enzyme activities and Vitamin A values in the Marmara and Black Sea Region Scad’s Liver

Abstract

The amount and number of pollutants causing marine pollution are increasing day by day. The use of antioxidant defense system components as biomarkers in aquatic species is preferred for early detection of contamination. In this study, the activities of GPx, SOD and CAT enzymes and vitamin A status in the liver of scad (Trachurus trachurus) from Black Sea and Marmara Sea pelagic fishes were investigated. Samples were collected March (1st period) and November (2nd period).

Compared to the Marmara Sea scads, the second term GPx activity was increased according to the 1st rotation (p ≤ 0.01) and the 2nd Term vitamin A level was found to be lower than the 1st rotation (p ≤ 0.05). In the comparison of Marmara and Black Sea, GPx activity was found higher in Black Sea scad’s compared to Marmara (p ≤ 0.05).

CAT activity and vitamin A level in both periods was lower in the Black Sea samples than in Marmara (CAT: 1st period $p \leq 0.01$, 2nd period $p \leq 0.05$; vitamin A: 1st period $p \leq 0.05$, 2nd period $p \leq 0.01$).

Antioxidant system parameters, which are biomonitor of pollution in marine fishes, the study of pelagic fish between carnivorous and herbivorous species, between surface and bottom fish species, migratory fishes and nonimmigrant sea fish both within and between them will enable us to obtain more detailed information about the antioxidant system of our marine fishes and to consume healthier seafood. In this study, the relationship between marine pollution and pelagic fish’s antioxidant metabolism has been investigated and in terms of the obtained data, it can be a source of work to be done in this respect.

**Key Words:** Scad, Liver, GPx, SOD, CAT, Vitamin A, Marmara Sea, Black Sea.

**Giriş**


Çevre kirliliğinin takibi ve tespitinde kullanılan birçok biyokimyasal teknik geliştirilmiş, canlı organizmaların daha önce tanılamama kapalı olan biosellüler bileşenler, sub-lethal biyolojik cevapla ilgili ölçülen tüm parametreler biyobelirteç olarak tanımlanmıştır (Napierska ve


Bu çalışmada, ekonomik açıdan yüksek değer taşıyan yüzey balıklarından İstavritlerde (Trachurus trachurus) kirlilik biyobelirteçlerinden GPx, SOD, CAT enzym aktiviteleri ve vitamin A düzeylerinin belirlenmesi amaçlanmıştır.

**Materiyal ve Metot**

Araştırmada, Carancidae familyasına ait İstavritler (Trachurus trachurus) kullanıldı. Örnekler, Marmara Ereğlisi’nin 2.5 mil açığında 41. kuzey enlemi ve 28. doğu boylamlarında kalan bölgeden, Karadeniz’de Sarıkum’un 1.26 mil açığında 42. kuzey enlem ve 34. doğu boylamları arasındaki bölgenin Gırgır yöntemi kullanarak mart ve kasım aylarında iki kez toplandı. Her iki bölgeden; her toplama dönemi her bir numune için ortalama 30-40 balığın karaciğerini içeren havuzlar oluşturularak 15 istavrit karaciğer örneği toplandı. İki dönemdeki toplam örnek sayısı 60’dır.

Yakalanan balıkların iç organları soğuk-buzlu ortamda çıkartıldı ve karaciğerleri fizyolojik tuzuyla suya yıkandı. Yakıma işlemi takiben 10 g’lık karaciğer örnek havuzlarından enzim analizleri için 5 g ve vitamin analizleri için ise, kalan diğer 5 g karaciğer örneği numaralandırılmış ayrı poşetlere konuldu ve alüminyum folyo ile sarılarak sıvı nitrojen tankında depolanır. Nakil işlemlerine sıvı nitrojen tankıyla gerçekleştirilir ve analiz işlemlerine kadar –80 °C soğutucu muhafaza edildi.


Gruplar arası farklılığın öneminin istatistik hesaplanmasında Eş yapma ve t-testi (Esin ve ark. 1997) kullanılmıştır.

**Bulgular**

Marmara ve Karadeniz’den mart ve kasım aylarında toplanan istavrit bahşı karaciğer dokusu SOD, CAT ve GPx aktiviteleri ile vitamin A düzeyleri Tablo 1’de verilmiştir.
Analiz sonuçlarına göre; dönemler karşılaştırıldığında, Marmara Denizi’nden toplanan örneklerin 2. Dönem GPx aktivitesi 1. Döneme göre artmış (p ≤ 0.01), 2. Dönem Vitamin A düzeyi 1. Döneme göre düşük (p ≤ 0.05) bulunurken, Karadeniz örneklerinde iki dönem arasında istatistik bir önem tespit edilememiştir.

Denizler karşılaştırıldığında; her iki dönemde de Karadeniz örnekleri vitamin A düzeyi (1.Dönem p ≤ 0,05, 2. Dönem p ≤ 0,01) ve CAT aktivitesi (1.Dönem p ≤ 0,01, 2. Dönem p ≤ 0,05) Marmara’dan düşük, GPx aktivitesi 1. Dönem Marmara’dan yüksek (p ≤ 0,05) bulunmuştur.

**Tartışma**


grubunda (kırleticiyeye maruz kalmayan balıklar) ise mevsimsel değişim antioksidan enzimlerin aktivitesini etkilemediğini tespit etmişlerdir. Ağır metallerin balıklarda oksidatif stres oluşturduğu artan, radikallerin balıkların antioksidan savunmasını yetersiz bıraktığını ve ısı artışının oksidatif stresi arttırıldığını bildirmişlerdir.

Bu çalışmada denizler karşılaştırıldığında; her iki dönemde de Karadeniz örnekleri vitamin A düzeyi ve CAT aktivitesi Marmara'dan düşük, GPx aktivitesi mart ayında Marmara'dan yüksek bulunmuştur. Mart ayında istatistiki önem GPx \( p \leq 0,05 \), CAT \( p \leq 0,01 \) ve vitamin A \( p \leq 0,05 \) olup, kasım ayında CAT \( p \leq 0,05 \), vitamin A düzeyi \( p \leq 0,01 \) olarak bulunmuştur. Çalışmada GPx aktivitesinin yüksek CAT aktivitesi ve vitamin A düzeyinin düşük olması Vinodhini ve Narayan (2009), Padmini ve ark. (2008)'ın çalışması ile benzerlik göstermektedir. Karadeniz’in oksijence zengin tabaka (10-50 m)'sında çözünmüş Cu ve Zn yüksek konsantrasyonlarda bulunur (Anonim 2000). Karadeniz'deki muhtemel petrol ve petrol ürünleri, herbisit ve ağır metaller istavritlerde oksidatif stresi indüklemiştir.


Marmara örneklerinde GPx aktivitesinin mart ayında Karadeniz'den düşük, her iki dönemde de CAT aktivitesinin ve vitamin A düzeyinin yüksek bulunması; çevresel faktör ya da kırleticilere indükленen oksidatif stresi Marmara İstavritleri antioksidan savunma sistemi Karadeniz istavritlerine göre daha iyi kompanse etmiş olabilir.

Kaynaklar


Stoliar, O.B., Lushchak, V.I. (2012). Environmental Pollution and Oxidative Stress in Fish.

Erişim tarihi: 15.02.2018


Tablo 1. Mart (Dönem 1.) ve Kasım (Dönem 2.) dönemleri Marmara ve Karadeniz (denizler ve dönemler arası) istavrit karaciğer örneklerine ait GPx, SOD, CAT aktiviteleri ve vitamin-A düzeyleri

<table>
<thead>
<tr>
<th>Dönem (Dönem 1)</th>
<th>GPx (nmol NADPH+H+/dakika/mg-protein)</th>
<th>SOD (U/g-protein)</th>
<th>CAT (k/g-protein)</th>
<th>Vitamin-A (µg/g-yaş ağırlık)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmara</td>
<td>Karadeniz</td>
<td>Marmara</td>
<td>Karadeniz</td>
<td>Marmara</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>x ± Sx</td>
<td>3,082 ± 0,61\text{a}</td>
<td>6,74 ± 1,91\text{*a}</td>
<td>0,0848±0,036\text{a}</td>
<td>0,0282±0,0052\text{-a}</td>
</tr>
<tr>
<td>Dönem (Dönem 2)</td>
<td>x ± Sx</td>
<td>19,33 ± 2,24\text{b}</td>
<td>14,83 ± 1,90\text{-a}</td>
<td>0,0576±0,0071\text{a}</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>a-b: p&lt;0,01</td>
<td>a-a: p&gt;0,05</td>
<td>a-a: p&gt;0,05</td>
<td>a-a: p&gt;0,05</td>
<td>a-b: p&gt;0,05</td>
</tr>
</tbody>
</table>

Denizler arası karşılaştırılmasında (yatay-satır) istatistiksel açıdan fark - (p ≥0,05), *: (p ≤ 0,05), **: (p ≤ 0,01) ile gösterilmiştir.

Dönemler arası karşılaştırılmasında (Dikey-her sütun için ayrı ayrı) istatiksel farklılıklar, farklı harfler ile gösterilmiştir.
Karadeniz barbunya balığının (*Mullus barbatus ponticus*) ilk üreme boyunun tahmini

Estimation of size at first maturity of Black Sea red mullet (*Mullus barbatus ponticus*)

**Abstract**

The objective of this study is estimation of the first maturity size of Black Sea red mullet (*Mullus barbatus ponticus*) that is commercially important fish species. In total, 229 of samples were collected and measured with their total length, body weight, sex and maturity during the period from September 2016 to March 2017. Sex ratio (F/M) is determined as 1:0.85. Average total lengths for females and males were estimated as 11.62 ± 0.114 cm and 11.07 ± 0.116 cm, average body weight as 16.30 ± 0.475 g and 13.96 ± 0.457 g respectively. Length weight relationships were estimated for females as \( W = 0.0112 L^{2.954} \), for males as \( W = 0.0098 L^{3.0045} \) and for all samples as \( W = 0.0102 L^{2.9903} \).

Size at first maturity was estimated as 10.73 cm for females and 10.95 cm for males while 10.88 cm for all specimens. Proportion of fish that are smaller than length of maturity was determined as 37.6 % (86 adet) of the sampled fish. The determining the larger minimum landing size both computed values for female and male, is recommended and it can be useful for sustainable fisheries management of red mullet stocks in Black Sea.

**Keywords:** Red mullet, size at first maturity, fisheries management, sustainable fisheries
Giriş
Avcılık yoluyla işletilen balık stoklarının korunmasında türün ilk üreme boyunun (LM) belirlenmesi önemli bir rol oynar. İlk üreme boynu balıkçılık yönetiminde önemli bir araç olup ebeveyn stok büyüklüğü, ilk avlama boyu (MLS) ve en küçük ağ gözü açıklığı gibi stokların durumunun değerlendirilmesi ve doğru işletilmesi açısından önemli bilgiler sağlar. Zira balık popülasyonları sınırlıdır ve stokların sürdürülebilirlikleri onların üretimine bağlıdır (Holden ve Raitt, 1974).

Dünyada avcılıkta elde edilen su ürünleri üretim miktarı son 30 yılda nispeten sabit kalmış olup stoklardan elde edilecek azami ürün miktarı sınırına ulaşıldığı iddia edilmektedir. Ancak balıkçı gemileri ve av araçlarından oluşan balık avlama çabası hala dünya çapında artmaya devam etmektedir (FAO., 2016).


Bu araştırmada Karadeniz barbunya balığı açısından çok önemli bir tür olan barbunya balığının bazı popülasyon parametreleri ve ilk üreme boynunun belirlenmesi yoluyla stokun sürdürülebilir bir şekilde işletilmesi için gerekli olan ilk avlama boyu ve minimum ağ gözü açıklığı çalışma oranları katkı sağlanması amaçlanmıştır.

Materyal ve Yöntem
Çalışmanın esas materyalini Sinop ve çevresinden uzatma ve trol ağlarıyla avlanan ve Sinop tezgâhlarında pazarlanan Karadeniz barbunya (Mullus barbatus ponticus Essipov, 1927) türünden Eylül 2016- Mart 2017 tarihleri arasında örneklenen toplam 219 birey oluşturmaktadır.

Örneklenen balıklarda total boy 1 mm hassasiyetli ölçüm tahtası kullanılarak, vücut ağırlığı ise 0.1 g hassasiyetli elektronik terazi ile ölçülmüştür. Daha sonra örnekler diseksiyona tabi tutularak cinsiyet ve eşeysel olgunluk durumu belirlenmiştir. Bu amaçla gonatların rengi, kesiti, vücut boşluğunda kapladığı alanı ve dokusu incelenmiş, tereddüt durumunda stereo mikroskop altında incelenmiştir. Örnekler içinde farklı cinsiyeteki bireylerin boy ortalamaları arasında görülen farkın istatistiksel değerlendirilmesinde T test (P=0,05) kullanılmıştır.

İlk üreme boyu (L₅₀) logaritmitk regresyon ile belirlenmiştir. Modelde göre bireylerin %50’inin eşeysel olgulunla eriştiği boy (L₅₀) ilk üreme boyuna (L₄₃) eşittir. Herhangi bir boy sınıfındaki olgun bireylerin oranı (P₅₀) ile balık boyu arasında; P₅₀=1/(1+exp (a+bTL)), şeklinde sigmoid bir ilişki vardır (Piñeiro ve Sainza, 2003). Denklemi doğrusal hale getirmek amacıyla Ln [(1/P₅₀) - 1] değerleri boy sınıflarına (TL) karşılık verilmiştir.
Burada elde edilen katsayılar aracılığıyla ilk üreme boyu hesaplanır.

\[ L_M = -a/b \]

Hesaplamalar, istatistiksel testler ve grafikler MS Excel yazılımı kullanılarak hazırlanmıştır.

**Bulgular**

**Populasyon özellikleri**

Araştırmada 124 adedi dişi, 105 adedi erkek olmak üzere toplam 229 balık örneklenmiş olup boy aralığı 8,7 – 14,4 cm arasında, vücut ağırlıkları 6,4 – 29,4 arasında değişmiş (Şekil 1.) ve cinsiyet oranı (dişi / erkek) 1:0,85 olarak hesaplanmıştır.

![Şekil 1: Örneklenen balıkların cinsiyete göre boy dağılımları.](image)

Ortalama tam boy dişi bireyler için 11,62 ± 0,114 cm, erkek bireyler için ve 11,07 ± 0,116 cm olarak hesaplanırken tüm bireyler için 11,37 ± 0,084 cm, ortalama vücut ağırlığı dişiler için 16,30 ± 0,475 g, erkek bireyler için 13,96 ± 0,457 g ve tüm bireyler için 15,23 ± 0,340 g olarak hesaplanmıştır. Farklı cinsiyetten balıkların ortalama boy ve ağırlıkları arasında gözlenen farklı istatistiksel açıdan önemsiz (P>0,05) olduğu belirlenmiştir.

Tüm bireylere ait boy ağırlık ilişkisi denklemi \( W = 0,0102 L^{2,9903} \) (R=0,979) olarak belirlenmiştir (Şekil 2.). Dişilere ait denklem \( W=0,0112 L^{2,954} \) (R=0,978) ve erkekler için \( W = 0,0098 L^{3,0045} \) (R=0,979) olarak tahmin edilmiştir (Şekil 3.).


**ŞEKİL 2:** Örneklenen tüm bireylere ait boy – ağırlık ilişkisi.

**ŞEKİL 3:** Dişi ve erkek bireylere ait boy ağırlık ilişkisi.

**İlk üreme boyu**

Farklı cinsiyetler için ilk üreme boyunda farklılık olup olmadığını belirleme amacıyla önce her iki cinsiyetten bireyler ayrı ayrı değerlendirilmiş ve yapılan t testi sonucunda üreme boyu bakımdan
cinsiyetler arasında gözlenen farkın istatistiksel açıdan önemsiz (P>0,05) olduğu belirlenmiştir (Tablo 1.).

Erişkin bireylerin toplam balık sayılarına oranları (P_L) hesaplandından sonra Ln [(1/P_L)-1] değerleri ile boy sınıfları arasındaki regresyon denkleminin kesme noktaları (a) ve eğimleri (b) belirlenmiştir (Şekil 4. ve Şekil 5.).

<table>
<thead>
<tr>
<th>Boy Sınıfı (cm)</th>
<th>Dişi Ge</th>
<th>Olg</th>
<th>Topl</th>
<th>Erkek Ge</th>
<th>Olg</th>
<th>Topl</th>
<th>Genel Ge</th>
<th>Olg</th>
<th>Topl</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>9</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>13</td>
<td>18</td>
<td>1</td>
<td>19</td>
</tr>
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<td>10</td>
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<td>25</td>
<td>4</td>
<td>29</td>
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<td>11</td>
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<td>14</td>
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<td>26</td>
<td>33</td>
<td>33</td>
<td>66</td>
</tr>
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<td>2</td>
<td>26</td>
<td>28</td>
<td>4</td>
<td>19</td>
<td>23</td>
<td>6</td>
<td>45</td>
<td>51</td>
</tr>
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<td>13</td>
<td>1</td>
<td>18</td>
<td>19</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td>1</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Toplam</td>
<td>43</td>
<td>81</td>
<td>124</td>
<td>55</td>
<td>50</td>
<td>105</td>
<td>98</td>
<td>131</td>
<td>229</td>
</tr>
</tbody>
</table>

**ŞEKİL 4:** Dişi ve erkek balıklar için ilk üreme boyunun tahmini.
ŞEKİL 5: Tüm bireyler için ilk üreme boyunun tahmini.

Tüm bireyler için balık boyu ile ergin birey oranı arasındaki ilişki;

\[ P_L = \frac{1}{1 + \exp (17,621 - 1,6219TL)} \]

(Şekil 6.).

ŞEKİL 6: Tüm bireyler için boy sınıflarına göre ergin bireylerin oranı.
Şekil 4 ve 5’den elde edilen katsayılar kullanılarak ilk üreme boyu dişiler için 10,73 cm, erkek bireyler için 10,95 cm olarak hesaplanırken her iki cins için ortak değer 10,88 cm olarak tahmin edilmiştir.

Örneklenen balıkların içerisinde 10,9 cm’den küçük balıkların oranı %37,6 (86 adet) olarak belirlenmiştir.

Tartışma ve sonuç
Karadeniz barbunya balığının (Mullus barbatus ponticus) diğer denizlerimizde yaşayan barbunya balıklarından hem morfolojik hem de biyolojik özellikleri bakımından farklı olduğu çeşitli araştırmalarla ortaya konmuştur (Turan, 2006; Keskin ve Can, 2009; Vasiljeva, 2012). Dolayısıyla türün avcılığına getirilecek düzenlenmelerin buna göre yapılması gerekmektedir. İlk üreme boyu Ege Denizinden avlanan barbunya balıkları (Mullus barbatus barbatus) için 12,9 cm çatal boy (Akyol vd., 2000), dişi ve erkekler için 11,9 cm ve 12,1 cm total boy (Arslan ve İşmen, 2014), 11,56 cm total boy (İlkyaz vd., 2018) olarak tahmin edilmiş olup değerler bu çalışmada elde edilen değerden (10,88 cm) büyüktür. Anılan çalışmalarla türün stoklarının sürdürülebilir şekilde işletilmesi için ilk avlama boyunun 12 ve 13 cm olarak önerilmiş olup Karadeniz barbunyasının pazarındaki fiyat oluşumu ve avcılık baskı dikkate alındığında yasal düzenlenmelerde asgari avlama boyunun 12 cm olarak uygulanması yararlı olacağını söylenebilir.

Örneklenen balıklar içerisinde belirlenen ilk avlama boyundan küçük balıkların oranı %37,6 olarak hesaplanmış olup, önerilen 12 cm’den küçük balıkların oranı ise %58,5 (134 adet) dir. Südürülebilir ve sorumlu balıkçılık açısından av araçlarının seçiciliğinin küçük balıkları avlamayacak şekilde düzenlenmesi ve denetimlerin artırılması zorunludur.

Teşekkür: Bu çalışmayı SÜF - 1901-15-02 numaralı proje olarak destekleyen Sinop Üniversitesi, Bilimsel Araştırma Projeleri Koordinatörlüğü’ne teşekkür ederim.

Kaynaklar


L-lactate levels in calves with pneumonia

Abstract

Reliable prognosis and rational treatments are needed to reduce the economic loss of the Pneumonia in beef cattle industries. This study was conducted with the assumption that blood L-lactate concentration could be an important marker in assessing the prognosis and physiopathological status of respiratory diseases (literature supported). In this context, the L-lactate levels of 22 calves with pneumonia (under field conditions and without etiology) were evaluated. It was observed that 4 patients with L-lactate levels above 4 mmol / L did not respond to treatment and those with a L-lactate below 4 mmol / L remained clinically resolved.

Key Words: L-lactate, pneumonia, beef calves.
Giriş

Olgu Öyküsü

Hasta hayvanlara sağaltım amacı ile flunixin meglofin (Flumed), Florfenikol (Nuflor), Vitamin C (Vit C Sanovel) uygulamaları yapıldı. Sağaltım uygulamalarına cevap veren hayvanlar da antibakteriyel sağaltım değiştirilerek Enrofloksasin (Baytril) uygulaması gerçekleştirilmiştir. Yapılan tüm sağaltım uygulamalarına yanıt vermeyen hastaların ise (n=4) öldüğü bildirildi.

L-laktat ölçümlerinin değerlendirilmesinde hastalıkların şiddet ve mortalite oranlarının düşürülmesi, irreverzible lezyonlar ile sağaltım maliyetlerinin de azaltılması önem arz ettirir (Luekeux, 1995). Bu kapsamda yapılan sınıflandırmalar grup-1 hastalığına benzer bir inflamatuvar yanıtın oluşturduğu, grup-3 kompanzo edilemeyecek belirgin bir inflamatuvar yanıtı oluştugu, grup-3 kompanzo edilemeyen klinik hastalık tablosunu gösteren ve ortaya çıkan inflamatuvar
reaksiyonların şiddetli etkisine bağlı fonksiyonel kayıbı ağırlaştıran (pozitif feed-back prensibi) ve son olarak grup-4 ise irreverzibl klinik hastalığı olan şeklinde değerlendirilmektedir. Patojenler tarafından oluşturulan pulmoner lezyonlar, inflamatuvar hücreler tarafından salınan serbest oksijen radikalleri ve/veya proteolitik enzimler veya proinflamatuvar mediyatörlerinin indüklediği mekaniksel bozukluklar hayvanların performans düzeylerini ve hatta hayatta kalmışını tehdit etmektedir. Ne yazık ki, bir hekim için, bu sınıflandırmayı kapsayan koşulları klinik muayene ile belirlememeyi yapmak yerine, belirlemek için daha objektif parametrede mevcut değerlendirmektedir (Coghe vd., 2000). Bu çalışmada bahsi geçen işletme bulunan hastaların klinik muayeneleri sonucunda hastalık semptomu gösteren ve göstermemeyen hayvanların varlığı ile yapılan bu nicel değerlendirmede hemen tüm gruplar içerisinde sınıflandılabilecek olguların bulunduğu belirlendi.

Kan L-laktat düzeyi anaerobik metabolizmayı destekleyen fizyolojik ve patolojik fenomenlerin etkisini değerlendirmek için yaygın olarak kullanılan bir biyobelirteç olup özellikle beşeri hekimlikte yaygın şekilde kullanılmaktadır (Evans vd., 1993; Harkins vd., 1993; Hartmann ve Mader, 1994; Pansold ve Zinner, 1994; Schwarz, 1994).

Tablo 1: Pnomonili danaların L-laktat seviyeleri
Table 1: L-laktat levels of Calves with pneumonia

<table>
<thead>
<tr>
<th>Vaka No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laktat (mmol/L)</td>
<td>2.05</td>
<td>2.04</td>
<td>5.10</td>
<td>6.64</td>
<td>2.87</td>
<td>2.06</td>
<td>1.03</td>
<td>1.35</td>
<td>2.66</td>
<td>2.81</td>
<td>1.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaka No</th>
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<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laktat (mmol/L)</td>
<td>2.67</td>
<td>5.63</td>
<td>1.6</td>
<td>3</td>
<td>5.57</td>
<td>2.88</td>
<td>1.33</td>
<td>7.73</td>
<td>2.05</td>
<td>1.31</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Kaynaklar


Harkins, J. D., Beadle, R. E., Kamerling, S. G. (1993). The correlation of running ability and physiological variables in


