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KOCATEPE VETERINARY JOURNAL, 2017: 10 (1) March

Page

RESEARCH ARTICLES

REVIEWS

CASE REPORT

LETTER TO THE EDITOR

Kocatepe Veterinary Journal

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RESEARCH ARTICLE

Determination of Changes in Some Biochemical Parameters and Oxidant-Antioxidant Balance After Food Intake in Sheep

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ABSTRACT

Daily variations in the concentration of urea, glutamine, glucose, cortisone, malondialdehyde (MDA) and antioxidant potential (AOP) were evaluated in the blood of sheep fed once a day. The study was carried out on 10 Anatolian Merinos sheep, average 1.5 years old and healthy. Animals were fed once per day with 61.3 % forage and 38.7 % concentrate mixture throughout the experimental period for 15 days. The blood samples were taken from the animals at first hour before and at 1, 3, 5, 7, 9, 12, 18 and 24th hours after feeding. Urea concentration decreased at first hour after feeding compared to before feeding and started augmentation after that and reached to levels before feeding at 12th hour after feeding. Glucose concentration increased at first hour after feeding compared to before feeding and started decline after that and reached to levels before feeding at 5th hour after feeding. Glutamine and cortisone concentrations decreased from at first hour to at 7th hour after feeding compared to before feeding and reached to levels before feeding at 7th hour after feeding. MDA levels decreased at first and 5th hours after feeding compared to before feeding and started increasing after 7th hour and reached significantly higher levels than before feeding in samples taken from at 12th hour to at 24th hour after feeding. While AOP increased at 3th and 9th hours after feeding, it decreased at 5, 7 and 12th hours after feeding. We concluded that the blood metabolic profiles and oxidant and antioxidant balance in sheep vary depending on time after feeding. In order to maximize the diagnostic value of these the blood metabolic profiles, the most suitable time for blood collection seems to be just before the feeding in sheep fed once a day.

Key Words: Antioxidant, Cortisone, Glutamine, Metabolic profile, Oxidant, Sheep.

Koyunlarda Yemlemeden Sonra Kan Oksidan-antioksidan Denge ile Bazı Biyokimyasal Parametrelerdeki Değişimlerin Belirlenmesi

ÖZ

Bu proje çalışması, koyunlarda beslemeden sonra geçen zamana bağlı olarak kan metabolik profili ile oksidan-antioksidan dengede meydana gelen değişiklikleri tespit etmek amacıyla yapıldı. Araştırma; ortalama 1,5 yaşlı, sağlıklı 10 Anadolu Merinosu koyunda gerçekleştirildi. Hayvanlar 15 gün süren deneme boyunca % 61.3 kaba yem ve % 38.7 karma yemden oluşan rasyonla günde bir kez beslendiler. Hayvanlardan besleme öncesi ve beslemeden sonraki 1, 3, 5, 7, 9, 12, 18 ve 24. saatlerde kan örnekleri alındı. Kan örneklerinde, üre, glikoz, glutamin ve kortizol düzeyleri ile malondialdehid (MDA) ve antioksidan potansiyel (AOP) belirlendi. Üre düzeyleri besleme öncesine göre besleme sonrası ilk saatte düştü, bu saatten sonra artmaya başladı ve besleme önceki düzeylerine beslemeden sonraki 12. saatte ulaştı. Glikoz düzeyleri besleme öncesine göre beslemeden sonraki ilk saatte önemli oranda arttı, bu saatten sonra düşerek beslemeden sonraki 5. saatte besleme öncesi düzeylerine ulaştı. Glutamin ve kortizol düzeyleri besleme öncesine göre beslemeden sonraki ilk saatten 7.saate kadar önemli düzeyde düşüş gösterdi ve beslemeden sonraki 7.saatte besleme öncesi düzeylere ulaştığı bulundu. MDA düzeyleri beslemeden sonraki ilk ve 5. saat örneklerinde önemli düzeyde düşüş gösterdi ve 7. saatten sonra artmaya başlayarak 12 saatten 24. saate kadar alınan örneklerde önemli düzeyde arttığı tespit edildi. AOP beslemeden sonraki 3. ve 9. saatlerde önemli oranda artarken, 5, 7 ve 12. saatlerde önemli oranda azaldığı bulundu.
Sonuç olarak, koyunlarda kan metabolik profil ile oksidan-antioksidan dengenin beslemeden sonra geçen zamana bağlı olarak değişmektedir.

Anahtar Kelimeler: Antioksidan, Kortizol, Glutamin, Metabolik profil, Oksidan, Koyun.

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INTRODUCTION

The costs of production related to nutrition in ruminant industry are among the problems of animal husbandry. Therefore, animal nutritionists suggest that an alternative to improve the profitability and the environmental sustainability of ruminant animal production is through the improvement of feed efficiency (Montanholi et al., 2010). This is also important to reduce feed costs, which make up a significant portion of the cost of animal production. A better understanding of the factors regulating feed efficiency and their potential as predictors of feed efficiency in ruminant animals is needed. Gonano et al. (2014) suggested that the characterization of blood metabolite concentrations over the circadian period and across physiological stages is important for understanding the biological basis of feed efficiency, and may culminate in indirect methods for assessing feed efficiency. For the feed to be economical for herbivorous animals, it needs to undergo microbial fermentation with a number of microorganisms in the rumen, which also counts for ruminants (Eryavuz, 2000; Kutlu and Serbester, 2014). Thus, because rumen fermentation increases during and after feeding, changes in rumen fermentation and the blood metabolic profile in the period after feeding can be seen in a 24-h period in animals fed once a day. Knowledge of the variation in the blood metabolic profile during a 24-h period is important for a better understanding of circadian cycle effects on metabolism and helps in assessment of production indices (Lee et al, 1978).

Since forage comprises up to 60 % of the diet for non-feedlot ruminants, fiber digestion in the rumen is an important factor in ruminant nutrition. Its digestion and subsequent fermentation by ruminal microbes provides much of the energy for the animal (Weimer, 1998). However, this leads to a long fermentation process of the diet in the rumen. As long as the fermentation continues in the rumen, the blood metabolite transition continues. This is why the ingredients of the feed consumed by animals and the regularity of the rumen fermentation constitute an important effect on the changes in blood metabolite levels (Kutlu and Serbester,2014). Hence, just like the levels (Herdt et al.,2000,Antunoviç et al.2004) of blood metabolite change depending on the breed, physiological status, age, and season, the levels also change depending on the feed consumed by the animals and the digestibility levels(Eryavuz et al. 2003). It can also enable an evaluation of the best time to collect blood in order to be able to correctly interpret metabolic mechanisms or status (Caldeira et al,1999).It also seems possible that the changes in the oxidant–antioxidant balance in the blood may occur during the rumen fermentation taken a long time. Thus, a constant metabolite transfer happens from the rumen into the blood. There is no study investigated the changes in the oxidant–antioxidant balance in the blood depending on the time after feeding in ruminants to date. Therefore, the purpose of this study was to evaluate daily variations in the concentration of urea, glutamine, glucose, cortisone, malondialdehyde and antioxidant potential AOP in sheep fed once a day. A better understanding of mechanistic processes altering the blood metabolite levels will help us to interpret metabolic mechanisms or status in animals fed a single daily meal.

MATERIALS and METHODS

Materials

A total of 10 male merino sheep, 1 years of age, having the same breeding and feeding conditions, weighing about 58 kg, were obtained from Afyon Kocatepe University Research and Application farm of Afyonkarahisar, Turkey. The animals were fed a 38.7 % concentrate and 61.3 % roughage rations for 15 days and feeding in the morning only one. The dry matter intake of sheep was 1.57 kg/day. The chemical analysis of the diet (NRC, 2007) is displayed in Table 1. The experimental protocols were approved by the Animal Care and Use Ethical Committee at Afyon Kocatepe University (91-09). On day fifteen, blood samples were collected from the jugular vein into tubes containing heparin as anticoagulant prior to feeding one hour and after 1, 3, 5, 7, 9, 12, 18, and 24 hours. All the animals were carefully monitored in a period.

Methods

The blood plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C. Whole blood malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hardley (1990). The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid with MDA. The plasma total antioxidant activity (AOA) was determined using the method described by Koracevic et al. (2001). Plasma glucose (201-07-1421, specific sheep kit, Sun RED, USA), glutamine (201-07-2039, specific sheep kit, Sun RED, USA), urea (201-07-1028, specific sheep kit, Sun RED, USA) and cortisol (201-07-0067, specific sheep kit, Sun RED, USA) were measured using an enzyme linked immunoassay (ELISA).

Statistical analysis

Statistical analysis were performed with the SPSS 16 computer program (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± SEM. Significant differences between groups were analyzed by one way ANOVA. Significant differences among the means were determined by using Duncan's multiple-range test at $p < 0.05$.

RESULTS

Daily variations in some biochemical parameters and oxidant-antioxidant balance of sheep fed once a day are given in Figure 1 for urea, Figure 2 for glutamine, Figure 3 for glucose, Figure 4 for cortisol, Figure 5 for MDA and Figure 6 for AOP.

DISCUSSION

The metabolite levels in the blood of ruminant animals are used as an indicator of basic metabolic processes (i.e. indicators of whole body, liver and muscle metabolism) and show differences depending on the ingredients of the diet (Eryavuz et al., 2003), number of meals (Froestchel et al., 1990), feeding time and physiological status (Antunovic et al., 2011). Blood parameters may also facilitate the indirect selection for feed efficient ruminants (Gonano et al., 2014). Hence, the detection of changes in biochemical and hematologic parameters is commonly performed to state the nutritional and health status of animals. In this study, the animals were fed at 8:30 in the morning with diet met all nutritional requirements specified by NRC (2007) for 15 days. On the 15th day of the study, blood samples were taken from the sheep 1 h before and 1, 3, 5, 7, 9, 12, 18, and 24 h after feeding to determine the AOP and the levels of urea, glutamine, glucose, cortisol, and MDA. The determination of the metabolite levels in the blood is a potentially useful application in practice to state the efficacy of the diet consumed by ruminant animals (Gonano et al., 2014). Therefore, attempts in this study were made to detect the changes in six hematological parameters related to metabolism throughout the day in sheep fed once a day. This information constituted relevant data about the levels of glucose, urea, and glutamine; parameters related to the energy and protein metabolism in the liver; changes in the MDA levels, AOP, and oxidant–antioxidant balance in the blood (Eryavuz et al., 2015); and effects of stress on the cortisone levels in the blood (Montanholi et al., 2013). Blood urea concentration is the result of the balance between urea uptake (from the gastrointestinal tract and from liver metabolism) and excretion (urine, feces, milk) and thus mostly responsive to feeding and digestive function. The blood urea levels in the present study increased significantly at 1 h after feeding and dropped until the seventh hour after feeding. As of this hour, the levels increased and reached, even exceeded, the levels before feeding at the 12th hour (Figure 1). This result supported the report of Nikkah et al. (2008) fund that blood urea rose at 2 hours after feeding in cows and indicated that the amino acid breakdown, which is used to create energy in the body, dropped right after the feeding but increased later on. Remod et al. (1993) observed a 100% increase in the urea flow into the rumen before feeding and 5 h after feeding in sheep fed two times a day. The increase in blood urea concentration as of the seventh hour in this study is accordance with the observation of Remod et al. (1993). The decrease in blood urea concentration as of the seventh hour also showed that the energy spent for urea synthesis in the liver decreased as well (Eryavuz et al.,2008). The plasma urea concentrations obtained in this study (15.3–25.8 mg/dL) were close to the levels found in the study performed by Altıntaş and Fıdancı (1993) (17.1–42.8 mg/dL) on sheep. This study showed that the blood glutamine levels, similar to the urea levels, significantly increased 1 h after feeding and dropped until the seventh hour after feeding. The levels increased as of this hour until they reached, even exceeded, the levels before feeding (Figure 2). Urea and glutamine syntheses are two ways to detoxify the ammonia levels in the mammalian liver, which occur in coordination, in form of an upper and lower pair, to eliminate the ammonia that escapes from ureagenesis. This is why glutamine and urea are harmless carriers of ammonia in the bloodstream (Newsholme et al., 2003; Wright et al., 2010). The findings of this study pointed out that the volatile fatty acid levels, which were transferred from the blood into the rumen after feeding as a result of digestion of carbohydrates in the rumen and fermentation, were very high until the seventh hour after feeding. The findings also showed that the amino acid breakdown began to increase as of the said hour to increase the glucose level in the blood. Moreover, a study conducted with newborn pigs showed (Van der Schoor et al., 2001) that amino acid oxidation decreased as soon as the oxidation of glucose in the intestines increased. This study demonstrated that the glucose levels significantly increased 1 h after feeding and decreased as of the fifth hour but then again increased as of the 12th hour until they reached the levels before feeding at the 24th hour (Figure 3). Huntington (1997) reported that the plasma glucose level of cattle, fed with a high content of concentrate feed, was formed to 44% via the absorption of organic acids from the rumen (especially propionic acid) and their conversion into glucose in the liver, to 33% through post-ruminal glucose absorption and to 23% through conversion of amino acids and other carbon sources into glucose. After feeding, the propionic acid levels increased as a result of the digestion of easily dissoluble carbohydrates in the feed and declined depending on the time elapsed after feding (Remond et al.,1993). The increase in the plasma glucose level right after feeding may be caused by the increased conversion of propionic acid, absorbed from the rumen, into glucose in the liver. The increase in the blood glucose level after feeding also leads to an increase in the insulin level; it weakens glucagon secretion, which in turn decreases gluconeogenesis. Thus, intravenous administration of glucagon to

sheep decreases feed intake (Deetz and Wangsness, 1981). The increase in the plasma glucose levels as of the 12th hour in this study might have resulted from the glucose derived from amino acids in the liver. The plasma glucose levels obtained in this study (29.7–51.4 mg/dL) are consistent with the normal levels observed in the study by Altıntaş and Fidancı (1993) (30–80 mg/dL).

Glucocorticoids play a key role in energy metabolism, influencing the animals' performance (Montanholi et al., 2010). The animals under stress have a sympathetic response causing a fight and flight response that increases the energy expenditures. A previous study referred to the existence of a potential relationship between cortisol metabolites excreted in the feces and feed efficiency (Montanholi et al., 2013) and also between feed intake and blood cortisol levels. This study showed that the plasma cortisol levels (Knott et al., 2010) significantly decreased after the feeding until the seventh hour and then started to increase until the samples attained the levels before feeding (Figure 4). It is also stated that the plasma cortisol levels may determine the effects of stress while extracting the samples from the animals (Möstl and Palme, 2002). The plasma cortisol levels obtained in this study were between the cortisol levels (18.7–38.0 ng/mL) of cattle (Colditz et al., 2007; Curley et al., 2008) and the levels (21.5–47.1 ng/mL) reported in sheep (Knott et al.,2010). MDA, which is formed as a result of free radical oxidation of polyunsaturated fatty acids in cell membranes, is also used as an indicator of oxidative damage caused by free radicals (Bulbul et al., 2008). In this study, the blood MDA levels significantly decreased right after feeding and reached the lowest level in the sample at the fifth hour after feeding. As of the seventh hour, the MDA level started to increase again, and in the sample of the 24th hour, it reached its most important and highest level (Figure 5). This finding showed that the production of free radicals in ruminant animals, which were fed once a day, decreased until the seventh hour and then started to increase again. If the level of cortisol, which is one of the hormones that display the effects of stress, is the lowest during the fifth hour, it can be said that the decrease in MDA levels influences the decrease in cortisol levels. Antioxidant defense mechanisms prevent damages caused by free radicals in tissues (Dündar and Aslan, 2000). This study showed that AOP significantly decreased at the 5th, 7th, and 12th hours after feeding and significantly increased in the 3rd and 9th hour samples (Figure 6). Because of rumen fermentation, the blood levels of metabolites and hormones changed after feeding. The observed change in the plasma antioxidant defense might have resulted from the changes in the levels of vitamins and minerals with antioxidant effect, absorbed during rumen fermentation in the lower digestive tract, and from differences in the metabolite and hormone levels (Nikkhah 2011; Gonano et al., 2014).In conclusion, the results in the present study demonstrated a change in metabolite and oxidative stress levels in the blood of sheep depending on the time after feeding and indicate that the altered postprandial rhythms of peripheral blood metabolites can closely link to timing after feeding. Daily variations in those metabolites may be of importance when interpreting these blood indicators of metabolic state. In order to maximize the diagnostic value of blood metabolites, the most suitable time for blood collection seems to be just before feeding in ruminants fed once a day. Conflict of interest. The authors declare they have no conflict of interest.

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RESEARCH ARTICLE

Gemsitabin Uygulanan Prostat Kanseri Hücre Hatlarında Oxaliplatin ve Sisplatinin Kemoterapötik Etkilerinin Belirlenmesi

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ÖZ

Ürogenital kanserler içerisinde erkeklerde görülme sıklığı açısından ilk sırada yer alan prostat kanseri, kanser ilişkili ölümler arasında ikinci sırada yer almaktadır. Mevcut araştırmalar, spesifik hasta popülasyonlarında ilave avantajlar sağlamak için platin-temelli kemoterapötikler ile hedeflenmiş tedavi kombinasyonlarına odaklanmıştır. Cerrahi veya radrasyonu takiben platin bazlı kombinasyon kemoterapisi kullanımı en fazla yararı sağlamaktadır. Bu çalışmanın amacı, platin temelli kemoterapötikler sisplatin ve oksaliplatin ile Gemsitabinin tek başına ve kombine dozlarının insan prostat kanserli hücre hatları olan DU-145 ve PC3 üzerindeki hücre proliferasyonu ve apoptotik yolaklar üzerine etkilerini belirlenmektedir. Prostat kanserli hücre hatlarında bu ilaçların tek başlarına veya kombine dozlarının, hücrelerin canlılığı üzerine olan etkileri WST-1 yöntemiyle ve CASP3, CASP8 ve CASP9 genlerinin mRNA düzeylerinde ifadelenme değişiklikleri RT-PCR ile belirlendi. Sonuç olarak, içsel ve dışsal yolakta görev alan *CASP8* ve *CASP9* genlerinin mRNA düzeyleri değerlendirildiğinde sisplatinin içsel yolaktan *(CASP9)* ve oxaliplatinin ise dışsal yolaktan *(CASP8)* apoptozu indüklediği tespit edildi. Bu göstermektedir ki; platin temelli kemoterapötik olan oxaliplatin ve sisplatin hedef yolakta farklı proteinler üzerinden etki etmektedir. Oxaliplatinin tek başına veya gemsitabin ile kombinasyonlarının apoptotik yolakta sisplatinden daha etkin olduğunu belirlendi.

Anahtar Kelime: Gemsitabin, Kaspazlar, Oxaliplatin, Prostat kanseri, Sisplatin.

The Determination of Chemotherapeutic Effects of Oxaliplatin and Cisplatin In The Prostate Cancer Cell Lines Administered Gemcitabine

ABSTRACT

While prostate cancer takes first place in terms of prevalence among genitourinary cancers in men, it is second among cancer related deaths. Current researches are focused on combining targeted therapy with platinum-based chemotherapy for achieve additional avantages for spesific patient populations. Use of platin-based combination chemotherapy followed by surgical resection and/or radiation ensures the most benefit terapy. The aim of this study was to compare the effectiveness of platinum-based chemotherapeutics Cisplatin and Oxaliplatin used single or combination doses with Gemsitabine on apoptosis and cell proliferation of DU145 and PC3 human prostate cancer cell lines. The effects of drugs alone or combinations on cell viability were determined by WST-1 method. The mRNA expression levels of Caspase-3, Caspase-8, Caspase-9 genes were analyzed by real time polymerase chain reaction (RT-PCR). As a result, when evaluated the mRNA levels of Caspase-8 and Caspase-9 genes that play role intrinsic and extrinsic pathway, it was faund that Cisplatin induced apoptosis by intrinsic pathway, and Cisplatin induced apoptosis by extrinsic pathway. It is determined that, only Oxaliplatin or along with Gemsitabin combinations are more effective on apoptotic pathway than Sisplatin.

Key Words: Gemsitabine, Caspases, Oxaliplatin, Prostate cancer, Cisplatin.

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GİRİŞ

Prostat kanseri erkeklerde sıklıkla teşhis edilen ve akciğer kanserinden sonra ikinci sırada görülen kanser türleri arasında yer almaktadır. Prostat kanserinin gelişim süreci için kesin bir sebep olmamasına rağmen etnik farklılık, yaş, beslenme ve genetik faktörler gibi bazı risk faktörleri etkili bulunmaktadır. Görülme sıklığının ileri yaşla artan prostat kanseri vakalarında hastaların % 65'i 70 yaşın üzerindedir. Buna göre 40 yaş altı erkeklerde görülme oranı 1/10000 olmasına rağmen 40 yaş ve üstü erkeklerde bu oran 1/38'e, 60 yaş ve üstü erkeklerde ise 1/15'e kadar yükselmektedir (Koochekpour 2011, Pienta ve Bradley, 2006; Rentsch, 2009). Sisplatin, oxaliplatin ve gemsitabin prostat kanseri başta olmak üzere çeşitli kanser türlerinin tedavisinde kullanılmaktadır (Curran, 2002, Zou ve ark. 2002). Gemsitabin kemoterapi ve radyoterapiye dirençli olmalarıyla bilinen solid tümörlere karşı önemli etki gösteren bir primidin analoğudur. Genellikle bölünen hücrelerde sitotoksik aktivite gösterdiği için hücre döngüsünde belirteçtir. Hücre döngüsü sırasında S-fazı ve G1 fazı aralığındaki hücrelerin programlarını inhibe etmektedir (Hertel ve ark., 1990). Sisplatin platin atomu etrafında klor ve amonyum atomları ile çevrili inorganik bir platin kompleksidir (Tüfekçi, 2009; Coşkun, 2011). Sisplatin uzun yıllardır kanser tedavisinde kullanılmasına rağmen biyokimyasal etki mekanizması tam olarak bilinmemektedir. İlacın sitotoksik özelliklerini nükleer DNA'ya bağlanıp replikasyon ve transkripsiyonu bozarak gösterdiği ve ayrıca çeşitli sinyal iletim yolaklarını aktive ettiği düşünülmektedir (Kelland, 2007; Wang ve Lippart, 2005). Diğer bir platin kompleksi olan oxaliplatin ise sisplatin yapısından ayrılan klor atomlarının yerine oksalat ligandının geçmesi ile oluşan bir bileşiktir (Desoize ve Modoulet, 2002). Oxaliplatin, sisplatine karşı direnç gösteren kanser türlerinde etkilidir (Desoize ve Modoulet, 2002). Özellikle kullanılan ilaçların tek başına ya da kombinasyonlarının yan etkileri dikkate alındığında prostat kanserinde daha etkili tedavi stratejilerinin geliştirilmesine ihtiyaç duyulduğu görülmektedir (Zou ve ark. 2002). Sisplatin ve oxaliplatin, kanser tedavisinde sıklıkla kullanılan ve birbirinin türevi olan kemoterapötikler olmasına karşın, özellikle sisplatinin istenmeyen yan etkileri gözönüne alındığında, ilaçların birbiri ile kombine edilerek sinerjistik etki gösterip göstermediklerinin belirlenmesi tedavide bunların daha düşük dozda kullanımı yolunu açması bakımından konuyu önemli hale getirmektedir. Böylece kemoterapide kullanılan bu ilaçların kombinasyonu ile apoptozise giden yolda aynı veya farklı yolaklar üzerinden sinerjistik etki göstermeleri ilacın tedavide istenmeyen yan etkilerinin hafifletilmesine neden olacaktır. Bu amaçla çalışmada, DU-145 ve PC3 prostat kanseri hücre hatları kullanılarak platin temelli kemoterapötik ilaçlar olan sisplatin ve oxaliplatinin hem tek başına hem de Gemsitabin ile kombinasyonu yapılarak uygulanmasının hücre proliferasyonu ve apoptoza giden yoldaki CASP3, CASP8 ve CASP9 mRNA düzeylerine etkisi belirlenmiştir. Bu bağlamda elde edilen veriler ile söz konusu ilaçların tek başına ya da kombinasyonlarının tedavide nasıl kullanılacağına ilişkin yapılacak diğer araştırmalara temel oluşturması hedeflenmektedir.

MATERYAL VE METOT

Hücre Hatları Araştırmada kullanılan DU145 insan prostat kanser hücre hattı Ege Üniversitesi Tıp Fakültesi Histoloji ve Embriyoloji Anabilim Dalı ve PC3 insan prostat hücre hattı ise İstanbul Kültür Üniversitesi Fen Edebiyat Fakültesi Moleküler Biyoloji ve Genetik Anabilim Dalı'dan temin edilmiştir.Çalışma Afyon Kocatepe Üniversitesi Merkez Araştırma Laboratuvarı Biyoteknoloji Ünitesinde gerçekleştirilmiştir.

Hücre Kültürü DU145 ve PC3 hücreleri, %10 FBS içeren RPMI 1640 besiyerinde % 95 nem ve % 5 CO2'li ortamda 37°C ayarlı etüv içerisinde kültüre edildi. DU145 ve PC3 hücreleri, (0.1- 50µM) gemsitabin ve sisplatin, (0.1-180µM) oxaliplatin ile ayrı ayrı ve gemsitabin+sisplatin ve gemsitabin+oxaliplatin kombinasyonları halinde 0-72 saat süreyle inkübe edildi. Gemsitabin, sisplatin ve oxaliplatin kemoterapötikleri için çözücü olarak dH2O kullanıldı. İnkübasyon sonrasında her bir doz ve süre için sitotoksik etkileri araştırıldı. Her bir konsantrasyon için 3 ayrı kültür yapılarak deney tekrarlandı.

WST-1 Sitotoksisite Deneyi DU145 ve PC3 hücreleri 96 kuyulu hücre kültür kaplarına 105 hücre/ kuyu olacak şekilde ekildi, bir gece besiyerinde inkübe edilerek hücrelerin yapışmasını takiben besiyeri uzaklaştırıldı. Daha sonra Gemsitabin, Sisplatin ve Oxaliplatin birlikte ve ayrı ayrı çeşitli konsantrasyonları ile 0-72 saat süreyle inkübe edildi. Belirtilen sürelerin sonunda hücre proliferasyonu WST-1 assay (Roche, Almanya; Kat. No: 001 644 807 001) ile belirlendi. Negatif kontroller kör olarak kullanıldı. Hücre canlılığı % hesaplamaları Microsoft Excel programı ile gerçekleştirildi.

Hücrelerden Total RNA İzolasyonu Belirtilen dozlarda DU145 ve PC3 hücrelerinden inkübasyon sürelerinin ardından Tripure Isolation Reagent ile total RNA izolasyonu gerçekleştirildi. Örnekler kullanılıncaya kadar -80°C'lik derin dondurucuda saklandı.

cDNA Sentezi RT2 HT First Strand cDNA sentez kiti (Qiagen, Almanya; Kat. No: 330411) kullanılarak total RNA'dan protokol doğrultusunda

gerçekleştirilmiştir. Real-Time PCR'da kullanılıncaya kadar -200C'lik derin dondurucuda saklandı.

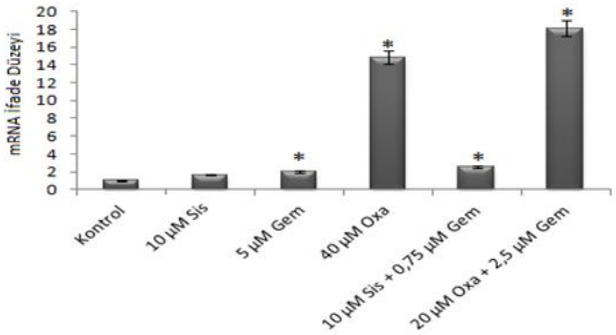
Genlerin İfade Edilmesinin Real-Time PCR ile Ölçümü CASP3, CASP8 ve CASP9 genlerinin mRNA miktarları, Real-Time PCR yöntemi ile RotorGeneQ cihazı kullanılarak yapıldı. Amplifikasyonlar 25 µL toplam tepkime hacmi içerisinde; cDNA, bölgeye özgü primerler ve RT2 SYBR Green Mastermix karışımı (Qiagen, Almanya; Kat. No:330500) ve steril H2O-PCR grade kullanılarak gerçekleştirildi. CASP3, CASP8 ve CASP9 genlerinin ifadelenmesini normalize etmek için GAPDH mRNA düzeyi referans olarak alındı. Reaksiyon sonucunda, CASP3, CASP8, CASP9 ve GAPDH genlerin mRNA düzeylerini gösteren Crossing point (Cp) değerleri belirlendi. CASP3, CASP8 ve CASP9 ifade düzeyleri GAPDH ifade düzeyine göre normalize edildi. Göreceli gen ifadesi sonuçları REST programı kullanılarak "Pfaffl" matematiksel yöntemi ile hesaplandı.

İstatistiki Analiz Doza bağlı olarak değişen, CASP3, CASP8 ve CASP9 ve GAPDH mRNA ifade düzeylerindeki farklılıklar "Qiagen Data Analysis Center" istatistik programı ile karşılaştırıldı. 0.05'den küçük olan P değerleri istatistiki açıdan anlamlı olarak kabul edildi.

BULGULAR

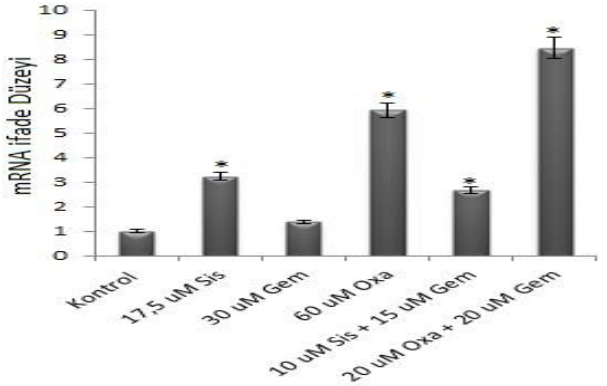
DU145 ve PC3 prostat kanser hücrelerinin gemsitabin, sisplatin ve oxaliplatin'in ayrı ayrı ve birlikte kombinasyonlarının uygulaması sonrasında tüm gruplardaki CASP3, CASP8 ve CASP9 geninin mRNA düzeyindeki değişimler Şekil 3.(1-6)'da gösterildi.DU145 prostat kanser hücrelerinde CASP3 geninin mRNA düzeyi kontrole göre 10 µM sisplatin uygulaması sonrasında 1.65 kat artmasına rağmen istatiksel olarak anlamlı bir artış gözlenmedi. Diğer gruplarda ise; 5µM gemsitabin ile muamele edilmesi sonrasında 2.04 kat, oxaliplatin uygulaması sonrasında 14.82 kat, 10 µM sisplatin + 0.75 µM gemsitabin uygulaması sonrasında 2.51 kat, arttığı belirlenmiş ve 20 µM oxaliplatin + 2.5 µM gemsitabin uygulanması sonrasında ise 18.12 kat artış istatiksel olarak anlamlı ($p<0.05$) bulunmuştur (Şekil 3.1).

PC3 prostat kanser hücrelerinde CASP3 geninin mRNA düzeyi kontrole göre 30µM gemsitabin ile muamele edilmesi sonrasında 1.37 kat artmasına rağmen istatistiki olarak anlamlı bir artış değildi. Diğer gruplarda ise; 17.5 µM sisplatin uygulaması sonrasında 3.22 kat, 60 µM oxaliplatin uygulaması sonrasında 5.93 kat, 10 µM sisplatin + 15 µM gemsitabin uygulaması sonrasında 2.65 kat, arttığı belirlenmiş ve 20 µM sisplatin + 20 µM gemsitabin uygulaması sonrasında 8.45 kat artışla istatistiki olarak önemli ($p<0.05$) bulunmuştur (Şekil 3.2)



Şekil 3.1. DU145 hücre hattında CASP3 geninin mRNA düzey değişimleri. Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

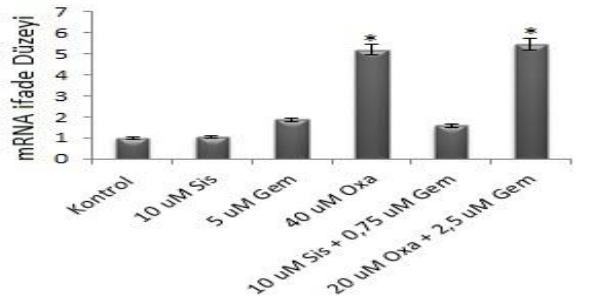
Figure 3.1. mRNA levels of CASP3 gene in DU145 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).



Şekil 3.2. PC3 hücrelerinin CASP3 geninin mRNA düzeyinde değişimler . Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

Figure 3.2. mRNA levels of CASP3 gene in PC3 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).

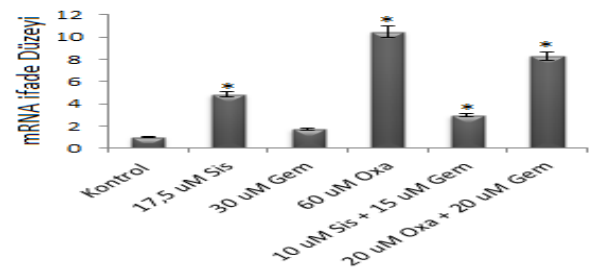
DU145 prostat kanser hücrelerinde CASP8 geninin mRNA düzeyi kontrole göre; 10 µM sisplatin uygulaması sonrasında 1.06 kat, 5µM gemsitabin ile muamele edilmesi sonrasında 1.86 kat, 10 µM sisplatin + 0.75 µM gemsitabin uygulaması sonrasında 1.59 kat artış göstermesine rağmen istatiksel olarak anlamlı bulunmadı. Diğer gruplarda ise; 40 µM oxaliplatin uygulaması sonrasında 5.2 kat arttığı belirlendi ve 20 µM oxaliplatin + 2.5 µM gemsitabin uygulaması sonrasında 5.46 kat artış istatiksel olarak anlamlı ($p<0.05$) bulundu (Şekil 3.3).



Şekil 3.3. DU145 hücrelerinin CASP8 geninin mRNA düzeyinde değişimler . Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

Figure 3.3. mRNA levels of CASP8 gene in DU145 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).

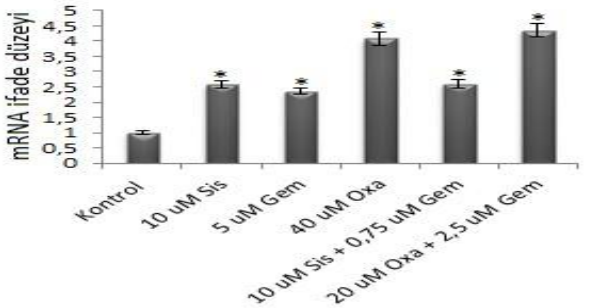
PC3 prostat kanser hücrelerinde CASP8 geninin mRNA düzeyi kontrole göre; 30μM gemsitabin ile muamele edilmesi sonrasında 1.72 kat artmış olmasına rağmen istatiksel olarak anlamlı bir artış değildi. 17.5 μM sisplatin uygulaması sonrasında 4.85 kat, 60 μM oxaliplatin uygulaması sonrasında 10.48 kat, 10 μM sisplatin + 15 μM gemsitabin uygulaması sonrasında 2.94 kat ve 20 μM oxaliplatin + 20 μM gemsitabin uygulaması sonrasında 8.28 kat artış istatiksel olarak anlamlı ($p<0.05$) bulundu (Şekil 3.4).



Şekil 3.4. PC3 hücrelerinin CASP8 geninin mRNA düzeyinde değişimler . Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

Figure 3.4. mRNA levels of CASP8 gene in PC3 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).

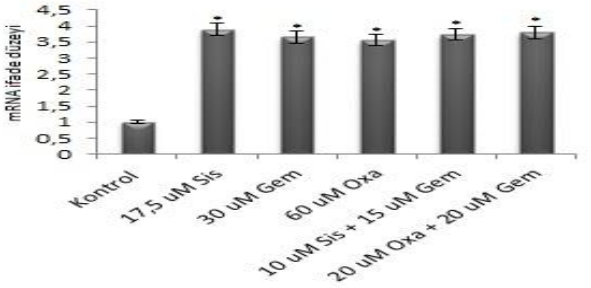
DU145 prostat kanser hücrelerinde CASP9 geninin mRNA düzeyi kontrole göre; 10 μM sisplatin uygulaması sonrasında 2.58 kat, 5μM gemsitabin ile muamele edilmesi sonrasında 2.36 kat, 40 μM oxaliplatin uygulaması sonrasında 4.08 kat, 10 μM sisplatin + 0.75 μM gemsitabin uygulaması sonrasında 2.6 kat arttığı belirlendi ve 20 μM oxaliplatin + 2.5 μM gemsitabin uygulaması sonrasında 4.34 kat artış istatistiki olarak anlamlı ($p<0.05$) bulundu (Şekil 3.5).



Şekil 3.5. DU145 hücrelerinin CASP9 geninin mRNA düzeyinde değişimler . Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

Figure 3.5. mRNA levels of CASP9 gene in DU145 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).

PC3 prostat kanser hücrelerinde CASP8 geninin mRNA düzeyi kontrole göre; 17.5 μM sisplatin uygulanması sonrasında 4.85 kat, 30μM gemsitabin ile muamele edilmesi sonrasında 3.65 kat, 60 μM oxaliplatin uygulaması sonrasında 3.55 kat, 10 μM sisplatin + 15 μM gemsitabin uygulaması sonrasında 3.73 kat ve 20 μM oxaliplatin + 20 μM gemsitabin uygulaması sonrasında 3.8 kat artış ile istatistiki olarak anlamlı ($p<0.05$) bulundu (Şekil 3.6).



Şekil 3.6. PC3 hücrelerinin CASP9 geninin mRNA düzeyinde değişimleri. Hedef genin ifade düzeyi GAPDH mRNA ifade düzeyi temel alınarak normalize edildi, *; ($p<0.05$).

Figure 3.6. mRNA levels of CASP9 gene in PC3 cell line. Target gene expression level was normalized based on GAPDH mRNA expression level, *; ($p<0.05$).

TARTIŞMA ve SONUÇ

Kemoterapötiklerin, farklı kemoterapötiklerle veya spesifik inhibitörlerle birlikte kullanımının tedavi etkinliğini arttırdığı ve bu bağlamda gemsitabin ile oxaliplatin ve sisplatin kombinasyonlarının da pankreas ve mesane gibi solid tümörlerin tedavisinde kullanıldığı bildirilmektedir (Kollmannsberger ve ark., 2004; Louvet ve ark., 2005; Santisteban ve ark., 2008; Lee, 2014). Ancak sisplatinin ciddi yan etkileri bulunduğundan bunun farklı türevlerinin kullanımı söz konusu olmaktadır. Bu araştırmada prostat kanseri DU145 ve PC3 hücre hatlarında, platin temelli kemoterapötik ilaçlar olan sisplatin ve

oxaliplatinin tek başına yada gemsitabin ile kombinasyonu yapılarak bunların hücre proliferasyonu ve bazı kaspazlar üzerine olan etkileri ortaya konuldu.Sisplatin DNA'nın yapısına kovalent bağlanmak suretiyle DNA replikasyonu ve translasyonunun bozulmasına yol açmakta ve hücrede sitotoksik etkiye neden olmaktadır (Lıu ve ark., 2007). Ancak ilacın böbrek, ürogenital ve sinir sistemi ile kemik iliği üzerine olan ciddi toksisitesi ve yan etkileri sisplatinin klinik kullanımını sınırlamaktadır (Daugaard ve ark., 1988; Hamer ve ark.,1991). Bu sebeple sisplatin dozunun azaltılması da klinik kullanımı için yeterli olmadığından ilacın farklı platin-temelli ilaçlarla kombinasyonu söz konusu olmaktadır.CASP3 mRNA düzeyleri açısından karşılaştırıldığında DU145 (10μM) hücre hattında 48 saatlik uygulamada sisplatin tek başına anlamlı artış göstermezken, tek başına gemsitabin ve sisplatin + gemsitabin kombinasyonu daha etkin görülmektedir ($p<0.05$). PC3 hücre hattında ise tek başına sisplatin ve sisplatin + gemsitabin kombinasyonu daha etkin görülmektedir ($p<0.05$). Elde edilen bu verilere göre gemsitabin ve sisplatinin tek başına kullanımından ziyade kombinasyonlarının hem daha düşük dozlarda ve hem de yüksek oranda CASP3 üzerinden apoptozise etkili olduğu görülmektedir. Yapılan çalışmalarda metastatik prostat kanserinde gemsitabinin tek başına kullanılması ile prostat spesifik antijene cevap oranı % 9 iken, diğer kemoterapötikler ile kombine edildiğinde bu oranın %23'e çıktığı bildirilmektedir (Morant ve ark., 2000; Di Lorenzo ve ark., 2007). Benzer bir çalışmada ise ileri evre prostat duktal adenokarsinomalı hastaların dosetaksel ilacına karşı cevap vermezken gemsitabin/sisplatin kombinasyonuna cevap vermesinin diğer bir tedavi seçeneği olduğu belirtilmektedir (Kamiyama ve ark., 2015).

Çalışmada DU145 (10μM) hücre hattında inkubasyon sonrası CASP3 mRNA düzeyi kontrolle karşılaştırıldığında sisplatin hariç tüm uygulamalarda istatistik olarak artış gösterirken en yüksek artışın (18.22 kat) 72 saatlik uygulama ile oxaliplatin + gemsitabin kombinasyonunda olduğu belirlendi ($p<0.05$). PC3 (17.5 μM) hücre hattında ise CASP3 mRNA ekspresyon düzeyinde gemsitabin hariç tüm gruplarda istatistik olarak anlamlı artış bulunurken, 72 saatlik uygulamada oxaliplatin+ gemsitabin kombinasyonundaki (8.45 kat) artış en yüksek bulundu ($p<0.05$). Bu sonuçlara göre prostat kanser hücrelerinde gemsitabin + oxaliplatin kombinasyonunun sisplatin ile kombinasyonuna göre daha CASP3 mRNA ekspresyonunu yüksek düzeyde indüklediği görülmektedir. Oxaliplatin açısından karşılaştırıldığında ise her iki hücre hattında da tek başına ve gemsitabin + oxaliplatin kombinasyonunun istatistiki olarak CASP3 mRNA düzeylerini artırdığı tespit edilmiştir. Elde edilen bu sonuçlar gemsitabinin bir diğer platin temelli kemoterapötik olan oxaliplatin ile kombinasyonunun güvenilirliği ve tolere edilebilirliğinin daha fazla olduğu bildirimleri ile uyumlu bulunmaktadır (Santisteban ve ark., 2008, Li ve ark,. 2011, Lee ve ark., 2014). Platin temelli kemoterapötikler olan sisplatin ve oxaliplatinin birbirlerinin türevi olmasına karşın etkilerinin aynı olmadığı ayrıca çeşitli kanser tiplerinde de etkinliklerinin farklı olduğu ortaya konmuştur (Marasco ve ark., 2015). Genital kanserlerde sisplatine göre oxaliplatinin daha etkin, güvenilir ve düşük nörotoksik profile sahip olduğu belirtilirken (Kolomeyevskaya Nonna ve ark., 2014), skuamöz hücre karsinomlarında ise etkinliğinin düşük olduğu ve rutinde sisplatinin yerini alamayacağı ifade edilmektedir (Fakhrian ve ark., 2014). Benzer bir çalışmada ise ileri evre mide kanserlerinde her iki ilacın da eşit aktivite ve toleransa sahip olduğu belirtilmektedir (Kim ve ark., 2013). Çalışmada DU145 hücre hattında inkubasyon sonrası CASP8 mRNA düzeyi kontrolle karşılaştırıldığında sadece oxaliplatin ile oxaliplatin + gemsitabin kombinasyonunda anlamlı artış bulundu ($p<0.05$). PC3 hücre hattında inkubasyon sonrası CASP8 mRNA ekspresyon düzeyi kontrolle karşılaştırıldığında gemsitabin hariç tüm gruplardaki artış ile birlikte, oxaliplatindeki artış en yüksek (10.48 kat) değere sahipti ($p<0.05$). DU145 ve PC3 prostat kanser hücreleri her ne kadar androjen bağımsız olsalar da farklı sinyal molekülleri aktiftir. Bu nedenle özellikle CASP8 mRNA düzeyini sisplatinin DU145 hattında etkilemezken PC3 hücre hattında istatistik olarak artırdığı görülmektedir. Bu sonuçlar göstermektedir ki, yalnızca oxaliplatin her iki hücre hattında da CASP8 mRNA üzerinden dışsal yolağı uyarabilmektedir. Çalışmada DU145 hücre hattında CASP9 mRNA düzeyindeki en yüksek artışın (4.34 kat) 72 saatlik uygulama ile oxaliplatin + gemsitabin kombinasyonunda olduğu belirlendi ($p<0.05$). PC3 hücre hattında ise , sisplatin (3.89 kat) ve oxaliplatin + gemsitabin kombinasyonundaki (3.8 kat) artış en yüksek bulundu ($p<0.05$). Bu verilere göre gemsitabin ve oksaliplatin kombinasyonunun CASP9 üzerinden etkili olarak daha düşük dozda sinerjistik etki gösterdikleri sonucuna varılmaktadır. Ayrıca her 3 ilaç ve kombinasyonlarının her iki hücre hattında da CASP9 üzerinden iç yolağı uyarabildiği görülmektedir. CASP3, CASP8 ve CASP9 mRNA düzeylerine bakıldığında sisplatin içsel yolak aracılığıyla CASP3'ü aktive ettiği ve oxaliplatin ise etkisini CASP8 üzerinden yani dışsal yolak üzerinden apoptozu indüklediği görülmektedir. Bu bulgular sisplatin ve oxaliplatinin hedef protein üzerinde farklı bölgelere bağlanmak suretiyle aktivitelerini gösterdikleri ifadesi ile desteklenmektedir (Marasco ve ark. 2015). Sonuç olarak ilk kez prostat kanseri hücrelerinde sisplatin ve oksaliplatinin tek başlarına ve gemsitabin ile kombinasyonunun apoptoz üzerine etkisinin araştırıldığı bu çalışmada, sisplatinin içsel

yolak ile oxaliplatinin ise dışsal yolak aracılığıyla apoptozu indüklediği ve oksaliplatinin sisplatine nazaran hem tek başına hem de gemsitabin ile kombinasyonunun apoptozu daha yüksek seviyede indüklediği belirlendi. Bunlara ilave olarak ciddi yan etkilerinden dolayı prostat kanseri tedavisinde kullanımı sınırlı olan sisplatinin yerine alternatif bir kemoterapötik ilaç olan oxaliplatinin ve bunun gemstabin ile kombinasyonlarının tedavi amaçlı uygulanmasına ilişkin daha ileri düzey araştırmalara ihtiyaç duyulduğu görülmektedir.

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RESEARCH ARTICLE

Effect of Mentha Piperita (Peppermint) Extract and its Juice on Egg Quality Traits during Different Storage Time in Laying Hens

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ABSTRACT

Much focus has been given on the use of herbs and herbal products to improve performance and to some extent the quality in freshly laid eggs but limited data are available regarding the impact of herbs on storage quality of eggs. The present study was designed to evaluate the effect of Mentha Piperita oil and mentha juice in feed and drinking water respectively, on egg quality traits in laying hens at different storage intervals. A total of 252 Babcock laying hens were divided into 7 groups and each group was further divided into 4 subgroups having 9 hens in each. Group A served as a control. Group A was fed basal diet without any supplementation. Group B, C and D were offered diets supplemented with mentha extract @ 50, 100 and 200 mg/kg of feed while groups E, F and G diets were having same doses of mentha juice in drinking water. At the end of the study (56 days), a total of 252 eggs (36 eggs from each group) were collected randomly. 84 eggs were analyzed at zero day of storage while other eggs were stored at 4°C temperature. Among these eggs, 84 were analyzed after 15 days and remaining 84 after 30 days of storage. The results revealed that egg quality traits like egg shell breaking strength (ESBS), yolk color (YC), haugh unit (HU) and egg weight showed non-significant difference (P>0.05) among all the groups at different storage intervals.

Key Words: Egg Quality, Mentha piperita, Haugh unit, Yolk color, Egg shell breaking strength.

Yumurtacı Tavuklarda Mentha Piperita(Nane) Ekstratının ve Özsuyunun Farklı Depolama Zamanlarında Yumurta Kalite Özelliklerine Etkisi

ÖZ

Yumurtacı tavuklarda bitkilerin ve bitkisel ürünlerin kullanımının performans ve taze yumurta kalitesine etkileri üzerine çalışmalar yapılmış olmasına rağmen depolama kalitesine üzerine etkileri hakkında oldukça sınırlı bilgi mevcuttur. Çalışma; Mentha Piperitaekstraktının ve özsuyunun sırasıyla yem ve su katkısı olarak kullanılmasının farklı depolama sürelerinde yumurta kalitesine etkisini araştırmak amacıyla planlanmıştır. Çalışmanın hayvan materyalini 252 Babcock ırkı yumurta tavuğu teşkil etmiştir. Hayvanlar 7 ana gruba ve devamında 4 alt gruba ayrılmış ve her alt grup 9 hayvandan oluşmuştur. A Grubu rasyonunda herhangi bir katkı kullanılmamış ve kontrol grubu olarak belirlenmiştir. B, C ve D grupları sırasıyla nane ekstraktını 50, 100 ve 200 mg/kg dozlarında yem katkısı olarak; E, F ve G grupları ise aynı dozlarda nane özsuyunu içme suyu katkısı olarak tüketmişlerdir. Çalışma sonunda (56 gün) 252 yumurta (her gruptan 36 yumurta olmak üzere) rastgele olarak toplanmıştır. 84 adet yumurta depolama öncesi analiz edilmiş, diğer örnekler +4°C'de depolanmıştır. Depolanan yumurtalardan 84 tanesinin analizleri depolamanın 15 gününde, geri kalan 84 yumurtanın ise analizleri depolamanın 30 gününde yapılmıştır. Çalışma sonucunda yumurta kalite parametrelerinden yumurta kabuğu kırılma mukavemeti, yumurta sarısı rengi, haugh birimi ve yumurta ağırlığı, farklı depolama sürelerinde gruplar arasında istatistiksel farklılık (P>0.05) göstermemiştir.

Anahtar Kelimeler: Yumurta Kalitesi, Mentha piperita, Haugh Birimi, Yumurta sarısı rengi, yumurta kabuğu kırılma mukavemeti.

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INTRODUCTION

Poultry sector is one of the vibrant sectors of livestock and plays a vital role in economy of any country. Poultry is a readily available source of protein food comparatively cheaper and easier to grow in short period of time. Although poultry sector is well developed but still there is need to strength it and make safer the poultry products for human health. Poultry products like eggs and meat are relishly consumed by all kind of people (adults and children). Layer farming is one of the major segments of poultry industry, raised for table egg production. Unlike the external quality of eggs, the internal quality of eggs starts to decline soon after laid by the hens. As eggs are produced in million numbers daily and it takes time till dissemination to the end consumers after undergoing the storage to avoid spoilage. The storage time and temperature have critical impact on the egg quality parameters (Samli et al. 2005) and the other important factors which affect the quality of egg can be genetics factors (Johnson and Merritt 1955) and age of the flock (Williams 1992) . The quality deterioration while storage, during distribution process, puts major loss on farmer and or less liking of eggs by the consumers. Quality of eggs can be measured by analyzing albumen quality as a standard criterion (Jin et al. 2011). Haugh unit is calculated by albumen thickness and weight of egg (Haugh 1937), can serve as another indicator of albumen quality (Jin et al. 2011). Albumen height or HU is maximum in the freshly laid eggs while decreases with increase in storage time (Jin et al. 2011). Changes in the egg quality parameters like HU, yolk color, egg shell breaking strength and others have direct link with moisture loss through shell pores by evaporation and also escape of carbon dioxide from egg albumen (Hinton 1968, Shenstone 1968, Robinson 1987). To prevent the egg quality losses during storage times, the use of herbal products could be a useful tool in the industry. Much focus have been given on the use of herbs and herbal products to improve performance and to some extent on the quality in freshly laid eggs but limited research data are available for the impact of herbs on the storage quality of eggs. Many herbal products (Extracts, essential oils or powders) have been use by many researchers and found positive effects of these products on performance parameters including egg production and quality (Aji et al. 2011, Rahimi et al. 2011, Khan et al. 2012). Effect of herbal extracts on egg quality traits, such as yolk composition, shell thickness or Haugh Unit, were reported in limited studies, whereas the majority of reports did not identify substantial effects (Nichol and Steiner 2008, Navid et al. 2013). Among these phytogenics, Mentha may have a good potential to be used in poultry. Mentha also known as mint belongs to family Lamiaceae (mint family) (Harley et al. 2004) and has many species (13-18) (Bunsawat et al. 2004). The chemical components of peppermint oil are menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate and pulegone (Leung 1980). Like other herbal products it has good impact on performance and immune status of poultry, especially in layers. Supplementation of mint in feed of broiler result in improved growth performance, FCR and immunity (Durrani et al. 2008) and spearmint extract may improve egg production, egg shell thickness and yolk weight. Pennyroyal (M. Pulegium) powder and its extract has positive influence on egg production performance, egg quality, blood biochemical and immunity parameters in laying hens (Paymard et al. 2013). Limited studies are available on the use of Mentha Piperita extract and its juice in laying hens regarding egg quality traits during storage conditions. Keeping in view the above points, the present study was designed to investigate the effect of Mentha Piperita extract and its juice on egg quality traits during storage in laying hens.

MATERIALS and METHODS

This study was conducted at the experimental animal farm of Afyon Kocatepe University under the Project approved by BAPK (15.SAĞ.BİL.23). The ethics committee of faculty of Veterinary Medicine approved the conduct of study under the case AKUHADYEK-455.15, 21.05.2015. For this study 252 Babcock white laying hens were procured from private Layer Farm. The age of the birds was 21 weeks. The birds were given 4 days adaptation and preparation period in the new research place. Total duration of study was 60 days (4 days adaptation period and 56 days experimental period). Birds were kept in cages and provided 16 hours light and 8 hours dark period during the whole study. Birds were divided into 7 random groups containing 36 birds in each group and then each group was further subdivided into 4 replicates containing 9 birds in each. A basal diet was formulated according the NRC (1994) recommendations to meet the bird's requirement. Group A served as control group and was provided basal diet. Groups B, C, and D were provided basal diet supplemented with mentha oil at the rate of 50mg/kg feed, 100mg/kg feed and 200mg/kg feed respectively. While the groups E, F and G was offered only basal diet but these groups were provided drinking water containing mentha juice at the rate of 50mg/L of water, 100mg/L of water and 200mg/L of water respectively. The mentha oil was procured from the commercial private company. Mentha juice was extracted by the blending of clean fresh mentha plant leaves and

filtered for the use in experiment. Mentha oil was mixed in the diet on the daily basis freshly along with sunflower oil to prevent any loss or oxidative spoilage. Similarly juice was also poured on daily basis in fresh drinking water. Ad libitum feed and fresh drinking water were provided during the whole trial period. Treatment protocol, ingredient composition of feed and its calculated and analyzed nutrient compositions are shown in table 1, 2 and 3 respectively. At the end of the study (56 days), 36 eggs from each group were collected randomly. Thus a total of 252 eggs were collected randomly. 84 eggs (12 from each group, 3 from subgroup) were analyzed on the same day (0day of storage) while remaining 168 eggs were stored at +4°C. Half (84) of the stored eggs (12 eggs from each groups by random collection, 3 from each subgroup) were analyzed for egg quality parameters like egg breaking strength (ORKA Egg Force Reader, EF 0468-2011), haugh unit, egg yolk color and egg weight (SANOVO Engineering Egg Analyzer, EA0333, Denmark) after 15 days of storage and remaining half of the eggs after 30 days of storage.

STATİSTİCAL ANALYSİS

Kolmogorov_Smirnov test was used to see the normality distribution of data. Logarithmic transformation was used on the data which did not show normality distribution. For independent variables, one way ANOVA was applied using Post Hoc with Bonferroni and Tamhane's T2 according to equality of variances. For dependent variables, to see statistical differences repeated measures ANOVA was used and post-hoc with Bonferroni and Tamhane's T2 according to equality of variances. To determine significance $P<0.05$ was used. Mean±SEM was showed in tables.

RESULTS

The results of the study showed that the egg shell breaking strength remained non-significantly different ($P>0.05$) in all groups at 0 day, 15th day and 30th day of storage, except group A which showed significantly reduced ($P<0.05$) value at day 15th of storage in among the group analysis with respect to time (Table 4). Similarly, the yolk color of eggs also showed no difference ($P>0.05$) in all groups during at 0 day, 15th day and 30th day of storage (Table 5). Haugh unit results also showed non-significant difference ($P>0.05$) between all the groups during 0 day, 15th day and 30th day of storage as compared with control group (Table 6). The results of egg weight also showed no change ($P>0.05$) in all groups as compared with the control group during the whole study at 0 day, 15th day and 30th day of storage (Table 7).

DISCUSSION

In the present study, egg shell breaking strength (ESBS) did not change significantly at 4°C storage temperature during 0, 15 and 30 days storage period as compared with control group between the groups as well as among the groups with reference to different time periods except in group A which showed significantly reduced ESBS at 15th day of storage while 0 day and 30th days ESBS remained same. This trend among group A might have no importance as other groups did not show any such pattern. It has been reported that egg shell weight and shell percentage decreased significantly with increase in storage time (Jin et al. 2011) at 5°C and 29°C temperature while no change was observed at 21°C temperature. Similar findings were published by Samli et al. (2005). In another study, it was reported that changes in shell weight were unclear with increasing storage time till 10 days (Silversides and Scott 2001). Likewise, in the present study no decrement in ESBS has been observed during different storage time at 0 day, 15th day and 30th day in supplemented groups. The yolk color (YC) results also showed no difference in mentha supplemented groups as compared with the non supplemented control group. It showed no effect of storage time on egg yolk color but in another study yolk color significantly decreased with increase in storage time in normal fed groups (Jin et al. 2011) at 5°C while similar to our results Maria Elena et al. (2006) demonstrated that yolk color was not change during different storage time periods at 4°C but changed negatively at 20°C. In the present study both control group and other mentha oil and juice supplemented groups did not show any change in egg yolk color during storage at different days. Haugh unit results also showed no significant difference in all the groups during different storage time periods of 0, 15 and 30 days. However, a prominent numerical difference in HU was seen at different storage times. The HU values showed numerically higher values among the groups during 15th and 30th day among the group when compared with reference to time of storage, but showed even no numerical or very less numerical difference in between the groups as compared with control group at different storage time periods. Similar to present study Jin et al. (2011) described that in normal fed laying hens, eggs HU did not change at 5°C with increase in storage time. Some other researchers (Samli et al. (2005, Tona et al. 2004, Akyurek and Okur 2009) also reported that HU did not change with increasing storage time at 5°C but can decrease dramatically at higher temperature. Egg weight also showed no change in all the treatments groups even between the groups and or among the group analysis at different storage time periods. Similarly, Jin et al. (2011) has reported no significant loss of egg weight with increasing

storage time till 10 days at 5°C and 21°C temperature in normal diet fed layers. Our results were also supported by the findings of Samli et al. (2005) and Akyurek and Okur (2009) who also reported no egg weight loss at 5°C temperature during storage of 10 days.The present study results showed no changes in egg quality traits. Like our study, available literature is also explaining that in normal diet fed laying hens, there is no effect of storage time at 4C temperature, but higher temperatures have detrimental effects on egg quality. Although enough data is available on the use of herbs and their products on egg quality traits of freshly laid eggs but no data is available for the use of herbal products on egg quality parameters analysis during different storage time periods. More research with multiple temperature and storage time frame is needed to investigate the effect of different dose levels of mentha oil and juice on egg quality parameters at higher temperature which is causing quality losses to eggs.

CONCLUSION

The result data from the current study indicated that supplementation of Mentha Piperita oil and its juice in the laying hen's diet had no significant effect on egg quality traits during storage for 15 and 30 days at 4°C. It is recommended to conduct more extensive research studies to explore the effect of this herbal product on egg quality parameters during prolonged storage at higher temperature which are more detrimental to egg quality traits.

Table 1. Dietary Treatment Protocol of different diets for different groups

Group	Treatment	Treatment
A (control)	Basal Diet	Normal water
B	Basal diet supplemented with mentha oil 50mg/kg feed	Normal water
C	Basal diet supplemented with mentha oil 100mg/kg feed	Normal water
D	Basal diet supplemented with mentha oil 200mg/kg feed	Normal water
E	Basal Diet	Drinking water supplemented with mentha juice 50mg/L
F	Basal Diet	Drinking water supplemented with mentha juice 100mg/L
G	Basal Diet	Drinking water supplemented with mentha juice 200mg/L

Table 2. Ingredient Composition of Feed (%)

Feed Ingredients	**Inclusion %**
Corn	52.0
Sunflower meal	8.1
Soybean meal	12.2
Full fat soya	12.0
Limestone	9.0
Meat and Bone meal	3.7
Sun flower oil	1.5
Vitamin -Mineral mix*	0.25
Methionine	0.15
Salt	0.3
Rovabio**	0.1
Rovaphos***	0.7

*Providedper kg of diet:Vitamin A:12.000.000 IU, Vitamin D3:3.000.000IU, Vitamin E:35.000, Vitamin K3:3.500 , Vitamin B1:2.750IU, Vitamin B2:5.500IU, Nicotinamid: 30.000IU,Ca-D Panthotenate: 10.000IU, Vitamin B6: 4.000IU, Vitamin B12-15IU, Folic acid:1.000IU, D-Biotin: 50IU,Cholin clorid:150.000IU, Manganese: 80.000mg, Iron: 60.000 mg, Zinc:60.000 mg, Copper:5.000 mg, Iodine:2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant:15.000 mg, **Provided per kg of diet: 10 million IU Beta xylanase, 17.5 million IU Beta glucanase, ***Provided per kg of diet: 500.000 mg phytase

Table 3. Analyzed and Calculated Nutrient Composition of Feed on Dry Matter Basis

Nutrient	Analyzed (%)	Calculated (%)
DM	89.88	88.50
Ash	14.29	13.50
Fat	6.85	6.50
CF	5.3	4.60
CP	18.60	18.00
Starch	28.11	
Sugar	3.75	
ME (Kcal/kg)	2721.87	2800
Ca		4.00
P		0.44
Na		0.17
NFE	44.84	

Table 4. Effect of *Mentha Piperita* oil and its juice on egg shell breaking strength (ESBS) at 0, 15 and 30 days of storage.

Groups	ESBS 0 day	ESBS Initial (15th day)	ESBS Final (30th day)	P
A	55.99±1.84[a]	44.42±2.56[b]	49.10±2.73[a]	0.02
B	49.60±3.41	47.59±3.66	47.81±2.58	0.92
C	44.70±2.33	39.90±2.99	41.00±2.71	0.40
D	46.72±2.11	44.29±1.41	48.21±4.09	0.58
E	48.68±2.55	50.78±3.34	46.35±2.37	0.49
F	46.83±2.62	44.32±1.84	47.13±3.28	0.44
G	47.96±3.06	43.92±1.53	49.64±1.20	0.21
P	0.09	0.15	0.42	

Superscripts a,b,c indicates the significant differences ($p<0.05$) among the same group with respect to time.
A control, B supplemented with 50mg/kg, C with 100mg/kg of feed, D with 200mg/kg of feed, E 50mg/L, F 100mg/L and G 200 mg/L of drinking water

Table 5. Effect of *Mentha Piperita*oil and its juice on yolk color (YC) at 0, 15 and 30 days of storage.

Groups	YC 0 day	YC 15th day	YC 30th day	P
A	11.18±0.58	11.60±0.78	12.09±0.49	0.70
B	11.67±0.58	11.30±0.94	13.22±0.22	0.07
C	11.67±0.57	12.50±0.68	12.73±0.43	0.24
D	12.09±0.37	12.33±0.40	12.73±0.41	0.50
E	10.42±0.51	12.20±0.81	12.64±0.41	0.06
F	11.92±0.29	12.33±0.55	12.42±0.53	0.31
G	11.67±0.55	13.00±0.17	12.73±0.33	0.11
P	0.28	0.64	0.72	

A control, B supplemented with 50mg/kg, C with 100mg/kg of feed, D with 200mg/kg of feed, E 50mg/L, F 100mg/L and G 200 mg/L of drinking water

Table 6. Effect of *Mentha Piperita*oil and its juice on haugh unit value (HU) at 0, 15 and 30 days of storage.

Groups	HU 0 day	HU 15th day	HU 30th day	P
A	63.53±8.49	77.13±3.55	79.91±1.29	0.07
B	72.48±3.84	72.51±6.87	76.56±1.96	0.56
C	63.50±8.01	70.89±3.85	78.48±1.85	0.40
D	70.15±5.85	79.00±1.64	70.85±4.17	0.29
E	69.52±5.71	76.24±1.30	73.94±1.80	0.41
F	50.40±8.36	72.13±4.89	73.45±1.90	0.11
G	57.43±9.74	78.17±3.71	73.58±1.63	0.06
P	0.32	0.69	0.08	

A control, B supplemented with 50mg/kg, C with 100mg/kg of feed, D with 200mg/kg of feed, E 50mg/L, F 100mg/L and G 200 mg/L of drinking water

Table 7. Effect of *Mentha Piperita*oil and its juice on egg weight at 0, 15 and 30 days of storage.

Groups	0 day egg weight	15th day egg weight	30th day egg weight	p
A	58.95±0.87	59.52±1.76	58.08±0.73	0.47
B	58.74±0.70	61.16±1.73	59.12±0.97	0.67
C	61.26±0.99	63.88±3.44	61.67±1.05	0.38
D	61.61±1.24	64.42±1.76	58.30±1.27	0.70
E	59.78±0.74	58.44±1.03	60.78±1.28	0.40
F	61.67±1.42	62.39±2.70	62.09±2.55	0.34
G	58.39±0.85	59.99±1.40	58.20±1.24	0.16
P	0.08	0.24	0.22	

A control, B supplemented with 50mg/kg, C with 100mg/kg of feed, D with 200mg/kg of feed, E 50mg/L, F 100mg/L and G 200 mg/L of drinking water

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RESEARCH ARTICLE

Tekirdağ'da "Koloni Kaybı Sendromu" Benzeri Kayıp Görülen Arılıklarda Bazı Patojenlerinin Araştırılması

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ÖZ

Dünya'da on yıldan fazla süredir dikkat çeken koloni kayıpları, farklı etken ve faktörlerin etkileşimi ile ortaya çıkan arı sağlığı sorunu olarak kabul edilmektedir. Bal arısı koloni sağlığını tehdit eden hastalık etkenleri arasında viruslar, *Nosema ceranae* ve *Varroa destructor* ciddi öneme sahiptir. Bu çalışmada 2015 ve 2016 yıllarında Tekirdağ ilindeki farklı arılıklardan gönderilen balarısı numuneleri incelenmiştir. Örnekler "koloni populasyonundaki beklenmeyen azalma veya ani koloni kaybı" şikâyetleriyle gönderilmiştir. Buna göre 17 arılıktan gönderilen 510 bal arısı örneği, *Deforme kanat virusu* (*Deformed Wing Virus* – DWV), *Nosema cerenae* ve *Varroa destructor* yönünden kontrol edilmiştir. Bu arılıkların tamamında varroosis tespit edilirken, DWV 15 arılıkta, *N. ceranae* ise 5 arılıkta tespit edilmiştir. DWV tespit edilemeyen iki arılıkta *N. ceranae* da tespit edilememiştir. Bu araştırmada, ani koloni kaybı sendromuna benzer şekilde görülen koloni kayıplarında DWV, *Nosema cerena* ve *Varroa destructor*'a rastlanma oranlarının araştırılarak bu konuda güncel veri sağlanması hedeflenmiştir.

Anahtar Kelime: Bal arısı, DWV, Koloni Kaybı, Nosema, Varroa

Investigation of Some Pathogens "Colony Loss Syndrome" Resembled Losses Apiaries in Tekirdağ

ABSTRACT

Colony losses, which are remarkable in the world for more than ten years, are regarded as bee health problems arising from the interaction of different factors and agents. The viruses, *Nosema ceranae* and *Varroa* destructor among the disease agents threaten the colony health, have significant importance. In this study, the honeybee samples delivered to our laboratory from different apiaries in Tekirdağ province between 2015 and 2016, were examined. Samples were sent by complaints with "unexpected decrease in colony populations or sudden colony loss". A total of 510 honey bee samples from 17 apiaries were tested for *Deformed Wing Virus* (DWV), Nosema cerenae and *Varroa destructor*. DWV and *N.cerena* were detected in 15 and 5 apiaries, respectively while varroosis was detected in all apiaries. *N. ceranae* was not be detected in the DWV negative two apiaries. In this study, it was concluded that considering the presence of DWV, *Nosema cerena* and *Varroa destructor* in sudden colony losses resemble case, and it would be advantageous the precaution and treatment strategies for bees against these pathogens.

Key Words: Honey bee, Colony Losses, DWV, Nosema, Varroa

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GİRİŞ

Bal arıları (*Apis mellifera* L.) ekosistemdeki dengeleyici ve eşsiz polinatör rolleriyle vazgeçilemeyecek ekonomik ve biyolojik öneme sahiptirler. Bal arıları bal, polen, propolis, arı sütü, balmumu, apilarnil ve perga gibi ticari ürünleriyle birlikte yetiştiriciliği yapılan türler arasında ekonomik açıdan en yüksek değere sahip böcek türüdür (Klein ve ark., 2007; Aizen ve ark., 2009; Gallai ve ark., 2009; Cakmak, 2014). Sosyal yaşam biçimiyle farklı bir varoluş mücadelesi sergileyen bal arıları, bazı patojenlere, pestisitlere ve stres faktörlerine (Karahan ve ark., 2015; Dussaubat ve ark., 2016) karşı da oldukça hassastır. Hastalık etkenlerine karşı duyarlılık, arı kolonisinin etkileşim halinde bulunduğu farklı ortam koşullarına, ırk ve ekotip özelliklerine, yetiştiricilik tekniklerine, karşılaşılan patojenin türüne, miktarına ve virulensine hatta kolonideki propolis miktarı ve kaynağına varıncaya kadar birçok faktörden etkilenmektedir. Başta parazitler olmak üzere virus, bakteri ve mantar gibi çeşitli patojen etkenler arı sağlığını pestisitler ile sinerjizma oluşturarak daha ciddi tehdit etmektedir (Evans ve ark, 2006; Simone ve ark, 2009; Evans ve Spivak; 2010; Richard ve ark, 2012; Simone-Finstrom ve ark, 2012; Mondet ve ark., 2016, Fine ve ark; 2017). Bilim adamlarını ve üreticileri yoğun şekilde meşgul eden bal arısı koloni sağlığı sorunları on yılı aşkın süredir dünya ile eş zamanlı olarak Türkiye'de de artan öneme sahiptir (Aydın ve ark., 2001; Akkaya ve Alkan, 2002; Çakmak ve ark., 2003; Muz ve Muz, 2009; Van der Zee ve ark., 2015; Brodschneider ve ark., 2016). Bal arısı koloni sağlığı sorunları arttıkça bu konuda yapılan *tıbbi* araştırmaların, enfeksiyöz hastalıklar üzerinde odaklandığı görülmektedir (Genersch, 2010; Genersch ve ark., 2010). Özellikle 2006 yılından itibaren çok sayıda araştırmada "honeybee colony collapse disorder (HCCD)" veya "honey bee depopulation syndrome (HBDS)" (Cox-Foster ve ark., 2007; Stoksad, 2007a; Stoksad, 2007b; Winfree ve ark., 2007) olarak tanımlanan bu sendrom kısaca farklı etkenlerin ortak etkileşimi neticesinde bal arısı kolonilerinde işçi arı nüfusundaki sıra dışı, ani kayıpları nitelemektedir. Ancak üreticilerin tam olarak bu tanımlamalara uymayan fakat oldukça benzer koloni kayıpları yaşadıkları da bilinmektedir. Günümüze kadar dünyada tanımlanan arı viruslarının çoğu *Picornaviridae* ailesindeki *Disictovirirus* ve *Iflavirus* genusları içerisinde sınıflandırılan, pozitif polariteli tek iplikcikli RNA viruslarıdır. Özellikle Deforme Kanat Virusu (DWV) ve Siyah Kraliçe Hücre Virusu (BQCV) gibi türler arılıklardan en çok izole edilen viruslardır. Bunun yanında arı virusları içerisinde özellikle DWV diğer arı patojenleri ile birlikte tespit edilme oranı dünyada en yüksek dolayısıyla en yaygın bal arısı virusudur (Zhang ve ark., 2012; Desai ve ark., 2016). *Iflavirus* sınıfında yer alan DWV 30 nm büyüklüğünde tek iplikcikli pozitif polariteli bir RNA molekülü içermektedir (Lanzi ve ark. 2006). Dünya'da ilk defa 1980 yılında Japonya'da tanımlanmıştır (Bailey ve Ball, 1991). Bu viruslar bal arılarının farklı gelişim aşamalarında akut ve persiste enfeksiyonlara yol açarak zayıf, güçsüz kolonilerin oluşmasına neden olurken, meydana gelen ölümlerle de beklenmeyen koloni kayıplarında (HCCD, HBDS) rol oynarlar (Benaets ve ark., 2017). DWV arılarda başlıca kanat anomalilerinin oluşumuna yol açar. Akut enfekte arılar eksik kanatlı, kanatsız, kısa veya buruşuk kanatlı olduklarından uçamazlar. Enfeksiyon sonucu ana arılarda meydana gelen ovaryum dejenerasyonuna bağlı patolojiler koloni nüfusunda kayıpların oluşumuna neden olur (Gauthier ve ark., 2011; Dainat ve ark., 2013). Patolojik durumdaki arı sayısı arttıkça kovan içi ve kovan dışı yapılması gereken görevler yerine getirilemediğinden koloni gelişimi yavaşlamaya başlar. Bu durum düzelmediği takdirde kolonilerde üretim durur, koloni populasyonu zayıfladıkça koloni nüfusunda gerileme başlar. Sosyal canlılar olan bal arıları ortak bağışıklık mekanizması ve savunma davranışı sergiledikleri için karşılaşılan tıbbi sorunlar hızla yıkıcı etki gösterebilir ve koloniler aniden sönebilir (Evans ve Pettis, 2005; Evans ve ark., 2006; Simone ve ark., 2009; Evans ve Spivak, 2010; Richard ve ark., 2012, Simone ve Spivak, 2012). Çoğu virusa vektörlük yapan ve arı hemolenfi dışında hiçbir besin kaynağı bulunmayan *Varroa destructor*, ekonomik ve tıbbi açıdan çok önemli arı akarıdır. Varroa enfeste kolonilerde DWV virulensinin ve koloni kayıplarının çok daha yüksek olduğu bildirilmiştir (Van der Zee ve ark., 2015; Nazzi ve Le Conte, 2016). Akarın, arı üzerinde açtığı yara odaklarından beslenirken tegument hasarına bağlı olarak değişik sekonder enfeksiyonlara kapı açtığı bildirilmektedir (Kanbar ve Engels, 2005). Farklı türleri bulunan Varroa akarının, Türkiye'deki tür tespiti ve yaygınlığı hakkında yapılan araştırmalarda mevcut türün *Varroa destructor* olduğu bildirilmiştir (Aydın ve ark., 2003; Warrit ve ark., 2004). Türkiye'de arılıklarda yaygınlığı yüksek olarak bildirilen Varroa'ya karşı mücadelede çeşitli organik asitlerin, sentetik insektisidlerin kullanımı ve etkinliği üzerine yapılan çalışmalar da mevcuttur (Akkaya, 1996; Girişgin ve ark., 2010; Akkaya, 2014). Bal arılarının son yıllarda gittikçe artan öneme sahip diğer bir patojeni *Nosema ceranae*, arıların bağırsaklarında neden olduğu hastalık tablosuna bağlı olarak arıların beslenme, uçma ve koloni içerisindeki görevlerini yerine getirmesine engel olmakta, koloni gelişiminde yavaşlama ve gerilemeye yol açmakta, temizlenmek veya dışkılamak üzere uçan işçi arıların çoğu zaman geri dönememesi sonucunda koloni populasyonunda beklenmeyen düşüşlere yol açmaktadır (Doublet ve ark., 2016). Gelişme ve üretim sorununun yaşandığı bal arısı kolonileri ile kış kayıplarının arttığı arılıklardan sıklıkla izole edilmektedir. Özellikle İspanya başta olmak üzere değişik merkezlerde yapılan araştırmalar kolonilerde görülen beklenmeyen

kayıplarda *Nosema cerenae*'nin da rolü olduğunu göstermiştir (Wenning, 2002; Higes ve ark., 2009; Bacandritsos ve ark., 2010; Dainat ve ark., 2012; Botias ve ark., 2013; Cepero ve ark., 2014). *Nosema cerenae* ve DWV miks enfeksiyonlarında, hedef hücrelere önce DWV'nin girmesi durumunda her iki patojenin sinerjizma gösterdikleri tespit edilmiştir (Doublet ve ark., 2015; Zheng ve ark., 2015). Bu araştırmanın amacı Tekirdağ ilinde "ani koloni kaybı" görülen arılıklarda söz konusu arı patojenlerinin araştırılması, üreticilerin şikayetleri hakkında kanıta dayalı şekilde bilgilendirilmesidir.

MATERYAL VE METOT

Bal Arısı Örnekleri Araştırmada kullanılan bal arısı örnekleri, 2015-2016 yıllarında Tekirdağ ilindeki arılıklarda beklenmeyen şekilde koloni gerilemesi veya ani kayıplar yaşayan üreticilere aittir. Örnekleme aşamasında üreticiler yönlendirilmemiştir. Bu örnekler arılıklarında sorun olduğunu düşünen üreticiler tarafından, problem yaşanan kovanlardan, anormal oldukları kanaatiyle toplanmıştır. Araştırmada kullanılan numuneler; gönderilen petek örnekleri, plastik pet şişe içerisindeki canlı ve/veya ölü arılardan oluşmaktadır. Çalışma materyali olarak canlı ve ölü işçi arılar kullanılmıştır. Çalışma materyalinin sağlandığı 17 arılığa ait 510 adet bal arısı örneğinden her bir arılığı temsilen 10 adet canlı, 10 adet ölü arı analiz zamanına kadar -80 °C'de muhafaza edilmiştir

Örneklerde Viral tanı Muhafaza edilen bal arısı örneklerinden, her bir arılığı temsilen beş adet canlı ve beş adet ölü arılardan toplam on tane alınarak tek tek homojenize edildi. Homojenizasyon daha önce bildirilen yöntemle (Muz ve Muz 2009) PBS içerisinde gerçekleştirildi. Santrifüj sonrası alınan 200 µl süpernatant viral RNA'nın eldesi amacıyla ticari bir kit (GeneJET Genomic DNA Purification Kit, Thermo) kullanılarak RNA ekstraksiyonu yapıldı. RNA ekstraksiyonu kitin kendi prosedürüne uygun olarak gerçekleştirildi. Elute edilen sıvıdan toplam 5 µl RNA RT-PCR amacıyla kullanıldı. cDNA sentezi ticari bir kit (First Strand cDNA Synthesis Kit, Thermo) kullanılarak, kitin kendi prosedürüne uygun olarak yapıldı. PCR testleri de, daha önce bildirilen DWV helikaz enzimini kodlayan gen spesifik primer çiftleri (Berenyi ve ark 2006) kullanılarak viral tanı gerçekleştirildi. Toplam 30 µl hacimde hazırlanan PCR karışımı içeriği; 5 µl cDNA, 2 ünite Taq polimeraz (TrueStart Hot Start Taq DNA Polymerase, Thermo), 10xTaq buffer, 2mM $MgCl_2$, 200 µmol dNTP miks ve her bir primerden 300 µmol oluşmaktadır. Hazırlanan karışım Muz ve Muz (2009) bildirilen ısı ve süre şartlarında reaksiyona tabi tutuldu. Çoğaltılan reaksiyon ürünleri 5µg/µl oranında ethidium bromide içeren %1'lik agaroz içerisinde yürütülerek UV ışığı altında görüntülendi.

Örneklerde *Nosema cerenae* ve *Varroa destructor* tanısı Homojenat sıvısından alınan 200 µl sıvı gDNA eldesi için kullanıldı. Bu amaçla ticari bir kit (GeneJET Genomic DNA Purification Kit, Thermo) kullanım rehberine uygun şekilde çalışıldı. Elde edilen gDNA *Nosema ceranae* spesifik oligonükleotid primer çifti kullanılarak (Martín-Hernández ve ark., 2007) PCR testi gerçekleştirildi. Hazırlanan PCR karışım içeriği ve reaksiyon ısı şartları Muz ve ark (2010) bildirildiği gibi yapıldı. Çoğaltılan reaksiyon ürünleri 5µg/µl oranında ethidium bromide içeren %1'lik agaroz içerisinde yürütülerek UV ışığı altında görüntülendi. Bal arısı numunelerinin gönderildiği şeffaf numune şişeleri önce *Varroa destructor* varlığı yönünden çıplak gözle makroskopik olarak incelenmiş ardından arılar bir pens yardımıyla tutulup stereomikroskop altında incelenmiştir.

BULGULAR

Örnek gönderilen 17 arılıktan 15'inde (%88,23) DWV, 5'inde (%29,41) *Nosema cerenae* ve tamamında *Varroa destructor* bulunduğu tespit edildi. Gönderilen bal arısı örnekleri arasında canlı ve ölü olarak dondurulan arıların DWV analizi sonuçlarında farklılık bulundu. Virus tespiti için analiz edilen bal arısı örnekleri arasında sadece canlı olarak dondurulan numunelerde DWV pozitifliğine rastlandı. Nosema pozitiflilik sonuçlarında ise aynı kolonideki canlı ve ölü arı numunelerinde fark olmadığı tespit edildi. *Nosema ceranae* pozitif bulunan örneklerin tamamında DWV pozitifliği saptandı. DWV bulunmayan iki arılıkta (%11,76) da *N. ceranae* tespit edilemedi. Tüm arılıkların *Varroa destructor* ile enfeste olduğu belirlendi. Örneklenen arılıklardan 5'i (%29,41) her üç patojen için pozitif iken 10'u (%58,82) iki patojen için pozitif olarak bulundu.

TARTIŞMA ve SONUÇ

Dünya genelinde HCCD olarak tanımlanan bu sendroma çok benzeyen semptomlarla seyreden koloni kayıpları yaşanmaktadır. Bu ikiz tablo bazı literatürlerde kısaca "*HCCD benzeri*" ifadesiyle tarif edilmektedir (Hristov, 2014; Parvanov ve Rusenova, 2014; Pries ve ark., 2016). Her iki soruna bağlı koloni kayıplarının arılıklarda birlikte görülüp görülmediği ve klinik epidemiyolojileri hakkında bazı araştırmalar bulunmaktadır. Hristov'un (2014) bildirdiğine göre, Bulgaristan'da görülen koloni kayıplarının HCCD ile bağlantısı araştırılmıştır. Meydana gelen ölümlerin gerçek nedenlerinin on vakada pestisit zehirlenmesi, on vakada varroosise bağlı kayıplar, altı vakada nosemosis bağlı kayıplar, altı vakada açlıktan dolayı ölüm ve üç vakada ise arıcıların teknik hatalarından dolayı ölümler yaşandığı ve HCCD nin tarifine kesin olarak uyan bir vakaya rastlanmadığını da bildirmiştir

(Hristov, 2014). Bunun gibi Pires ve ark. (2016)'da HCCD benzeri kayıplar yaşandığını ancak bunun tam olarak HCCD şeklinde tabir edilemeyeceğini belirtmiştir. Türkiye'de kolonilerde hastalık oluşturan etkenler hakkında yapılan saha çalışmalarında arı populasyonlarındaki bal arısı virusları, Nosema türleri ve *Varroa destructor* enfestasyonlarının yaygınlığı araştırılmış, bu patojenlerin varlıkları hakkında farklı sonuçlar elde edilmiştir. Türkiye'de *Varroa destructor*'a % 6,2 ile %100 arasında değişen oranlarda rastlanıldığı bildirilmiştir (Balkaya ve ark, 2016). DWV varlığı Türkiye'de ilk defa 2006 yılında çok yoğun koloni kayıpları yaşanan Doğu Akdeniz bölgesinde tespit edilerek 2008 yılında resmen tanımlanmıştır (Muz ve Muz, 2008). Söz konusu çalışmada kolonilerde DWV'ye rastlanma oranı %100 olarak belirtilmiştir. Bunun dışında DWV hakkında yapılan araştırma sayısı oldukça sınırlı olup Hatay ve Ordu illerinde 2009 yılında (Muz ve Muz, 2009; Gülmez ve ark., 2009) ve ayrıca 2015 yılına ait araştırmalarda DWV varlığı tespit edilmiştir (Tozkar ve ark., 2015). Nosema türlerinin moleküler tanısı amacıyla Türkiye'de farklı çalışmalar gerçekleştirilmiştir (Muz ve ark., 2010; Ütük ve ark., 2010; Whitaker ve ark., 2010; Muz ve ark., 2012; Ütük ve ark., 2016; Büyük ve ark., 2017). Türkiye'deki kolonilerden *Nosema ceranae* ile *Nosema apis*'in ilk moleküler teşhisi ve genetik ayrımı 2010 yılında bildirilmiştir (Muz ve ark., 2010). Koloni kayıpları hakkında 2010 yılında gerçekleştirilen bir araştırmada, Hatay ve Adana yöresi arılıklarında *Varroa destructor*'a rastlanma oranı %98, *Nosema ceranae*'ya rastlanma oranı %12,9 olarak tespit edilmiş, kolonilerin %52,3'ü ise sağlıklı olarak bildirilmiştir (Yalçınkaya ve Keskin, 2010). Farklı açıdan yapılan bir anket çalışmasında ise kışlatma nedeniyle yaşanan koloni kayıpları, 2008-2009 yılları arasında %14,5, 2009-2010 yılları arasında %18,9 ve 2010-2011 yılları arasında ise %12,6 olarak bildirilmiştir (Tunca ve ark., 2016). Mevcut araştırmada, koloni kaybı benzeri sorunlar yaşayan 17 arılıktan gönderilen 5 örnekte (%29,41) *Nosema ceranae*'ya rastlanırken gönderilen örneklerin hepsinin *Varroa destructor* ile enfeste oldukları tespit edilmiştir. Türkiye'de bal arısı virusları hakkında yapılan bir araştırmada (Gülmez ve ark., 2009) DWV nin sadece *Varroa destructor* ile enfeste kolonilerde bulanacağı bu nedenle diğer kolonilerin DWV ari sağlıklı olarak kabul edilebileceği bildirilmiştir oysa koloniler arasında DWV'nin bulaşma yolu sadece *Varroa destructor* olmadığı farklı kaynaklarda bildirilmektedir. Kolonilerde DWV bulaşıklığı için *Varroa destructor* enfestasyonu ön şart değildir. Bununla birlikte, DWV ve *Varroa destructor* birlikteliğinin arı sağlığındaki riski arttırdığı farklı yönleriyle tartışılarak açıklanmıştır (Yue ve ark., 2007). DWV kovan içindeki farklı arı ürünlerini de kontamine etmektedir. Örneğin arı sütü, polen ve bal DWV ile kontamine olabilmektedir. Yine sağlıklı arılar tarafından kovan dışından getirilen polen örneklerinde de DWV bulunabilmektedir (Singh ve ark., 2010; Mazzei ve ark., 2014). DWV'nin bulaşmasında taşıyıcı ana arı, erkek arı sperması, taşıyıcı vektör varroa, virus kontamine besinler rol oynamaktadır (Yue ve Generich, 2005; Fievet ve ark., 2006; Yue ve ark., 2006, 2007; Yanez ve ark., 2012; Amiri ve ark., 2016). Dünya'da koloni kaybı görülen ya da buna benzer sorunlar yaşanan arılıklarda tespit edilen DWV ve *Nosema ceranae* oranları değişiklik göstermektedir. Bazı araştırmalarda DWV ve *Nosema ceranae* birlikteliğinin koloni kayıplarında daha ciddi sorunlara yol açtığı iddia edilirken (Johnson ve ark., 2009; Bromenshenk ve ark., 2010), hedef hücrelere önce DWV'nin girmesi durumunda her iki patojenin sinerjizma gösterdiği de tespit edilmiştir (Doublet ve ark., 2015; Zheng ve ark., 2015). Sonuç olarak bu araştırmada bal arısı koloni kaybı (HCCD) benzeri sorunların yaşandığı arılıklardan gönderilen bal arısı örnekleri bazı hastalık etkenleri açısından incelenmiştir. Arılıkların tümünde (%100) *Varroa destructor* enfestasyonu, %88,23'ünde DWV ve %29,41'inde ise *Nosema ceranae* tespit edilmiştir. Buna göre koloni kaybı benzeri sorunlara karşı tedbir olarak mevcut patojenlerin varlığı göz önünde tutularak başta *Varroa destructor* mücadelesi olmak üzere, *Nosema ceranae*'ye karşı koruma ve tedavi tedbirlerinin yanı sıra viral hastalıkların yayılmasına engel olacak şekilde "iyi arıcılık uygulamalarına" dikkat edilmesi önerilmektedir.

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REVIEW

Süt Sığırlarında Paratüberküloz

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ÖZ

Paratüberküloz, *Mycobacterium avium subsp. paratuberculosis* tarafından oluşturulan enfeksiyöz bir hastalıktır. Başlıca evcil sığırların, koyun ve keçilerin bir hastalığıdır. Yanı sıra etken vahşi ve egzotik ruminant türlerinde de enfeksiyona neden olabilmektedir. Paratüberküloz, özellikle süt sığırı işletmelerinde, önemli ekonomik kayıplara neden olan hastalıkların başında gelmektedir. Hastalığın klinik formu kronik ishal ve kilo kaybı ile karakterizedir. Bununla birlikte paratüberküloz, klinik hastalık dönemi öncesi, uzun bir subklinik evreye sahiptir. Bu dönemde sürüdeki sağlıklı bireyler enfekte hayvanlar tarafından kontamine edilir. İnsanlarda gözlenen Crohn's hastalığının etiyolojisindeki olası rolü nedeniyle hastalığın potansiyel zoonoz karakterde olduğu da rapor edilmektedir.

Anahtar Kelimeler: Crohn's Hastalığı, ELISA, Johne's Hastalığı, Paratüberkülozis, Süt Sığırı, Tüberkülozis.

Paratuberculosis in Dairy Cattle

ABSTRACT

Paratuberculosis is an infectious disease caused by *Mycobacterium avium subsp. paratuberculosis*. It observed mainly in domestic cattle, sheep and goats. Besides, the disease can lead to infection in the wild and exotic ruminant species. Paratuberculosis, especially in the dairy business, is one of the diseases which cause significant economic losses. Clinical form of the disease is characterized by chronic diarrhea and weight loss. However, paratuberculosis has a long subclinical phase prior to clinical disease. During this period, healthy individuals in the herd are contaminated by infected animals. Because of the possible role in the etiology of Crohn's disease in humans that the disease is reported to be potentially zoonotic character.

Key Words: Crohn's Disease, ELISA, Johne's Disease, Paratuberculosis, Dairy Cattle, Tuberculosis.

GİRİŞ

Paratüberküloz (pTB) ilk kez sığırların ince bağırsağında 1895'de tespit edildi (Johne ve Frotingham 1895). Başlangıçta, tüberküloz etkenine benzeyen bu bakteri (MAP) tüberkülozun atipik bir formu olarak tanımlandı. 1906 yılında etkenin tüberküloz etkeninden farklı olduğu tespit edilmiş olup, *Pseudotüberculosis Enteritis* olarak yeniden isimlendirilmiştir (Bang 1906). Asit-faz nitelikte bir bakteri olan MAP sığır dışkısında ortalama bir yıl süreyle canlı kalır (Baumgartner ve Khol 2006).

Paratüberküloz (pTB) süt sığırı işletmelerinde ekonomik kayıplara neden olan en önemli hastalıklardan biridir (Hasanova ve Pavlik 2006). Hastalık sığırlarda uzun bir subklinik evreye sahiptir. Dışkıda etken çoğu enfekte sığırda iki yaşından önce tespit edilemez (Eddy 2004, Osterstock ve ark. 2010). Bu dönemde hasta sığır sürüdeki sağlıklı hayvanları enfekte etmeye devam eder (Baumgartner ve Khol 2006). Bahsedilen bu erken enfeksiyon döneminde, ruminantlarda klinik semptom gözlenmez (Allaker ve Kapas 2003). Hastalık kronikleştikçe etken lenf yumrularına yayılır ve klinik semptomlar ortaya çıkar. Hastalığın başlıca bulguları arasında ishal ve kilo kaybı sayılabilir (Eddy 2004).

Paratüberkülozun konak dağılımı oldukça geniştir. Başlıca süt sığırlarında görülen bir etken olarak bilinmektedir. Ancak Muflon (Machackova ve ark. 2004), Manda (Sivakumar ve ark. 2005) ve Deve'lerde de (Selbitz 2002) bu hastalık rapor edilmiştir. Yanı sıra, ruminant olmayan bazı hayvan türlerinde de bu hastalığa rastlanmıştır (Judge ve ark. 2005). Son yıllarda, insanlarda görülen Crohn's hastalığının etkeninin de MAP olduğu rapor edilmektedir (Pickup ve ark. 2004).

Yaygınlık

MAP'ın bölgesel ve ülkesel prevalansı değişiklik göstermektedir. Dünya üzerinde birçok ülkede pTB varlığı tespit edilmiştir. Paratüberküloz prevalansı Almanya için %84.7 olarak rapor edilirken, Avustralya'nın bir bölümününde ise bu hastalığa rastlanmadığı bildirilmektedir. İsveç'te yapılan bir çalışmada ise pTB prevalansının asgari düzeyde olduğu belirtilmiştir. Süt sığırları üzerinde yürütülen araştırmalar paratüberküloz prevalansının Danimarka için %47, Kanada için %43, Amerika Birleşik Devletleri için ise %50 olduğunu ortaya koymuştur (Collins ve ark. 1994, Jakobsen ve ark. 2000, Van Leeuwen ve ark. 2001, Hacker ve ark. 2004).

pTB süt sığırlarında ciddi verim kayıplarına neden olan en önemli enfeksiyöz nedenlerden biridir. Ülkemizde de pTB ile ilgili çalışmalar yapılmakta ve güncelliğini korumaktadır (Civelek ve ark. 2009, Öztürk ve ark. 2010, Yıldırım ve Civelek 2013, Makav ve Gökçe 2013).

Bulaşma

Hastalığın yayılmasındaki en önemli faktör, enfekte hayvanların dışkıları ile bulaşık yem ve suların tüketilmesidir (Smith 2001). Doğum sonrası, anneden yavruya olan fekal ve oral bulaş, hastalığın yayılmasındaki en önemli faktördür (Whitlock ve ark. 1986). Yanı sıra, buzağıların doğum sonrası anne ile beraber barındırılması ve direk anneyi emerek süt alması enfeksiyonun geçişinde önemlidir (Çetinkaya ve ark. 1997). MAP enfekte dişi hayvanların sütünde de bulunabilir. Yanı sıra, yeni doğum yapmış sığırların kolostrumunda da MAP etkenine rastlanmıştır. Yeni doğan buzağılar etkeni kontamine anne memesinden direk alabilir. Süt ve kolostrumun pastörizasyonu bulaş ihtimalini azaltmaktadır. Fakat MAP yayılımı tam olarak önlenemez (Baumgartner ve Khol 2006). Yapılan çalışmalar, genç buzağıların dışkıları ile de bakterinin yayabildiğini ortaya koymuştur (Weber ve ark. 2005).

Besleme hataları, gebelik dönemi ve yüksek süt verimi vb stres faktörleri, uzun süreçli kortikosteroid kullanımları paratüberkülozun klinik formunun ortaya çıkmasında tetikleyici unsurlardır (Eddy 2004). Hastalık üç devrede incelenebilir. *Sessiz dönem*; alınan dışkı örneklerinde etken tespiti zordur. Bununla birlikte sindirim kanalı doku kültürlerinde etken tespit edilebilir. Bu dönem, iki yaşından itibaren, uzun bir süre devam edebilir (Smith 2001). *Subklinik dönem*; kilo kaybı ve ishal bu evrede gözlenmez (Strıcklands ve ark. 2005). Bu dönemdeki enfekte hastalar dışkıları ile enfeksiyonu yayar. Sürünün tamamı subklinik dönemde enfekte olabilir. Bu evre birkaç yıl sürebilir. *Klinik dönem*; klinik semptomlar bu evrede görülür. Kilo kaybı ve şiddetli ishal ile karakteristiktir (Eddy 2004).

Tanı

Klinik paratüberkülozun tanısı semptomlar, anamnez ve nekropsi sonuçları ile konur. Bulgu göstermeyen vakalarda teşhis amaçlı laboratuvar testlerden yararlanılır. Bununla birlikte özellikle genç sığırlarda kullanılabilecek spesifite ve sensitivitesi yüksek bir test ise bulunmamaktadır (Baumgartner ve Khol 2006). pTB tanısında günümüzde başlıca kültür, PCR, immunite testleri ve serolojik testlerden yararlanılmaktadır (Baumgartner ve Khol 2006, Civelek ve ark. 2009, Mecitoğlu ve Demir 2012). ELISA tanıda en sık kullanılan metottur.

Otopsi Bulguları

Enfekte sığırlarda gastrointestinal sistem ve lenf nodüllerinde lezyonlar gelişir. İleum cidarındaki kalınlaşma tipiktir. Barsak kıvrımlarının beyin benzeri görünüm alması bu hastalık için karakteristiktir. Ayrıca, vücut boşluklarında seröz effüzyon da tespit edilir (Osterstock ve ark. 2010).

Ekonomik Önem

pTB ekonomik açıdan önemli kayıplara yol açan ve tedavisi ise bulunmayan bir hastalıktır. Hastalığın infertilite, mastitis vd. subklinik problemleri tetiklemesiyle birlikte maddi kayıp daha da artar. ABD'de enfeksiyonun ekonomiye senelik zararının 250 milyon dolar civarında olduğu rapor edilmiştir (Sharma ve ark. 2008). Türkiye'de ise farklı çalışmalar yapılmakla birlikte, hastalığın ülke genelindeki prevalansı tam olarak belirlenmemiştir. Dolayısıyla bu hastalığın neden olduğu etkiler ülkemiz açısından net olarak bilinmemektedir (Mecitoğlu ve Demir 2012).

Koruma ve Kontrol

Kesin tedavisi günümüz koşullarında olmayan bu hastalıktan korunma ve kontrolde, MAP pozitif olduğu tespit edilen hayvanların sürüden eliminasyonu önem arz etmektedir. MAP negatif sürülerde ise olası bir bulaşın önlenebilmesi için gerekli tedbirler alınmalıdır. MAP şüpheli sürülerde, farklı zamanlarda, birkaç farklı serolojik test ile etken varlığının araştırılması ve olası subklinik vakaların erken teşhisi önerilir (Baumgartner ve Khol 2006).

Sürülerde pTB'nin varlığı; >1.5 yıl yaşlı hayvanlarda altı ay aralıklarla gerçekleştirilecek ELISA, bakteriyel kültür ve dışkı PCR tarama yöntemi gibi metodlar kullanılarak araştırılmalıdır. pTB şüpheli veya pozitif çiftliklerde ek hijyen tedbirleri alınarak, sürü içi ve dışı yayılımın önüne geçilmelidir. Subklinik enfekte hayvanlar sağlıklılardan ayrılmalı, verim kaybı/klinik semptom gösteren subklinik enfekte hayvanlar sürüden elimine edilmelidir. Alınacak tedbirler başlıca yeni doğanlara hastalığın bulaşmasını engellemeyi amaçlar. Doğum sırasında uygulanan hijyen prosedürü ve yeni doğan buzağıların anneden ayrılması kontaminasyonu kısmen azaltmaktadır. Ayrıca, antijen ve antikor negatif ve pozitif annelerden doğan yavruların ayrı yerlerde barındırılması ile hastalıktan kısmen de olsa korunulmuş olunur. Yeni doğan beslenmesinde antijen-antikor negatif annelerden alınan kolostrumun kullanılması önerilir. Damızlık amaçlı negatif bireyler tercih edilmelidir. Eradikasyon uygulanan çiftliklerde, iki yıl boyunca besleme sırasında genç ve yaşlı hayvanlar mutlak ayrılmalıdır. Genç sığırlarda kullanılan ekipman ayrı olmalı ve dezenfeksiyon ve biyogüvenlik kurallarına riayet edilmelidir. Hayvan hareketlerine sınır getirilmesi ve mevcut sürülerin pTB varlığı yönünden araştırılması ile MAP'ten ari bölgeler oluşturulabilir ve paratuberküloz yayılımı önlenebilir (Baumgartner ve Khol 2006, Yıldırım ve Civelek 2013).

SONUÇ

Subklinik pTB'li hayvanlar hastalığı sağlıklı olanlara bulaştırma eğiliminde olmaları nedeniyle önemlidir. Hastalığın zoonotik potansiyeli ve pastörizasyona dayanıklılığı göz önünde bulundurulduğunda, özellikle süt sığırı yetiştiriciliğinin yoğun yapıldığı bölgelerde subklinik enfeksiyon varlığının ortaya konması önem arz eder (Yıldırım ve Civelek 2013). Subklinik enfekte sığırların başlıca süt ve dışkısı ile ortama yayılan etkenler insan sağlığı için olası tehdittir (Pickup ve ark. 2004, Yıldırım ve Civelek 2013).Süt sığırlarında hastalığın prevalansın tespitinde sürü bazlı kullanılabilecek en ucuz ve en uygulanabilir test günümüzde ELISA'dır.

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REVIEW

Effects of Bacteriocin Applications For *Clostridium botulinum* and *Listeria monocytogenes* in Seafood Products

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ABSTRACT

Food related diseases areon the rise and the safety of food is still an increasingly important public health issue in worldwide. Seafoods are particularly suitable for both microbiological spoilage and biochemical deterioration so it is essential to develop proper strategies to protect these products' safety, maintain the higher quality and also extend their shelf life. There are number of techniques for protecting the seafoods safety but especially biopreservation is pointed out to maintain the higher quality and minimum effects on nutritional values, extending shelf life and stabilizing the organoleptic properties. With the increasing antibiotic resistance problem and awareness of the risks of chemical preservatives for public health, bacteriocins have attracted a considerable attention. Due to their naturally produced structure, bacteriocins are much more admissible by consumers. The inhibition of the foodborne pathogens by bacteriocins has been studied by several researchers. Eventually, nisin and sakacin P are the most studied bacteriocins, but the other bacteriocins like curvaticin, carnocin, bavaricin, and divergicin also studied for potential applications in seafood products. In this review, it is aimed that to summarize the bacteriocin applications as an antimicrobial agent in seafood products.

Key Words: Bacteriocin, Biopreservation, Foodborne Infections, Nisin, Seafood.

Deniz Ürünlerinde Bakteriyosin Uygulamalarının *Clostridium botulinum* ve *Listeria monocytogenes* Üzerine Etkileri

ÖZ

Günümüzde, gıda kaynaklı hastalıklar artış göstermekte ve güvenilir gıdanın önemi gittikçe daha önemli hale gelen bir halk sağlığı problemi haline gelmektedir. Diğer yandan, deniz ürünleri hem mikrobiyolojik ve hem biyokimyasal bozulma ajanlarına karşı oldukça duyarlı ürünler olmaları nedeniyle deniz ürünlerinin güvenliği ve kalitesini sağlamak, aynı zamanda raf ömrünü uzatmak amacıyla etkin muhafaza teknolojilerinin geliştirilmesi önemlidir. Deniz ürünlerinde kullanılabilecek alternatif gıda muhafaza yöntemlerini arasında, raf ömrünün uzatılması ve hijyenik kalitenin sağlayan, besinsel ve organoleptik açıdan ürünü en az etkileyen biyolojik muhafaza yöntemleri özelikle ilgi çekmektedir. Antibiyotik dirençliliği endişelerinin yanı sıra, tüketicilerin kimyasal koruyuculardan kaynaklanabilecek potansiyel sağlık risklerine olan duyarlılığı dolayısıyla bakteriyosinlere olan ilgi artış göstermiştir. Bakteriyosinler doğal olarak üretildikleri için tüketiciler tarafından daha kolay kabul edilmektedir. Bakteriyosinlerin gıda kaynaklı patojenleri inhibe edici etkisi birçok araştırmacı tarafından çalışılmaktadır. Sonuç olarak, nisin ve sakacin P üzerinde en fazla çalışılan bakteriyosinler olmakla birlikte, curvaticin, carnocin, bavaricin ve divergicin bakteriyosinlerinin de deniz ürünlerinde potansiyel uygulamaları üzerinde çalışılmaktadır. Bu derlemede, bakteriyosinlerin antimikrobiyal ajan olarak deniz ürünleri üzerine uygulamalarının özetlenmesi amaçlanmıştır.

Anahtar Kelimeler: Bakteriyosin, Biyokoruma ,Gıda Kaynaklı İnfeksiyonlar, Nisin,Deniz Ürünleri.

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INTRODUCTION

The growing interest for advanced product quality and longer shelf life of seafoods and products has led the consumers consume safer and higher qualified foods (Cortesi et al. 2009).In this review, it is aimed that to summarize the bacteriocin applications as an antimicrobial agent in seafood products. Despite of the new techniques and safety assessments, there are wide range of preservation techniques are available but the number of foodborne illnesses are on the rise and the safety of food is still an increasingly crucial public health concern. It is declared by Centers for Disease Control and Prevention (CDC) that each year about 48 million people get ill, 128,000 of them are hospitalized, and approximately 3,000 of them lose their lives as result of the foodborne diseasesinthe United States (Anonymous 2016, Karthik et al. 2013).On the other hand, due to the various nutrient composition of seafood and their products, they are particularly suitable for both microbiological spoilage and biochemical deterioration so it is essential to develop proper strategies to protect these products' safety, maintain the higher quality and also extend their shelf life(Cakli and Kisla 2003, Jamuna et al. 2005, Ghanbari and Jami 2013).The processes, using for the control of foodborne bacteria, are generally not adequate for eliminating these bacteria that can survive during traditionally processing such as salting, canning or smoking of the seafoods and products (Ghanbari and Jami 2013).Since seafood products are consumed without heat treatment or they are minimally heat-treated, it is very significant to improve suitable preservation techniques to provide their safety, quality and also improve their shelf life (Soomro et al. 2002, Cortesi et al. 2009). Biological preservation means afood safety development by means of using a natural microflora or antimicrobial metabolites. With the increasing antibiotic resistance problem and awareness of the risks of chemical preservatives for public health, bacteriocins have attracted a considerable attention. Due to their naturally produced structure, bacteriocins are much more admissible by consumers (Galvez et al. 2010, Ghanbari and Jami 2013).Polypeptide structured bacteriocins are synthesized ribosomally by bacteriaand they are assimilated by the digestionsystem to human body and they have bacteriocidal activity. Since food-related diseases are on the rise and the safety of food is still an increasingly important public health concern, using of bacteriocins, inhibit foodborne pathogens without any adverse effects, has aroused great interest. Only nisin is approved for using as afood preservation agent by Food and Agriculture Organization (FAO), but also there are plenty of bacteriocins generated vialactic acid bacteria have possible applications in foods (Cleveland et al. 2001, Chen and Hoover 2003, Delves-Broughton 2005, Kisla and Unluturk 2003).Especially Gram-negative microorganisms cause the spoilage in fresh fish and there are not a plenteous number of studies related to using bacteriocins as a biological preservation agent in these products. Spoilage and foodborne pathogens like *Clostridium botulinum* and *Listeria monocytogenes* may create apublic health danger in these products. Several researchers have studied for decreasing the risks related with these pathogens. Eventually nisin and sakacin P are the most studied bacteriocins, but the other bacteriocins like curvaticin, carnocin, bavaricin, and divergicin also studied for potential applications in seafood products (Degnan et al. 1994, Einarsson and Lauzon 1995, Nilsson et al. 1997, O'Sullivan et al. 2002). Limitation of *L. monocytogenes* in smoked and vacuum packaged salmon with using bacteriocins has been observed via different researchers. Sakacin P has been demonstrated for its potential effects to eliminating *L. monocytogenes* and use of this bacteriocin is familiar with seafood products (Katla et al. 2002, Blom et al. 1997, Brurberg et al. 1997, Eijsink et al. 1998, Ganzle et al. 1999, Aasen et al. 2000).For the elimination of *L. monocytogenes* in smoked rainbow trout, nisin and sodium lactate concentrations were analized in another study. Nisin and sodium lactate concentrations were inoculated into the trout before and after the smoking. Effects of the treatments for organoleptic properties and shelf life were also tested. The study showed that nisin and sodium lactate are both effective for inhibition of the *L. monocytogenes* when they were used separatelyin smoked trout, however the treatment was much more efficient when nisin and sodium lactate using together. Whenever they were used together, *L. monocytogenes* counts diminished from 3.3 to 1.8 log cfu/g in 16 days. Besides, the study showed that the organoleptic properties of the trout was not effected after the treatments and also the treatments extended the shelf life for additionally 7 days of storage (Nykanen et al. 2000). Aasen et al. (2000) analyzed the efficiency of nisin and sakacin P treatments in smoked salmon and chicken meat. They pointed out that due to their amphiphilic structure, bacteriocins may be adsorbed by food structure and exposed to proteolytic deterioration, so this can restrict their use in preservation. They observed that muscle proteins adsorbed less nisin than sakacin P, however there was not a statistically significant difference between nisin and sakacin P according to their bacteriocin activity. Both in chicken meat and smoked salmon,inhibition of *L. monocytogenes* continued for 3 weeks, but salmons exposed to proteolytic deterioration. They also stated that nisin and sakacin P activities didn't show a difference in heat processed foodsat least 4 weeks. In another study, sakacin P, nisin, and two strains of *L. sakei* (one of them sakacin P producing and the other is not) were analyzed for *L. monocytogenes* limitation in

vacuum-packaged smoked salmon samples. Salmons were observed during 4 weeks. In salmon samples added only sakacin P, limitation of *L. monocytogenes* was determined for one week. However, in salmon samples added *L. sakei* strain that can produce sakacin P, limitation of *L. monocytogenes* was determined. Also, both of the *L. sakei* strains showed bacteriostatic effect on *L. monocytogenes* throughout the whole storage time. Whilst, in samples which were added with the combination of sakacin P and *L. sakei* strain That can produce sakacin P, bacteriocidal effect was observed (Katla et al. 2002). *L. curvatus* SB13 produce a bacteriocin called curvaticin 13. Curvaticin 13 and nisin activities for repressing the redevelopment of *L. monocytogenes* are analized by Bouttefroy and Milliere (2000) in their study. They observed that the combination of curvaticin 13 and nisin caused higher inhibition effect than their use in separately. In another study, treatments of sprayed nisin to cod fillets, herrings and smoked mackerels were studied for the inhibition of *C. botulinum*. In the study, sporesof *C. botulinum* Type E injected into packaged fishes and observed statistically important delay for toxin production at the temperatures of 10 and 26°C(Delves-Broughton 2005). Nisin was also effective for *L. monocytogenes* in a carbon dioxide atmosphere packaged smoked salmon. Besides, heat treatment with the combination of nisin was not harm to lobster meat's organoleptic properties and achieved better inhibition of *L. monocytogenes* than when nisin and heat treatment were used separately (Delves-Broughton 2005). As a means of extending shelf life, benzoic and sorbic acids are usually added for the production of brined shrimps. Concerns about using organic acids have given rise researchers to study about the potential effect of bacteriocins for brined shrimp preservation. Einarsson and Lauzon(1995) analyzed the efficiency of carnocin UI49, bavaricin A, and nisin Z for shelf life improvement. According to the study, carnocin UI49 was not effective for the improvement of shelf life, whilst bavaricin A, produced by *Lactobacillus bavaricus* MI 401, and nisin Z prolonged the shelf life up to 16 and 31 days, respectively. When brined shrimps were treated with carnocin UI49, bavaricin A and a control group with no preservatives, Gram-positive bacteria dominated in the microflora. However in nisin Z treated group Gram-negative bacteria dominated during thewhole storage time (Einarsson and Lauzon 1995). Divergicin M35, bacteriocin generated by *Carnobacterium divergens* M35, was isolated by Tahiri et al. (2004) from a commercially availablefrozen smoked mussels. According to their study, divergicin M35 was effective for inhibiting both *L. monocytogenes* and carnobacteria. Consequently, divergicin M35 can be used as preservation agent in seafood products for *L. monocytogenes*.

CONCLUSIONS

As a result, since food related diseases are on the rise and the safety of food is still an increasingly important public health concern in worldwide, using of bacteriocins, inhibit foodborne pathogens without any adverse effects, has aroused great interest. The effectiveness of bacteriocins for preservation of foods is proved. Only nisin is approved for using as a food preservation agent by FAO, but also other bacteriocinslikesakacin P, curvaticin, carnocin, bavaricin, and divergicin have applications in seafood systems. With the increasing antibiotic resistance problem and awareness of the risks of chemical preservatives for public health, bacteriocins have attracted a considerable attention about their use in seafood products. Addition of the bacteriocin producing culture or just the bacteriocin presents an attractive alternative for food preservation. A probable drawback associated with using bacteriocins as biopreservatives in foods is the improvement of bacteriocin resistance in spoilage or pathogenic bacteria. Consequently, the opportunity of using multiple bacteriocin producing bacteria or only the bacteriocins for the inhibition of foodborne pathogens in seafoods can be studied in further studies and it will be a important improvement for food safety in worldwide.

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CASE REPORT

Camallanus spp. in Aquarium Fish (Poecilia reticulata)

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ABSTRACT

The aim of this study was to determine the species of parasites in Guppy fish (*Poecilia reticulata***)** which was brought to Parasitology Laboratory of Faculty of Veterinary Medicine, Afyon Kocatepe University. The owner of the aquarium informed that sudden and mass deaths were observed in the aquarium and brought some of the dead fish to the laboratory for the examination. In addition to abdominal swelling in the fish, reddish parasites crawling out of the anus were also seen. Some of dead fish underwent examination for diagnostic purposes, the parasites were collected and fixed in 70% alcohol, then cleaned with glycerin and examined under a microscope. Collected parasites are described as *Camallanus* spp. according to related (Stromberg ve Crites, 1974). No other parasitic infection were found during the examination.

Key Words: Fish, *Camallanus*, Guppy **(***Poecilia reticulata***)**, Parasite

Akvaryum Balıklarında (*Poecilia reticulata*) *Camallanus* spp. Olgusu

ÖZ

Bu çalışmada Afyon Kocatepe Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim dalı laboratuvarına getirilen Lepistes balıklarında **(***Poecilia reticulata***)** gözlenen parazit türlerinin belirlenmesi amaçlanmıştır. Akvaryum sahibi akvaryumdaki balıklarda ani ve toplu ölümlerin olduğunu bildirmiş ve ölen balıkların bir kısmını laboratuvara getirmiştir. Balıklarda karın şişkinliğinin yanı sıra anüsten dışarı çıkmış kırmızımsı renkte parazitlerin olduğu görülmüştür. Parazitlerin teşhisi amacıyla balıklar açılarak parazitler toplanmış ve %70 alkolde fikse edilmiş daha sonra glyserin ile temizlenerek mikroskopta incelenmiştir. Toplanan parazitler ilgili literatür (Stromberg ve Crites, 1974) ışığında *Camallanus* spp. olarak teşhis edilmiştir. Yapılan incelemede başka herhangi bir paraziter enfeksiyona rastlanmamıştır.

Anahtar Kelimeler: Balık, *Camallanus*, Lepistes **(***Poecilia reticulata***)**, Parazit

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INTRODUCTION

Ornamental animal care and feeding take an important place among many methods which is developed to blow off steam. About 60 million people are engaged in aquarium fishery in the world. As well as being a hobby, aquarium fish farming is a quite commercially important (Hekimoğlu, 2006). However, many metabolic, viral, bacterial and parasitic diseases in freshwater fish can lead to clinical symptoms ranging from developmental failure to death, together with various pathological lesions in aquarium fish (Henker, 1975, Michel, 1981).

There are some cases about parasites of ornamental fish in China (Kuo et al., 1994), Germany (Moravec et al., 1999), Australia (Evans and Lester, 2001), Korea (Kim et al., 2002), Sri Lanka (Thilakaratne et al., 2003). *Camallanus* species belong to the nematode class and their females are ovoviviparous. *Camallanus* spp. has an indirect development which uses larval copepods and crustaceans as an intermediate host. Third-stage larvae develop in the intermediate host which receives first-stage larvae from faeces of infected fish. Definitive hosts are infected by digesting intermediate hosts which carries infective third-stage larvae. As an exception, *Camallanus cotti* only has direct development (Anderson, 1992). *Camallanus* species are red coloured and can be easily diagnosed while protruding from anus of the infected fish (Yanong 2011).

MATERİALS AND METHODS

Case Report

Anamnesis was taken from the owner of the aquarium to determine the species of parasites in the Guppy fish which were brought to Parasitology Laboratory of Faculty of Veterinary Medicine, Afyon Kocatepe University. The owner of the aquarium informed that sudden and mass deaths was observed in the aquarium and brought some of the dead fish to laboratory. Abdominal swelling in the fish, reddish parasites protruding from anus were also seen. Some of dead fish underwent examination for diagnostic purposes, the parasites were collected and fixed in 70% alcohol, then cleaned with glycerin and examined under a microscope. Collected parasites were described as *Camallanus* spp. according to related literature (Stromberg and Crites, 1974) with anterior end (Figure 1) and posterior end (Figure 2). No other parasitic infection were found during the examination.

RESULTS AND DİSCUSSİON

Kakar et al. (2013) reported that *Camallanus* species are widely spread, especially among Guppy fish in the world according to different authors. There are not many studies about parasites of aquarium fish in Turkey. However, Doğanay et al. (1989) reported that they found 0.4 % of *Camallanus lacustris* in guppy fish. Although absence of certain information on the pathogenicity of *Camallanus* species, it has been reported that adults can cause destruction and ulcerative lesions in the intestinal epithelium despite the fact that larvae of some species do not cause significant tissue damage. It has also been noted that severe infections can sometimes lead to death due to intestinal obstruction from time to time especially in small fish (Kim et al. 2002). In cases such as this one, it has been detected that sudden mass deaths can be caused by *Camallanus* spp. in the aquarium.

Parasitic infections which causes large economic loss for aquarium fish farming should not be ruled out. There are scarcely any reports in Turkey about *Camallanus* spp. which causes significant loss especially in small fish such as guppies. This case is the first report in Afyonkarahisar.

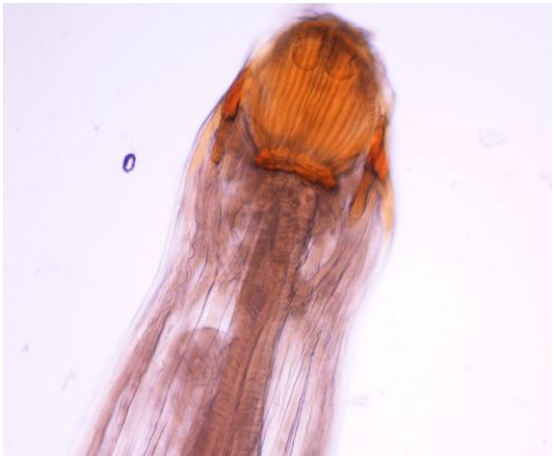

Figure 1: Anterior end of *Camallanus* spp.
Resim 1: *Camallanus* spp. ön uç

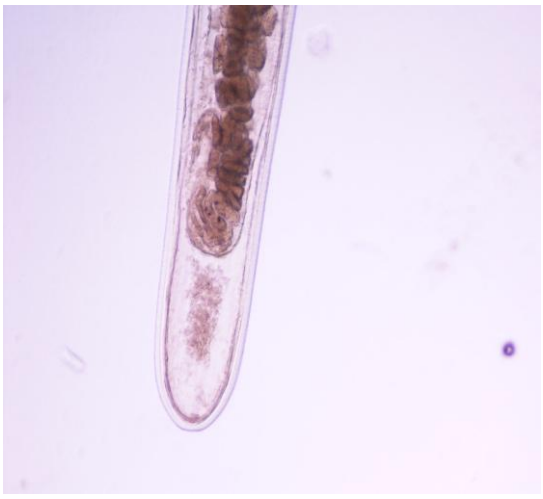

Figure 2: Posterior end of *Camallanus* spp. (Female).
Resim 2: *Camallanus* spp. (Dişi) arka uç.

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CASE REPORT

Efficiency of Eprinomectin for the Treatment of Naturally Infested with Sarcoptes scabiei in Rabbits (Oryctolagus cuniculus)

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ABSTRACT

Bu çalışma, Afyon Kocatepe Üniversitesi Veteriner Fakültesi Hayvan Hastanesine göz, burun, ağız etrafında ve ayaklarda kaşıntılı yara şikâyetleri ile getirilen on iki beyaz Yeni Zelanda tavşanı (*Oryctolagus cuniculus*) üzerinde yürütüldü. Tavşanların lezyonlu bölgelerinden bisturi yardımıyla alınan deri kazıntıları %10'luk KOH ile ezildikten sonra ışık mikroskobunda incelendi ve tavşanların *Sarcoptes scabiei* ile infeste oldukları belirlendi. Tedavide eprinomektin 0.2 mg/kg ve deri altı yolla 14 gün arayla iki kez uygulandı. Eprinomektinin etkinliği; deri kazıntısının parazitolojik muayenesinde *Sarcoptes scabiei* akarlarının varlığı ve/veya klinik belirtilerin devam edip etmediği durumuna göre değerlendirildi. Sonuçta eprinomektinin tavşanlarda *S. scabiei*'nin doğal enfestasyonlarına karşı etkili olduğu belirlenmiştir.

Key Words: Afyonkarahisar, *Sarcoptes scabiei*, Rabbit, Eprinomectin, *Oryctolagus cuniculus*.

Sarcoptes scabiei ile Doğal Enfeste olan Tavşanlarda (*Oryctolagus cuniculus*) Eprinomektin Tedavisinin Etkinliği

ÖZ

This study was carried out on twelve white New Zealand rabbits (*Oryctolagus cuniculus*) which brought with itchy wounds symptoms around eye, nose, mouth and feet to Afyon Kocatepe University, Veterinary Faculty, Animal Hospital. The skin scrapings that taken from the lesion areas of rabbits with a scalpel, were examined in the light microscope then crushed with 10% KOH and it was determined the rabbits infested with *Sarcoptes scabiei*. At treatment, Eprinomectin was administered 0.2 mg/kg subcutaneously twice with 14 days interval. The efficacy of eprinomectin was assessed either clinically or parasitologically examination by the absence of *Sarcoptes scabiei* mites due to skin scraping. The results of the present study determine that eprinomectin is effective against naturally infestations of *S. scabiei* in rabbits.

Anahtar Kelimeler: Afyonkarahisar, *Sarcoptes scabiei*, Tavşan, Eprinomektin, *Oryctolagus cuniculus*.

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INTRODUCTION

Sarcoptes scabiei is a mite causing mange in many mammals such as horses, cattle, sheep, goats, dogs, and rabbits (Kettle 1995). Zoonotic agents exist in living beings as obligatory parasites (Bornstein et al. 2001, Baker et al. 2014). The agents in infested rabbits dwell in the regions like face, ears and legs (Schoeb et al. 2007). Although the infection is widely observed in rabbits, it is rather difficult to eradicate the agent ultimately (Meredith 2008). Female agents dig tunnels through the epidermis and lay their eggs there (Baker 1998).

The first clinical symptom of the infection is itching (Soulsby 1982). The lesions, occurring on the skin due to excessive itching, lead to pyoderma and hair loss by creating a disposition to secondary infections. Lesions around the mouth and nose result in anorexia, weight loss, cachexia and death (Soulsby 1982, Baker et al. 2014). As biochemical changes in the blood serum occur in severe infestations, anemia and leukopenia are the most common symptoms (Baker et al. 2014).

There have been numerous studies to determine the effect of the drug against various parasites in pets and farm animals (Shoop et al. 1996, Shoop et al. 2001, Cringoli et al. 2003, Aguirre et al. 2005, Rehbein et al. 2005, Habela et al. 2006, Geurden and Vercruysse 2007, Kozan et al. 2008, Bilgin et al. 2010, Visser et al. 2013). Eprinomectin was applied topically in cats to determine its efficacy against *Dirofilaria immitis* (Baker 1998). There have also been several studies on lab animals for which eprinomectin was applied orally (Sevimli et al. 2009), topically (Ulutas et al. 2005, Rambozzi et al. 2014) and in an injectable way (Baoliang et al. 2006).

This study aimed to determine the efficiency of eprinomectin in the treatment of 12 New Zealand rabbits naturally infested with S*arcoptes scabiei*.

MATERIAL AND METHOD

12 New Zealand rabbits were brought to Afyon Kocatepe University Animal Hospital with itchy sores around the eye, nose, mouth and legs constituted the material for this study. After having being crushed with 10% of KOH, the rabbit skin scrapings obtained from the areas with lesions were examined under a light microscope (Nikon Eclipse 80i - DS-5M-L1 imaging system). The patients identified with *Sarcoptes scabiei* in the microscopic examination were subcutaneously administered 0.2 mg/kg of eprinomectin twice with an interval of 14 days.

RESULTS

In the microscopic examination of the skin scrapings derived from rabbits with lesions around eyes, mouth, nose and legs revealed mature *Sarcoptes scabiei* mites (Image 1) and eggs (Image 2). Apart from skin lesions, severely impaired general condition was detected in the patients (Image 3, Image 4).

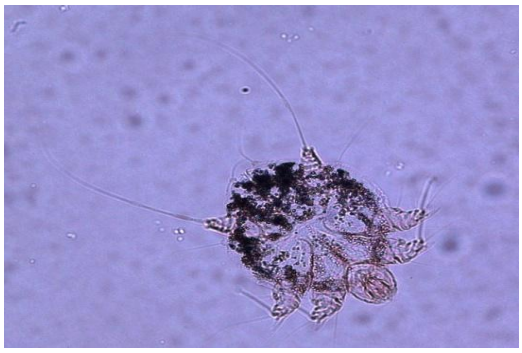

Image 1. Adult of *Sarcoptes scabiei* (x10)

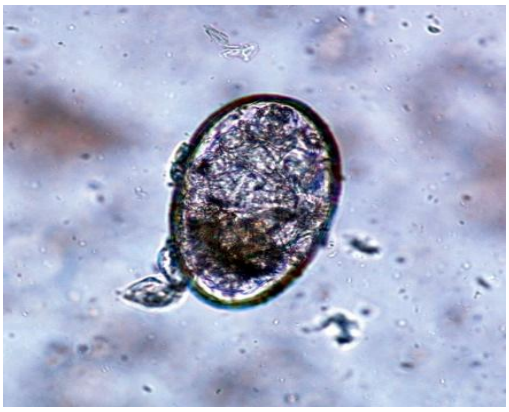

Image 2. Egg of *Sarcoptes scabiei* (x40)

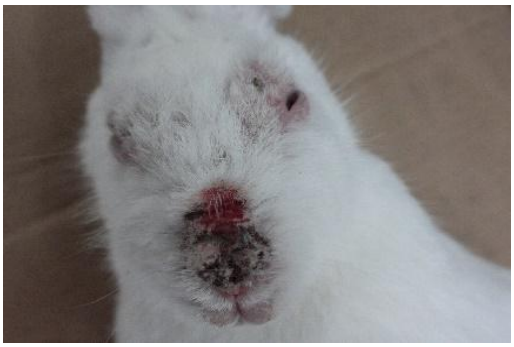

Image 3. Mouth, nose and eyes injuries of the infected rabbit

Image 4. Foot injury of the infected rabbit

Following the eprinomectin administration, clinical recovery of the rabbits started; no agent was detected in the skin scrapings derived on the 7th and 14th days (Image 5, Image 6).

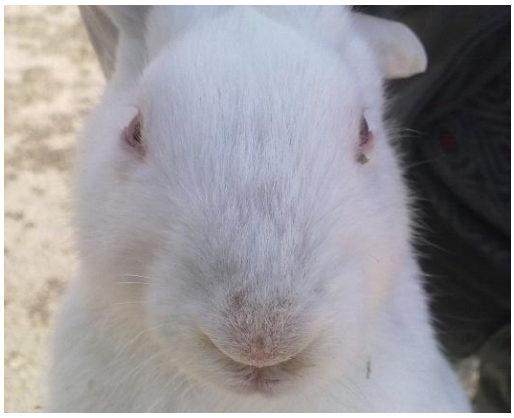

Image 5. View of the head region post-clinical improvement

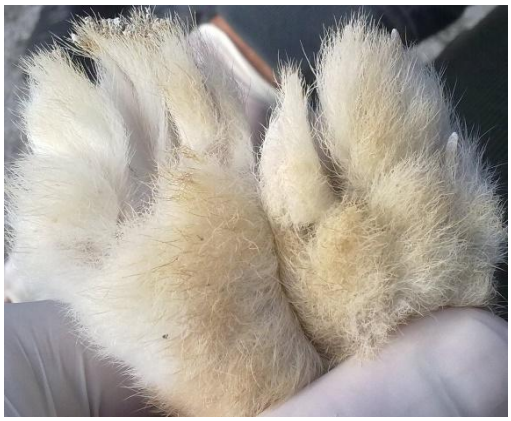

Image 6. View of the foot post-clinical improvement

DISCUSSION

Sarcoptic mange in rabbits primarily starts around the mouth and then lesions spread all around the face and eyes (Schoeb et al. 2007). Ivermectin group of antiparasitic drugs are used topically, orally and through parenteral routes for mange treatment (Bornstein et al. 2001). Ulutas et al., (2005) administered eprinomectin topically to rabbits naturally infested with *Psoroptes cuniculi* as two doses with an interval of 14 days, and recorded a clinical recovery starting from the third day following the first application. It was reported in another study that one dose of 200-300 µg/kg of eprinomectin administered subcutaneously sufficed for the treatment of the rabbits infested with *Psoroptes cuniculi*; 100µg/kg of the drug did not cure the infestation (Baoliang et al. 2006). Rambozzi et al., (2014) topically administered a dose of 5 mg/kg of eprinomectin to rats infested with *Myocoptes musculinus* and achieved an efficient cure. In a study aimed to determine the effectiveness of doramectin, eprinomectin and selamectin against *Syphacia muris* in rats, Sevimli et al., (2009) reported that on the second day following the administration eprinomectin was 100% effective; doramectin and selamectin were effective at the rates of 99.32% and 98,72%, respectively. However, this impact occurred on the sixth day. Kurtdede et al., (2007) reported that a dose of topical selamectin was effective in the treatment of New Zealand rabbits naturally infested with *Psoroptes cuniculi* (6-18 mg/kg) and Angora rabbits naturally infested with *Sarcoptes scabiei* (10-12 mg/kg). Although various antiparasitic drugs against Sarcoptic mange in rabbits were administered via various administration techniques, there is not a single study on subcutaneous administration of eprinomectin. Medication started right away on the day of mange diagnosis and followingly clinical recovery was observed.

As a consequence, it was observed that sarcoptic mange in rabbits could be successfully cured with 0.2 mg/kg of subcutaneous eprinomectin administration.

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LETTER TO THE EDITOR

Pulpitis Case in a Dog: Is Dirofilariosis the culprit?

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ABSTRACT

In the presented article, an interesting case was defined by the color change in the teeth. The case material consisted of 3.5 old female dogs was refered to the clinic for routine vaccination. In our study, it was determined that the patient had exercise intolerance, intermittent cough and shortness of breath. Patient; Complete blood count, serum biochemistry measurements, chest X-ray and detailed physical examination. The results of the rapid diagnostic test kit (SNAP 4DX) analysis revealed that the dog was infected with Drofilaria immitis. During the patient's oral cavity examination, a pinkish color change was detected in the teeth as an unusual finding (Figure 1). The findings are discussed below.

Key Words: Dirofilariosis, Dog, Pulpitis.

Bir Köpekte Pulpitis Olgusu: Dirofilaria Olağan Şüpheli midir?

ÖZ

Sunulan makalede dişlerde renk değişimi ile karakterize ilginç bir vaka tanımlandı. Olgu materyalini rutin aşılama için kliniğe getirilen 3.5 yaşlı dişi köpek oluşturdu. Alınan anamnezde hastada egzersiz intoleransı, aralıklı öksürük ve nefes darlığı olduğu belirlendi. Hasta; tam kan sayımı, serum biyokimya ölçümleri, akciğer grafisi ve detaylı fizik muayene ile değerlendirilmiştir. Hızlı tanı test kiti (SNAP 4DX) analiz sonuçları, köpeğin Drofilaria immitis ile enfekte olduğunu ortaya koydu. Hastanın ağız boşluğu muayenesi sırasında, olağan dışı bir bulgu olarak, dişlerde pembemsi renk değişikliği tespit edildi (Şekil 1). Elde edilen bulgular aşağıda tartışılmıştır.

Anahtar Kelimeler: Dirofilaria, Köpek, Pulpitis.

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INTRODUCTION

In the veterinary practice or namely, clinical setting veterinary surgeons often rely on oral examination in an attempt to detect whether a dental procedure is required (Allione 1999). Indeed subgingival pathology (Allione 1999) or pulpal diseases may go undiagnosed. Endodontic disorders denote dental pulp damage, namely pulpitis. Pulpitis may be reversible or irreversible due to the severity of the illness. Minor trauma may be related to reversible pulpitis, where as inflammation related swelling and prevention of blood entering the root canal might cause irreversible pulpitis. Besides tooth fractures expose pulp tissue to bacteria located within the oral cavity. There was no tooth fracture in the present case, whereas probable hypothesis for probable bacterial infection might involve *D. immitis* harboured *Wolbachia sp*. This may be briefly explained with the relationship between Dirofilaria species and *Wolbachia sp*. *Dirofilaria immitis* is the main filariasis agent in dogs and cats, causing heartworm disease. *D. immitis*, like other filarial nematodes, harbours intracellular endosymbiotic bacteria belonging to the genus Wolbachia (Rickettsiales) (Bandi 2001). The association between filarial nematodes and *Wolbachia* is considered an obligatory (perhaps mutualistic) symbiosis (Casiraghi 2002). *Wolbachia*-associated molecules may interact with cells of the innate immune system such as macrophages and neutrophils (PMNs), thus contributing to the pathogenesis and immunology of filarial diseases (Bandi 2001, Brattig 2001, Taylor-Robinson1994). Another hypothesis interestingly causing discoloration of the teeth in the present case might be related to nitric oxide. Nitric oxide (NO) as a chemical compound, has long been recognized to possess pleiotropic effects, involving vasodilatation, immunomodulation and neurotransmission (Bogdan 2001). In immune responses, NO is known to be produced mainly by activated macrophages and to modulate Th1/Th2 balance, as well as to induce immunosuppression (Allione 1999, Taylor-Robinson1994, Dai 1999). In bacterial or protozoon-infected hosts, NO also serves as a toxic molecule against these pathogens (Casiraghi 2002). In contrast, macrophage NO production driven by living helminth parasites or their products is considered to be involved in immunosuppression through the prevention of worm-specific T-cell responses (Dai 1999, Atochina 2001, O'Connor 2000, Oliveira 1999)

Given the data that adult *D. immitis* worms located primarily within the pulmonary arteries of infected dogs, the vasodilatation initiatedvia NO may serve to the maintenance of habitat spaces for the invading Dirofiaria species (Hiroyuki 2002). It may be safely suggested that *D. immitis*-derived factorsexist to facilitate parasitism of the worm through immunosuppression and arterial relaxation via NO (Hiroyuki 2002). In the present article an interesting case of discoloration of the teeth was reported. A 3.5 years old female dog was referred to clinic for routine vaccination. The owner reported exercise intolerance, intermittent coughing and dyspnea time to time. On initial referral complete blood count, serum biochemistry, chest x-ray and detailed physical examination were all performed. A point of care rapid diagnostic test kit (Snap 4dx plus) revealed that the dog was infected with *Dirofilaria immitis*. Routine blood work analysis were at normal physiological intervals. The only abnormality in haematological analysis was eosinophilia 16%.(Table 1) In parasitological diseases eosinophilia is known to be the expected blood result. Biochemical analyzes revealed that the values were within the reference range. In the clinical examinations was, a pinkish discoloration of the apical of the tooth detected in the detailed examination of the mouth. The presence of microfilariae was microscopically observed in the microfilm examination performed by the thick-drop method.To the present authors' knowledge the latter hypothesis might be confounding, as NO production via *D. immitis*, might be associated with pulpitis in this dog.Independently from the hypothesis withdrawn above, it may be safely suggested that tooth discoloration, nemaly pink teeth might be observed during parasitological/bacterial infections, which must be taken into consideration in veterinary practice, promptly requiring therapy application for preventing irreversible pulpitis.

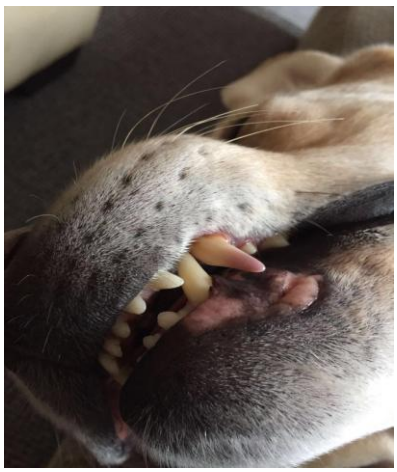

Figure 1. Pulpitis in a dog. Discolored (pink) teeth.
Şekil 1. Bir köpekte Pulpitis olgusu. Dişlerde renk değişimi.

Table 1. Haematological analysis (Eosinophilia 16%).
Tablo 1. Hematolojik Analizde (%16 ezinofili).

WBC	LENF	MON	GRAN	LENF%	MON%	GRAN%	RBC	HGB	HCT
$16.3X10^9$	$4.7X10^9$	$1.8X10^9$	$9.8X10^9$	28.8%	10.9%	60.3%	$7.10X10^{12}$	13.9	45.7
MCV	**MCH**	**MCHC**	**RDW%**	**PLT**	**MPV**	**PDW**	**PCT**	**EOS%**	
64.4	19.5	30.4	13.2%	$412X10^9$	7.9	16.2	0.325%	**16.0%**	

KAYNAKLAR

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Thesis:
Yeni D. Some andrological parameters and biochemical properties in relation toseason in rams. PhD thesis, Afyon Kocatepe University Health Science Institute, Afyonkarahisar,2010.

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Titles of tables, graphics and figures should be both Turkish and English.

Brief Communications: Briefcommunications should be consise but complete description of a limitedivestigation, which will not be included in a later publication. They shouldnot exceed 1600 words. They should bear no more than two tables or figures. AnABSTRACT should be given but no other sections. Typescripts should be clearlymarked Brief Communication.

Review Articles: Review articles related to allmedical topics are welcome for publication. They should give an update onrecent advances in a particular field and be targeted at research veterinariansor clinicians who are not necessarily working in the same field. The lengthshould not exceed 4500 words. It should have a precise abstract. Author ofreview should have at least two citations. For each issue maximum 2 reviews arepublished.

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- To be a substantially novel presentation
- To be a technique or treatment that wouldsubstantially alter management and prognosis of the described condition
- The first clinical report or first case(s) ofdiseases in a particular location where epidemiology is an important factor

– To exemplify bestpractice in medical science.

Letters to The Editor: Letters describingcase reports or original material may be published in the KVJ and will bepeer-reviewed prior to publication. Letters making critisisms on recentlypublished papers in the KVJ will also be considered and the correspondingauthors of the original paper will be invited to respond accordingly.

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