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Kocatepe Veterinary Journal, Afron Kocatepe University, Faculty of Veterinary Medicine, 03200, Afronkarahisar, TURKEY.

Tel: +90 272 214 9309 Fax: +90 272 214 9309 E-mail: kvj@aku.edu.tr

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RESEARCH ARTICLE

The Effect of Some Environmental Factors To Race Performance of Purebred Arabian Horses

Fatih YILDIRIM*

Department of Animal Science, Faculty of Veterinary Medicine, University of Atatürk, Erzurum-25240, Turkey

ABSTRACT

This study evaluated the relationships between some environmental factors and race performance of purebred Arabian racehorses in the Turkish Jockey Club. For this purpose, the belonging 60 purebred Arabian horses (45 males and 15 females) which registered to Turkish Jockey Club was benefited from 3087 races records. In the research, the effect of gender, age, race distance, dam age, and the city in which the race took place to race performance on the turf and dirt track was investigated. In the environmental factors affecting race speeds of horses were found statistically different. The turf track, dirt track and general average of the race speed were calculated as 14.45 ± 0.02 m/sec, 13.52 ± 0.01 m/sec and 13.77 ± 0.01 m/sec, respectively. The horses's race performance results; it was better than others the turf track, male, short distance, 9-12 dam age, 3 age, and Istanbul, for the race track, gender, race distances, dam age, race age, and race city, respectively. In conclusion, it is thought that performing of similar studies with more horses and more comprehensive studies may be more useful in examining the race performance of horses.

Keywords: Arabian horse, environmental factors, race, performance

Safkan Arap Atlarının Yarış Performansına Bazı Çevresel Faktörlerin Etkisi

ÖΖ

Bu çalışma, Türkiye Jokey Kulübü'ndeki Safkan Türk Arap atlarının bazı çevresel faktörler ve yarış performansları arasındaki ilişkilerin belirlenmesi amacıyla gerçekleştirilmiştir. Bu amaçla Türkiye Jokey Kulübü'ne kayıtlı 60 safkan Arap atına ait (45 erkek ve 15 dişi) 3087 yarış kaydından yararlanılmıştır. Araştırmada çim ve kum pistteki koşu performansına cinsiyet, yaş, mesafe, ana yaşı, ve yarışın yapıldığı şehrin etkisi incelenmiştir. Atların yarış hızlarını etkileyen bu çevresel faktörlerde istatiksel olarak farklılıklar bulunmuştur. Yarış hızının çim pist, kum pist ve genel ortalaması sırasıyla 14.45±0.02 m/sn, 13.52±0.01 m/sn ve 13.77±0.01 m/sn olarak hesaplanmıştır. Atların yarış performans sonuçlarında; çim pist, erkek, kısa mesafe, 9-12 ana yaşı, 3 yaş ve İstanbul verileri diğerlerinden, sırasıyla yarış pisti, cinsiyet, yarış mesafesi, ana yaşı, koşu yaşı ve yarış şehri bakımından daha iyi sonuçlar vermiştir. Sonuç olarak, daha fazla at sayısına sahip ve daha kapsamlı benzer çalışmaların yapılması atların yarış performansının incelenmesinde daha faydalı olabileceği düşünülmektedir.

Anahtar Kelimeler: Arap atı, çevresel faktörler, yarış, performans

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ORCID ID; FY: 0000-0002-9402-4008

*Corresponding author e-mail: fatihyildirim@atauni.edu.tr

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INTRODUCTION

Arabian horses, known as one of the oldest breeds in the world, have been raised in the Arabian Peninsula and surrounding regions since 2000 BC (Glazewska 2010). Because the Arabian horse has a symmetrical body, an elegant, is resistant to various weather conditions, perform well in long-distance races and a high pass down hereditary power, it has played a significant role in hybridization studies for the development of new breeds (Özbeyaz and Akçapınar 2003).

Nowadays, because horse breeding is usually made for racing and sports purposes, the yields in these two breedings are running ability and spring. At races, horses' running ability (speed) can be defined as racing performance. In horses, racing performance is a quantitative character. Therefore, genetic and environmental factors control this feature (Paksoy and Ünal 2010).

In horseracing and race performance, breeders use horses' records as well as the records of their offspring, relatives and ancestors (Ricard et al. 2000). Also, racehorse time is one criterion used to quantitatively evaluate horseracing and race performance that is to say the success of the horse according to other horses (Ekiz et al. 2005b, Mota et al. 1998). Race performance of horses is influenced by genotype, body structure, and various environmental factors. These factors include care and feeding, exercise, gender, age of the horse and dam, distance, handicap weight, rider status and previous medical condition of the racehorse illnesses. It is crucial for the success of the breeding programs that these factors are taken into account to improve race performance (Paksov and Unal 2010). In another study conducted on Arabian horses, when factors affecting race time were examined, it was found that horse's gender and race track were significant, but the horse' age was significant only at certain race distances (Ekiz et al. 2005a). It is also important for their training in the interaction of horses with the rider (Yıldırım et al. 2017).

Some researchers have studied the age of the racehorse and dam, the year of the race, race distance, racing categories and surface of the race track in order to evaluate possible correlations between these factors and the race performances of horses (Köseman and Özbeyaz 2009). Others have reported that the age of the horse, race distance, structure of the track, amount of the jackpot, running season, number of running horses, handicap weight of the horse and the horse's position on the track were significant factors in race performance (Martin et al. 1996).

In Turkey, official horseracing is organized by the Turkish Jockey Club (Turkish abbreviation: TJK), a member of the World Arabian Horse Organization. Races are held at nine tracks throughout the country. The tracks have either turf or dirt surfaces. Arabian horses are mostly used in endurance racing throughout the world, in Turkey, they are commonly used in flat racing (Özen and Gürcan 2017).

The aim of the present study was to investigate the relationships between some environmental factors and race performance of purebred Arabian horses in the TJK. It is hoped that this research will contribute to a better understanding of the effect of some environmental factors on race performance.

MATERIALS and METHODS

The racing records of Arabian horses in this study were provided by the TJK and included official races that took place from May 2007 to December 2016. The dataset in this period was comprised of records from 3087 races. These records were taken from 60 purebred Arabian horses (45 males and 15 females) registered by the TJK. Race information included the horses' genders, ages, ages of dams, race track, race distance, race speeds, and cities. The turf and dirt track are composed of structurally different materials, and so affects the running results. For this reason, while the race results are examined, the races made on the turf and dirt track considered differently and the performances of the horses are evaluated separately for each track. Therefore, in order to better observe the performances (speed-m/sec) of the horses on the turf and dirt track, horses performances were evaluated both as separate and as a whole. The race speed of the horses was determined according to the distance in each run was calculated individually. The effects of gender, race distance, dam age, horse' age and race city were fitted to model used in analyses for each track type (turf and dirt).

Factors related to the horse race performances were evaluated by the formula:

 $Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklmn}$

where Y_{ijklmn} = each parameter value discussed, μ = population average, a_i = effect of the horse' gender (male, female), b_j = effect of the race distance in meters (1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200), c_k = effect of the age of dam in years (5-8, 9-12, 13-16, 17-20, 21+), d_l = effect of the age of the horse at the time of the race, in years (3, 4, 5, 6, 7, 8+), f_m = effect of the city in which the race took place (Istanbul, Izmir,

Bursa, Ankara, Adana, Elaziğ, Sanlıurfa, Diyarbakır) and e_{iiklmn} = random error effect.

While analysing the data, the General Linear Model was used in SPSS Statistic 20. Tukey's multiple comparison test was used to evaluate the significance of the differences among averages of the sub-groups.

RESULTS and DISCUSSION

The results of fixed effects of gender, race distance, dam age, race age and race city in which the race took place of horse on race performances or speeds were shown in Table 1. In the evaluation of entire data set, statistically, significant differences were found in all of the fixed effects (P < 0.001). The race performance or race speed (m/sec) average of the horses was obtained as 14.45±0.02 m/sec for turf track, 13.52±0.01 m/sec for the dirt track and 13.77±0.01 m/sec for the general average. According to the results obtained, the race speed of the horses running on the turf track was found to be higher than the horses running on the dirt track. Similar to the results of this study, some researchers stated that the speed of horses running on the dirt track is less than the horses running on the turf track (Köseman and Özbeyaz 2009, Paksoy and Ünal 2010, Paksoy et al. 2018). However, as Paksoy and Unal (2010) stated in his studies, there may be a change in the race times of horses according to the wetness and dryness of the turf or dirt track. Therefore, to examine horses's pedigree and race performance records may provide better results.

In horse races organized in Turkey, horses of both genders are organized together, and in some races, separate races are organized for each gender. Therefore, in this study, both together and separate results are examined in terms of gender. According to the analyses results, the effect of gender on the horse's racing speed was not significant in turf and dirt track (P > 0.05). However, it was found that male horses running on the turf track run faster than female horses. In general, researchers reported male horses exhibited better racing performance than female horses in horse races due to both morphological and hormonal reasons (Özbeyaz and Akçapınar 2003). In this study, It was thought that this result may be due to lack of population number of female horses.

Race distances are considered short if they are less than or equal to 1300; middle-distances are 1400 to 1700 m and long-distance races are longer than 1800 m (Anonymous 2018). Racing horses perform better at their own distance. In addition, the well-known horses of the structure and features by the owners are run in proper distance runs, and so the

performances of the horses are well exhibited (Paksov and Ünal 2010). It is not recommended that horses compete at distances that are not suitable for their body structures (Özen and Gürcan 2016). In Turkey, between 1991-2000 years, running in the open race organized for male Arabian horses, which the race speeds (calculated from the relevant source) were examined at various distances, the race speed increased as the race distance increased (Köseman and Özbeyaz 2009). However, in this study, the horses's speed of the race generally decreased as the race distance increased. In addition, the race speed results for all race distances (1200 to 2200 m) obtained in this study showed a better performance than the race speed results of the Köseman ve Özbeyaz (2009). This result can be considered as a result of the selection and maintenance-feeding operations carried out over the years. In the present study, the effect of race distance on race performance or speed was significant for each track type (P < 0.001). Especially, at 1200 and 1500 mt of the turf track, and at 1300 mt of the dirt track, the speed of the horses was better than at other distances. In other words, it was determined that horses performed faster than 1500 mt or less.

In the studies on the effect of the dam age on the horses's racing performance are reported that there is a relationship between the two characteristics (Barron 1995, Köseman and Özbeyaz 2009). In this study, similarly, racing performances were influenced by dam age. And even, the race performances on the turf (P \leq 0.05) and dirt (P \leq 0.001) track were statistically significant. In the UK, the race performance of the foals born from young mothers in the Throughbred horses is higher, but those from mothers older than 11 years has been reported that the race performance decreased (Barron 1995). In a study conducted on Arabian horses, the effect of dam age on race time was insignificant, but 9-12 year-old dams had less race time (Köseman and Özbeyaz 2009). In the present study, when the effect of dam age on the race performance was examined, the highest speed on the turf track was 9-12 dam age, and the highest speed on the dirt track was 21+ dam age.

The age of onset for purebred Arabian horses to race in Turkey was determined to be 3 years. In a race time study conducted on Arabian horses (Köseman and Özbeyaz 2009), the differences between the average age of racehorses (3 to 9+ age) at different distances were not statistically insignificant; however, in this study, the horses's race speed (3 to 8 age) was found to be significant. Others have reported that the race performance of horses is significantly affected by the race age of the horse (Chrzanowski and Koeboke 1993, Chrzanowski et al. 1996). In this study, similarly,

race speeds were influenced by race age. The race performances or speeds of the horses according to their age at the races were determined as 3 years for the fastest horses on both turf and dirt tracks. In this study, the speed of the horses running on the turf track at the age of 8+ is remarkable. According to some researchers, there is a proven relationship between the race performance of the horses and the race lives (Sobczynska 2007), and thus, the horses running on the turf track at 8+ age may be seen as the reason for the high speed of racing. Because, it is thought that the best running horses are kept to make more profits by the breeders.

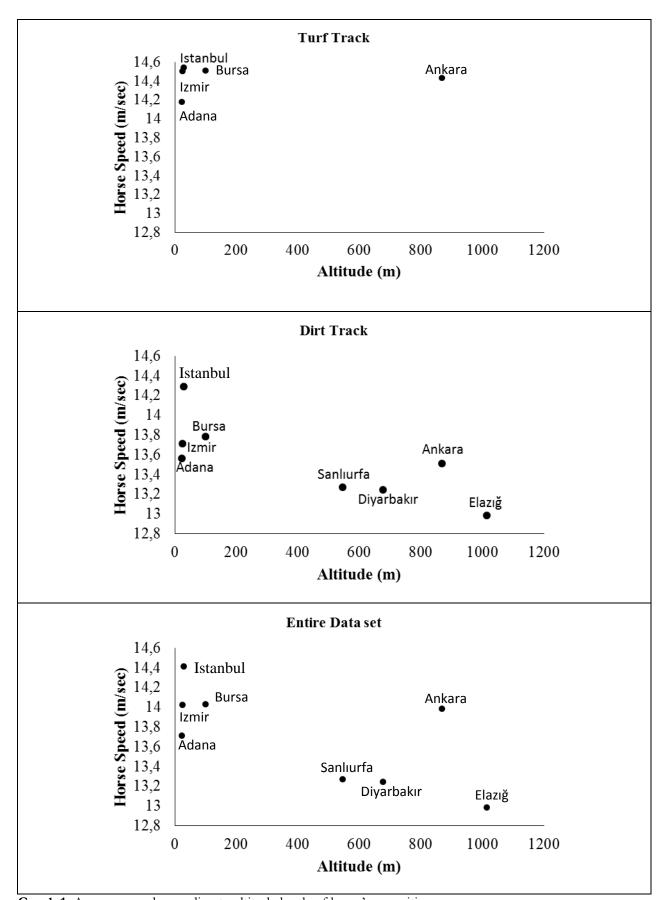
Considering the race city of track, Istanbul, Izmir, Bursa, and Ankara at turf track; Istanbul at dirt track of horses's race performances or speeds were found to be significantly larger than at other race cities. In addition, in regard to the effect of the race city on the horse's performance, the speed of the horses running on both the turf track and the dirt track in Istanbul has the highest values. There are

very few studies on the effect of racing cities on horses's race speeds in Turkey. In the studies conducted, the average speed for Arabian horses was reported to be 13.70 m/sec in Elazig and 14.41 m/sec in Istanbul (Duru 2017). In this study, 12.98 m/sec racing speed found for Elazığ was higher than the racing speed found by Duru (2017), but 14.41 m/sec racing speed for Istanbul was similar to the findings of the same researcher. In conducting the research, the relationship between the altitude levels of cities and the horses's race speeds was remarkable. Therefore, this relationship was presented in Graph 1 as separate data. When the Graph 1 was examined, it was observed that the horses's race speeds in the provinces with altitude above 500 m are generally lower than the provinces with less than 500 mt. As a result, it was concluded that the altitude levels of the racing places may be an impact on the horses's race speeds. However, this relationship (between racing speed and altitude) can be examined in more detail.

Table 1. The averages (m/sec), significant and comparison test results relevant to the turf and dirt track of the horses's race speeds examined as the gender, race distance, dam age, race age, and race city.

Parameters _			Turf			Dirt			Entire D	ataset
		n	$\overline{\mathbf{X}} \pm S\overline{\mathbf{X}}$	Sig	n	$\overline{\mathbf{X}} \pm S\overline{\mathbf{X}}$	Sig	n	$\overline{\mathbf{X}} \pm \mathbf{S}\overline{\mathbf{X}}$	Sig
Gender	Male	529	14.46±0.02	NS	1815	13.52±0.02	NS	2344	13.73 ± 0.02	***
Gender	Female	318	14.42 ± 0.03	INS	425	13.52 ± 0.03	113	743	13.91 ± 0.03	4.4.4
	1200	147	14.71 ± 0.04^{a}		333	13.83 ± 0.03 ab		480	14.10 ± 0.03^{ab}	
	1300	69	14.41±0.06bc		95	13.94 ± 0.06^{a}		164	14.14 ± 0.05^{a}	
	1400	152	14.57 ± 0.04 ab		404	13.82 ± 0.03 ab		556	14.02 ± 0.03 ab	
	1500	20	14.70 ± 0.11^{a}		194	13.69 ± 0.04^{b}		214	13.79 ± 0.05 ^{cd}	
Race Distance	1600	84	14.56 ± 0.05 ab		98	13.36 ± 0.06 ^{cd}		182	13.92 ± 0.05 bc	
	1700	95	14.34±0.05bc	***	142	13.13 ± 0.05 ^d	***	237	13.62 ± 0.05 de	***
(m)	1800	51	14.31±0.07bc		210	13.33±0.04 ^{cd}		261	13.52±0.04e	
	1900	85	14.25±0.05c		314	13.17±0.03 ^{cd}		399	13.40±0.04e	
	2000	61	14.24±0.06c		271	13.33±0.04 ^{cd}		332	13.50 ± 0.04^{e}	
	2100	45	14.12±0.07c		138	13.32±0.05cd		183	13.52±0.05e	
	2200	38	14.23±0.08c		41	13.38±0.09c		79	13.79 ± 0.08 ^{cd}	
	5-8	191	14.39±0.04ab		770	13.40±0.02b		961	13.60±0.02c	
	9-12	294	14.50 ± 0.03^{a}		523	13.63±0.03a		817	13.94 ± 0.03^{a}	
Dam Age	13-16	190	14.47±0.04a	*	492	13.56 ± 0.03^{a}	***	682	13.82±0.03b	***
(years)	17-20	47	14.26±0.07b		260	13.42±0.04b		307	13.55±0.04c	
	21+	125	14.43±0.05ab		195	13.69±0.05a		320	13.98 ± 0.04^{a}	
	3	108	14.61±0.05a		90	13.78 ± 0.07^{a}		198	14.23 ± 0.05^{a}	
	4	275	14.39±0.03b		532	13.62±0.03b		807	13.88±0.03b	
Race Age	5	190	14.40±0.04b		504	13.57±0.03b		694	13.80±0.03bc	
(years)	6	125	14.50 ± 0.05^{ab}	**	399	13.52±0.03b	***	524	13.76±0.03bc	***
() cars)	7	78	14.38±0.06b		326	13.53±0.04b		404	13.70±0.04c	
	8+	71	14.49 ± 0.06^{ab}		389	13.24±0.03c		460	13.43±0.03d	
	Istanbul	122	14.54 ± 0.05^{a}		146	14.29 ± 0.05^{a}		268	14.41 ± 0.04^{a}	
	Izmir	179	14.50 ± 0.04^{a}		274	13.71±0.03bc		453	14.02±0.03b	
Race City	Bursa	212	14.51 ± 0.03^{a}		411	13.78±0.03b		623	14.03±0.03b	
	Ankara	218	14.43 ± 0.03^{a}		204	13.51±0.04 ^d		422	13.98±0.03b	
	Adana	116	14.18±0.05b	***	361	13.56±0.03 ^{cd}	***	477	13.71±0.03 ^c	***
	Elazığ	-			265	12.98±0.04 ^f		265	12.98±0.04e	
	Sanlıurfa	_	_		457	13.27±0.03e		457	13.27±0.03d	
	Divarbakır	_	-		122	13.24±0.05°		122	13.24±0.06d	
General A	-	847	14.45±0.02		2240	13.52±0.01		3087	13.77±0.01	

a-f: Differences among groups indicated with different letters in the same column are significant, ***: P < 0.01, *: P < 0.01, *: P < 0.05, NS: P > 0.05.



Graph 1. Average speed according to altitude levels of horse's race cities

CONCLUSION

Racing performances or speeds of horses was impressed by the horse's gender, race distance, dam age, race age, and city in which the race took place.

The horses's race performance results; it was better than others the turf track, male, short distance, 9-12 dam age, 3 race age, Istanbul, for race track, gender, race distances, dam age, race age, race city, respectively. When the race performances of horses are examined, it is thought that performing of similar studies with more horses and more comprehensive studies may be more useful. In addition, it is recommended that more detailed research is carried out on the effect of the race places altitude to the horses's race performance.

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RESEARCH ARTICLE

Establishing Near Infra Red Spectroscopy (NIR) Calibration for Starch Analysis in Corn Grain

Ercan TOMAS1, İsmail BAYRAM2*

¹Veterinary Medicine, Ministry of Agriculture and Forestry-Turkey, ²Department of Animal Nutrition, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar-Turkey,

ABSTRACT

This study has been carried out to calibrate the starch values of 320 corn samples taken from Soil Products Offices (TMO) in seven different geographical regions of by using an NIR instrument. The corn samples used in the study were selected from the regions where corn production is intensive in Turkey and brought to the laboratory. The corn samples brought to the laboratory were milled and then spectra were formed. Subsequently, the starch values were determined in the laboratory with the use of wet chemical analysis methods. The calibration values generated were R = 0.6410; $R^2 = 0.4109$ Standard Deviation = 4.4208, R = 0.5854 from the validation set; $R^2 = 0.3427$ Standard Deviation = 4.5662. The calibration interval in the study was 44.53 and 45.72, respectively. This study has concluded that more samples representing the 7 different regions in Turkey for the starch contents of corn are needed in order to generate scientifically reliable results with the FT-NIR device.

Keywords: Corn, Starch, NIR, Calibration

Mısır Tahılında Nişasta Analizi İçin Near Infra Red Spectroscopy (NIR) Kalibrasyonu Oluşturulması

ÖZ

Bu araştırma Türkiye'nin yedi farklı coğrafi bölgesindeki TMO ofislerinden getirilen toplam 320 adet mısır numunesindeki nişasta değerlerinin NIR cihazı kullanılarak kalibrasyonunu yapmak amacıyla gerçekleştirilmiştir. Araştırmada kullanılan mısır numuneleri Türkiye'de mısır üretiminin yoğun olarak yapıldığı bölgelerden seçilmiştir ve laboratuvara getirilmiştir. Laboratuvara getirilen mısır numunelerinin öğütülmesi işlemi yapılmış, daha sonra spektraları oluşturulmuştur. Akabinde, laboratuarda yaş kimyasal analiz yöntemiyle nişasta değerleri bulunmuştur. Oluşturulan kalibrasyon setinin R=0.6410; R²= 0.4109 Standart Sapma = 4.4208 şeklinde değerleri alınmış, validasyon setinden ise R=0.5854; R²= 0.3427 Standart Sapma = 4.5662 değerleri elde edilmiştir. Araştırmada, kalibrasyon aralığı 44.53 bulunurken, validasyon aralığı ise 45.72. olarak bulunmuştur. Sonuç olarak bu araştırma ile Türkiye'nin 7 farklı bölgesini temsil edebilecek mısır örneklerindeki nişasta miktarı FT-NIR cihazı kullanılarak elde edilen kalibrasyonların bilimsel açıdan daha doğru sonuçlar vermesi için daha fazla miktarda numune ile çalışmaların yapılması gerektiğine kanaat getirilmiştir.

Anahtar Kelimeler: Mısır, Nişasta, NIR, Kalibrasyon

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ORCID ID; ET: 0000-0002-6368-3801, İB: 0000-0002-9993-7092

*Corresponding author e-mail: <u>ibayram1965@gmail.com</u>

INTRODUCTION

The maize plant is an annual cultivated field crop. It has an advanced root system. Grain maize is a significant role in both human and animal nutrition. Due to its high energy and dry matter, maize has recently been planted as a silage plant as a major feed source for ruminant animals (Anonymous, 2016). Corn is the most produced cereal in the world because of its high energy and nutritional value. Corn production in the world has reached 780 million tons in recent years (Özcan et al. 2009). USA and China are leading countries in the world in corn production (Anonymous 2016). There is a high level of energy in the grain. Corn contains less protein and β-carotene compare to other grains. Corn is used at high levels in poultry and swine rations. In order to raise the energy level of rations, it is used more frequently in the first period of lactation together with grain feed such as wheat and barley. Depending on the animal species and the yield period of the animal, corn grain can be added up to 65-70% of the ration (Anonymous 2016). Near infrared spectroscopy (NIRS) technology is a near infra-red spectroscopy method based on the absorption of electromagnetic radiation in the wavelength range of 400-2500 nm. The nutritional values of the feed raw materials previously calibrated to the device are determined and used to analyze the feed raw materials, and the nutrient content of the feeds is determined quickly. Knowing the values of the feedstuffs involved in the ration directly affects the accuracy of the ration. The tendency towards devices and technologies that make it fast and reliable in determining food values without the need for many chemicals is steadily increasing. Nowadays, nearinfrared (NIR) devices have been introduced that give fast results to replace traditional wet chemical analyzes (Cen and He 2007). The NIR device was first used in the world to determine the amount of moisture contained in cereals (Osborne and Feam 1986).

The NIR device is a method of analyzing nutrients such as protein, fat, carbohydrate, ash, moisture (Osborne et al. 1983). NIR technology can also be used to determine the amino acids in industrial feeds (Gonzalez-Martin et al. 2006). Using the NIR device, the results of the analysis were evaluated in corn and the reliability of the results increased after a certain period of time (Jarvis and Walker 1993). NIR technology has also been used to determine the amount of tocopherol in animal feeds (Gonzalez-Martin et al. 2006). Due to the use of external calibrations, nutritional value of feed raw materials produced in Turkey gives misleading results. This situation causes errors in ration calculations (Güngör et al. 2007).

In the NIR device, R² value is regarded as a reference value. The R² values of 0.95 and above are considered to be fairly good results, whereas those of 0.90 and 0.95 are moderate and those below 0.90 are poor results. For standard error values, the value of 0.3-0.5 is quite good, 1-1.5 is medium, 2-3 is poor (Shenk et al. 2003).

Starch is found in wheat, barley, rye, corn grain in large quantities. Grains are used in abundant quantities so that the amount of energy can be balanced during the feeding of high milk-yielding cows. Generally the optimum starch ratio in dairy cows is 25-30% in dry matter (Dryden 2008). Campbell et al. (1999) estimated the content of starch and cereal amylose in maize with the NIR device. The results show limited sensitivity of this method, whereas the NIR technique can be used as a rough screening method for starch amylose content. In a study (Jiang et al. 2007) to determine protein, starch, and fat content of corn by using NIR device, protein starch and fat contents were found to be the same as those determined with wet chemical method. Same Researchers concluded that the NIR spectroscopy can be used to determine protein, starch and fat content. NIR spectroscopy has been used for the rapid analysis of selected starch samples and the classification of starch molecules is possible with NIR technology (Írudayaraj et al. 2002). In another study, differences between wheat and corn starch were determined using NIR spectroscopy (Hodsagi et al. 2012). There is a certain cost due to the use of time and some chemicals during the chemical analysis. Near infrared reflectance (NIR) can be used to analyze economically in a short period of time without destroying the samples. For example, the NIR technique can be used to analyze complex structures such as starch (Foley et al. 1998). The NIR spectroscopy method has revolutionized the method of analyzing animal feeds quickly and accurately, and the method is becoming more and more popular. Particularly in the field of livestock, when the feed values are determined, the raw materials of feed are vital in predicting nutrients (water, protein, starch, oil, sucrose). In the near future it will be widely used as a feed evaluation method (Givens et al. 1997).

The objective of this study was to establish a national calibration method in Turkey. Another objective was to calibrate the corn starch contents with the use of NIR device.

MATERIAL and METHOD

This study was carried out on 320 grain corn samples. The samples used in the study were collected from Toprak Mahsulleri Ofisi (Soil Products Office) (TMO) in 7 different cities to

represent seven different regions in Turkey. Samples were taken from corn producers with the aid of agencies in seven different provinces such as Adana, Diyarbakir, Gaziantep, Iskenderun, İzmir, Konya and Şanlıurfa. During the sampling process, the identification information of each producer and the information of the region where the corn was grown were recorded. Each corn sample was placed into an impermeable plastic bag after the relevant records were taken and then rapidly transported to Feed Analysis Laboratories. After the samples were delivered to the laboratory, they were milled (ZM200, Retsch Ltd., Düsseldorf, Germany) with ultra-centrifugal rotor mill and passed through a 1 mm sieve. Immediately after this procedure, the ground and eluted corn samples were again placed in clear, impermeable plastic bags and separated. After the grain corn was milled, spectra were taken individually from each sample. During spectral picking, the milled corn samples put on a clear glass petri dish were placed in the automatic rotor of the NIR instrument and the starch-related spectra were collected three times for each corn sample. All collected spectra were stored in electronic form in an appropriate form on the integrated personal computer connected to the NIR device by the same computer program provided by the manufacturer company. The collected samples were again placed into separate plastic bags and stored at -20 ° C until analysis. Milled corn samples were stored in desiccators overnight at room temperature for starch analysis chemometrically and solubilized without rehydration. Starch analysis was carried out with the aid of a digital polarimeter (ADP410 Polarimeter) in accordance with the Starch analysis method (Method No: 996: 11) reported in the AOAC (2018) method for dissolved corn samples. The registration of the wet chemistry results obtained for each sample was carried out separately into the operator program in the NIR Master.

Calibrations and statistical evaluations based on spectral and chemometric analyses (wet chemistry) data obtained with the aid of the NIR instrument in the study were evaluated with the help of the NIRCAL program (Büchi Labortechnik AG, Flawil, Switzerland). Calibration and validation sets within spectra were separated with the help of the program. The obtained spectra were evaluated on the second derivative by PLS (Partial Least Square) method. The SNV (Standard Normal Variate) method was applied to the data for the normalization study. Subsequently, a first grade derivative was obtained (1st Derivation B Cap 5

Points Gap 2) and the data was readied for regression. The outlier values were subtracted from the calibration set and linear regression was applied to the normalized spectra and the calibration quality parameters were manifested. At this stage, the standard deviations of the R2 value, validation and calibration set were calculated. Graphical outputs were generated with Regression Coefficients according to the reflectance. Furthermore, the squared total value (V-Set PRESS) of the estimation residual error of the validation set was derived. Outlier values were not included in the study because they were determined by the program and reduced the calibration quality.

RESULTS

Reflectance images (1 / log) after preliminary applications applied on the spectra and normalized spectra are shown in Graph 1.

It was observed that the functional spectra obtained after the application were obtained at 9000-4000 nm / cm wave length. The Principal Components of the validation set (V-Set PRESS) has been around 12. The basic component values via the V-Set PRESS are shown in Graph 2.

The resulting regression consistency was obtained as the standard error of the calibration (SEC) divided by the estimation standard error (SEP). The consistency value was determined as between 80 and 100. Consistency analysis results are shown in Graph 3.

The output of the linear regression values obtained from the calibration and validation sets is shown in Graph 4.

Regression analysis included 623 in the calibration set and 205 in the validation set. The model for the Calibration and Validation sets formed as a result of the analysis is indicated below:

Calibration set

f(x) = 0.4109x + 38.4569

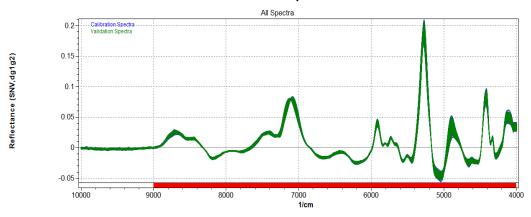
R=0.6410; $R^2=0.4109$ Standard Deviation = 4.4208

Validation set

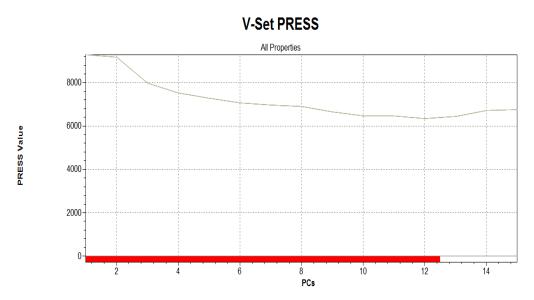
f(x) = 0.3659x + 41.3845

R=0.5854; $R^2=0.3427$ Standard Deviation = 4.5662

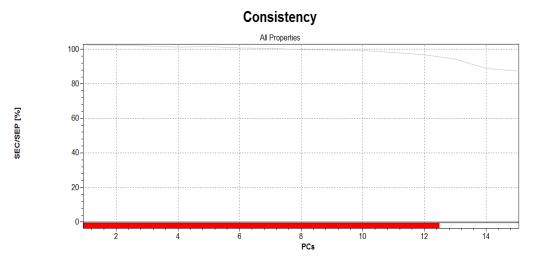
Pretreated Spectra



Graphic 1. Normalized spectra set

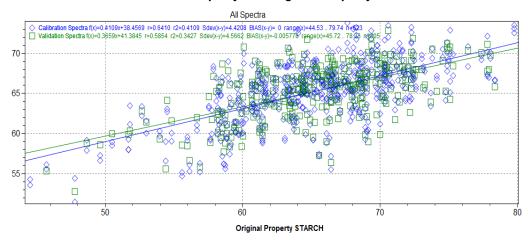


Graphic 2. Squared total value of the estimation residual error.



Graphic 3. Consistency analysis of calibration set.

Predicted Property vs. Original Property



Graphic 4. Calibration and validation set models

DISCUSSION and CONCLUSION

A total of 960 spectra from a total of 320 samples (3 spectra from each sample) collected from regions in which corn growing predominates in Turkey have been used in the study. 623 spectra were used in the calibration set and 205 in the validation set. 132 spectra were excluded from the evaluation. The number of spectra and samples obtained in some other studies related to this topic and the values of the data showing the estimation strength and the literature reports are shown in table 1. The results of the chemometric (wet chemical) starch analyzes in the study are shown in Table 2.

As indicated in the table 2, the data set values in this study have been determined with a rather wide interval. This wide range of variation in the values obtained after wet chemical analysis carried out on maize grain samples from different regions of Turkey may be due to starch content values. The starch content of corn in the world also displays quite a comprehensive range interval (Lardy 2013). The grain corn starch content values obtained by wet chemical (chemometric) method in this study were determined as 65.10 %. At the same time these detected values rate among normal starch values for maize in the world and in Turkey (Wehling et al. 1993). Chemometric analyzes were performed on 320 samples in this study and 0.3408 standard error results were obtained. The values we have obtained are the same as the results found in the other study (Wehling et al. 1993). Wehling et al. (1993) determined standard error values for corn starches at an interval of 1.41-2.07% in maize grains. Likewise, Melchinger et al. (1986) reported standard error results with protein, cellulose and water soluble carbohydrates in corn grain, protein and ADF values in corn cobs with NIR analysis

which were 0.16%, 0.11%, 0.21%, 0.28% and 0.46% respectively and these values were 6% to 22% higher than the results of chemometric analysis. In a similar study on the subject, the R² value in an NIR calibrated study in which corn starch was added to onion salt in different ratios was determined to be in the range of 0.92-0.93 and the standard error calibration value was determined in the range of 2.52-2.65 (Lohumi et al. 2014). Paulsen and Sing (2004) determined that the average extractable corn value in their study of 2267 corn samples carried out between 1997 and 2001 was in the range of 63.4-68.5%. The same authors determined a standard error of 1.24 while the R² was determined as 0.79. Baye et al. (2006) reported a corn starch value in the interval of 33.1-75.9% with a standard deviation value of 9.2 and an R² value of 0.86. Likewise, Burgers (2009) reported a corn starch value of 60.1% and a standard deviation value of 1.1. The average starch value of sweet potato was found to be 89.79% while the R² value was found to be in the range of 0.85-0.92 (Lu et al. 2006), while the mean value of the starch content of corn was reported to be 58.5-74.4% with an R2 value of 0.77 (Paulsen et al. 2003).

Spectral values were applied as a pretreatment and the first derivate gap2 was applied to the standard normal variation (SNV) and multiplicative scatter correction (MSC) in the study. It is reported that these variables (parameters) are used extensively in the evaluation of spectra (spectrum) (Panero et al. 2013, Zhao et al. 2015). It has been reported that multiplicative scatter correction (MSC) standard normal variation (SNV) implementations are highly successful in avoiding erroneous path length and light scattering, which can not be determined, by erroneous spectrophotometry errors (Panero et al. 2013).

Variations in ambient temperature variations, sample differences or ambient spectra originating variables due to the sensor may vary (Bokobza 1998). Therefore, in order to avoid this situation formed by the initial impact and minimize the level of the chemometric line variable established by the chemometric analysis and avoid an erroneous evaluation, it is recommended to evaluate the first and second derivatives (Mark and Workman 2003).

The partial least squares method (PLS) was used to evaluate the spectra obtained in this study. This method is widely used in NIRS calibrations and it is reported to be one of the most functional ones (Blanco and Peguero 2012). With this PLS method, explanatory variables with multiple linear correlation between them can be obtained with the help of Harezmi path (algorithms) as well as change in explanatory variables (Via et al. 2014).

The R² (coefficient of determination) value is used to calculate the estimation value. This value is the regression coefficient, which is the coefficient of determination of the association between variables and is calculated by taking the square value of R (Sohn et al. 2006). This R² value is between 0 and 1. The closer this value is to the number 1, the stronger the result is (Sohn et al. 2006). In the presented this study, the calibration value set for the starch value was 0.6410 and the R² value was 0.4109, the R value of the validation set was 0.5854

and the R² value was 0.3427. Wehling et al. (1993) found the R² value for starch in the range of 0.872-0.892 in their study with maize kernels using the NIRS technique. Likewise, Fu (2004) determined the R² value for corn grain starch to be 0.991. In another study (Holder 2012) the aim was to determine the starch amylose and amylopectin ratio with NIRS and the R2 value was determined as 0.832. The literature reports have significantly higher mean values for corn starch and R2 values than the R² for starch in our study. This conclusion can be attributed to the variety and type of soil in the area where the maize plan was grown, differences in climate and geographical properties and most importantly differences in the races of the maize plants.

This study was carried out with the intention of establishing a national calibration method for maize kernels grown in our country. To this end, maize samples were collected from seven different provinces to represent all regions which were processed with an FT-NIR. It has been concluded that it is possible to use an NIR device to determine the corn starch and set a calibration of a national starch amount to represent the whole country, however in order to have more reliable and usable results a more comprehensive study involving at least 500 and more maize samples must be carried out.

Table 1. Data from a limited number of studies involving R² values of nutrients in grain maize.

Corn	Number of Sample	Analyses of Feedstuffs	R ²	Reference
Grain	100	Starch	0.83-0.94	Plumier et al. 2013
Grain	90	Starch	0.98, 0.99	Zhong et al. 2016
Grain	304	Starch	0.77	Paulsen et al. 2003
Grain	90	Starch	0.86	Baye et al. 2006
Grain	2267	Starch	0.79	Paulsen and Singh 2004
Grain	2160	Starch	0.86	Baye et al. 2006
Grain	87	Protein, fat, sugar	0.75, 0.29, 0.02	Tallada et al. 2009
Sorghum, Grain	140	Starch	0.91	Wolfrum et al. 2013
Grain	320	ADF	0.30	Gümüştaş and Bayram 2018

Table 2. Wet chemical starch results in maize rains.

Descriptive statistics of Starch chemistry analysis results				
Average	65,1090			
Standard Error	0,3408			
Standard Deviation	6,0384			
Median	65,4090			
Variance	36,4619			
Relative standard deviation	0,09274 (9,27%)			
Optimum value	79,74			
Lowest value	42,58			

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RESEARCH ARTICLE

Effect of 7-Dehydrocholesterol on Ram Semen Spermatological Parameters on Short Term Preservation

Muhammed Enes İNANÇ1*, Şükrü GÜNGÖR1, Ayhan ATA1

¹Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-15030 Burdur – TURKEY

ABSTRACT

The aim of this study was investigated to effect of 7-dehydrocholesterol loaded cyclodextrin (7-DCLC) on ram semen spermatological parameters on short term preservation at +4 °C. Collected semen with electroejaculator at non-breeding season. Each animal native ejaculates were mixed and divided into 4 equal groups, diluated with tris/egg yolk extender with included different rates of 7-DCLC (1.5 mg, 3.0 mg and 5 mg/120x10⁶) and control (C). After extended semen samples were preservation at +4 °C and evaluated motility, membrane integrity and abnormal spermatozoa rate until 96. hour. There were no significant differences between the groups on abnormal spermatozoa rate (P>0.05); there were a significant differences between the groups on motility at all storage period (P<0.05). The highest motility were observed in 7-DCLC 1.5 mg at 72. and 96. hour (P<0.05). The highest dose (5 mg/120x10⁶) 7-DCLC were decreased the motility and membrane integrity compared to other groups. Besides, 7-DCLC 3.0 mg group membrane integrity was lower than control at 72. hour (P<0.05). In conclusion, tris/egg yolk extender which is contain 7-DCLC 1.5 mg were protected to ram semen motility and membrane integrity up to 96. hour at +4°C preservation.

Keywords: 7-dehydrocholesterol, cyclodextrins, motility, membrane integrity, short term storage.

Kısa Süreli Saklanan Koç Spermasında 7-Dehidrokolesterolün Spermatolojik Parametrelere Etkisi

ÖZ

Bu çalışmanın amacı, 7-dehidrokolesterol yüklenmiş siklodekstrinin (7-DCLC) +4 C⁰'de kısa süreli saklanan koç spermasının spermatolojik parametrelerine etkisinin araştırılmasıdır. Her bir hayvandan spermalar elektroejakülatör yardımı ile sezon dışında alınarak dört eşit gruba ayrıldı, farklı oranlarda 7-DCLC (1,5 mg, 3,0 mg and 5 mg/120x10°) içeren ve içermeyen kontrol (C) tris/yumurta sarısı sulandırıcı ile sulandırıldı. Sulandırılan spermalar, +4 C⁰'de saklanarak 96 saat süresince motilite, membran bütünlüğü ve anormal spermatozoa yönünden incelendi. Saklama süresi boyunca anormal spermatozoa yönünden gruplar arasında bir farklılık bulunmazken (P>0,05); motilite açısından gruplar arasında farklılıklar tespit edildi (P<0,05). 72. ve 96. zaman dilimlerinde en yüksek motilite 7-DCLC 1,5 mg/120x10° grubunda tespit edildi (P<0,05). En yüksek dozlu (5 mg) 7-DCLC grubu diğer gruplarla karşılaştırıldığında spermatozoa motilitesini ve membran bütünlüğünü düşürdüğü tespit edildi. Ayrıca, 72. saatte 7-DCLC 3,0 mg grubunun membran bütünlüğü kontrol grubuna göre daha az olduğu tespit edildi (P<0,05). Sonuç olarak, 7-DCLC 1,5 mg içeren tris/yumurta sarısı sulandırıcısı koç spermasında motiliteyi ve membran bütünlüğünü +4 C⁰'de 96 saat süresince koruduğu belirlendi.

Anahtar Kelimeler: 7-dehidrokolesterol, siklodekstrin, motilite, membran bütünlüğü, kısa süreli saklama

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ORCID ID; MEİ: 0000-0001-6954-6309, ŞG: 0000-0003-3460-522X, AA: 0000-0003-0590-5995

*Corresponding author e-mail: enesinanc@hotmail.com

INTRODUCTION

The artificial insemination technology can simplify sheep industry (O'Hara et al. 2010) and one of the important factors for spread to superior genotypes (Falchi et al. 2018). In this technology, semen cryopreservation is the most used preservation type in farm animals (Bucak et al. 2008). Some authors reported that cervical artificial insemination with frozen-thawed semen generally results in lower pregnancy than fresh or short therm preservation (Maxwell et al. 1999, Paulenz et al. 2002). The low fertilization from cervical artificial insemination with frozen-thawed semen is based on the irreversible damage at cryopreservation process (Salamon and Maxwell 1995, Bailey et al. 2000). The low proportion of ram semen biologically protect to their cell membrane and can capable to pass cervical barrier and reach to fertilization site at the cervical artificial insemination (Salamon and Maxwell 2000, O'Hara et al. 2010). In cryopreservation high proportion of ram spermatozoa cells have under goes damage during freezing-thawing process. Especially spermatozoon viability, membrane integrity and motility has been (Salamon and Maxwell 1995, De decreased Lamirande et al. 1997). These problems caused by freezing resulted in the keeping of the ram's sperm at +4 °C. The loss of motility, membrane integrity and fertilization is observed more gradually in the storage of semen at +4 °C (Maxwell and Watson, 1996, Avdatek et al. 2018).

Ram semen has a lower cholesterol/phospholipid and higher polyunsaturated/fatty acids rate than other animal species (Moce et al. 2010). Cholesterol has a multi type effect on the plasma membrane; acts as a antioxidant, decreases membrane membrane permeability, ensures a suitable chemical and/or physical microenvironment for proteins in the membrane and reduces phase changes, regulates morphological characteristics (Aksoy et al. 2010, Moce et al. 2010). On the other hand, cyclodextrins are commonly defined as cyclic oligosaccharides produced by the breakdown of starch (Crini 2014). There are also some reports supplemented cholesterol loaded cyclodextrin (CLC) into different animal species for semen preservation and increased phospholipid-cholesterol ratio (Purdy and Graham 2004, Murphy et al. 2014). This increase has been protected to morphological integrity (Mansour 2009), DNA damage (Katanbegzadeh et al. 2014), motility, viability and membrane integrity (Purdy et al. 2004). 7-dehydrocholesterol is intermediate product of cholesterol in biochemical shema and it's one of the cholesterol conjugate. 7 dehydrocholesterol is formed prior to cholesterol is produced. In previous studies have demonstrated the protective effects of 7-DCLC supplementation on in vitro spermatological parameters of frozen ram semen (Inanc et al. 2018a)

and chilled bull semen (Inanc et al. 2017). But there are no information about ram semen short term preservation studies with 7-DCLC in tris extender. Thus, the aim of this study was investigated to effect of 7-DCLC on ram semen spermatological parameters on short term preservation at $+4 \, \text{C}^0$.

MATERIAL and **METHOD**

Cyclodextrin preparation

Methyl-beta-cyclodextrin (MßC, C4555)- 7-dehydrocholesterol (CAS number: SC 214398) complex prepared by Purdy and Graham (2004) method. Briefly, 1g of MßC was solved in 2 ml of methanol in test tube. 200 mg 7-dehydrocholesterol was solved in 1 ml chloroform in another test tube. After, 450 ml 7-dehydrocholesterol was mixed with MßC; solvents were evaporated with nitrogen vapor and finally 7-DCLC powders were obtained. 7-DCLC working solutions were prepared with 50 mg 7-DCLC; these were dissolved in 1 ml of a stock tris solution in different test tube at 37 °C and the solution was mixed with vortex.

Animal and Study Design

In this study three mature Pirlak-Merino crossbred rams were used. The animals were submitted to special farm with uniform breeding conditions at the Callica in Burdur, Turkey. 18 ejaculates were collected with electroejaculator (Minitube, e320). Every were examined macroscobic eiaculates microscobic evaluation and good quality ejaculates (concentration: $\geq 2 \times 10^9$ /ml, volume ≥ 0.5 ml, motility: ≥ 80%) were mixed. Mixed ejaculates were diveded equal four groups and control group without the additive into a tris-based extender (Tris, T1503; 3.63 gr; Citric acid, C0759; 1.82 gr; Glucose, G7528; 0.5 gr/100 ml distilled water, 15% (v/v) egg volk); other three groups which is contain 7-DCLC (1.5 mg, 3.0 mg, 5.0 mg/120x106) were firstly extended with 7-DCLC solutions and incubated for 15 min at 25 °C, then the groups were extended with a tris-based extender to a final concentration of 500 × 106 spermatozoa/mL. Extended semen groups were loaded to straws and they were considered as 0. hours and stored at +4 °C until 96. hours. This study was approved by Mehmet Akif Ersoy University Animal **Experiments** Local Ethics Committee (06.12.2017/260).

Spermatological Evaluation

Subjective motility was analyzed using a phase-contrast microscope (400× magnification), at 37°C. Sperm motility scores were performed in at least five different parts for each sample. The mean of the five successive evaluations was kept as the motility score. Evaluation of membrane integrity, Hypo-Osmotic Swelling (HOS) test was used and based on curled and swollen tails. This was determined by incubating

10 µL of semen with 100 µL of 100 mOsm hypoosmotic fructose (D-Fructose F0127) solution at 37 °C for 60 min. Totally 200 spermatozoa were checked under 400× magnification and spermatozoa with coiled or swollen tails were recorded (Revell and Mrode 1994). Abnormal spermatozoon rate was determined by liquid fixation method. Spermatozoon were fixed with Hancock solution (Hancock 1952) and abnormalities detected under 1000x magnification with phase-contrast microscopy by evaluating at least 200 sperm cells (Schafer and Holzman 2000).

Statistical Analysis

The study was replicated six times. The results were expressed as Mean (X) ± Standard Error (SEM). Means was analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine the significant differences in all parameters among all groups using the SPSS/PC computer program (version 22.0). Differences with

values of P < 0.05 were considered statistically significant.

RESULTS

There were no significant differences between the control and 7-DCLC 1.5 mg; 7-DCLC 3.0 mg and 5.0 mg on motility at 0. hour (P<0.05). The highest motility were observed in 7-DCLC 1.5 mg at 48. Hour (Table 1). There were a significant differences between the groups on motility at 72. and 96. hours (P<0.05); the highest motility were 53.26 \pm 1.23%; 48.03±1.88% in 7-DCLC 1.5 mg respectively. The highest dose (5 mg/120x106) 7-DCLC were decreased the motility and membrane integrity compared to other groups. Besides, 7-DCLC 3.0 mg group membrane integrity 33.65± 5.88% was lower than control at 72. hour (P<0.05) (Table 2). Also, there were no significant differences between the groups on abnormal spermatozoa rate (P>0.05) at the storage period (Table 3).

Table 1. Mean (±SEM) ram semen motility (%) of short term preservation with 7-DCLC

Groups	0. hour	24. hour	48. hour	72. hour	96. hour
Control	84.16±0.83a	79.16 ± 2.38^{a}	52.03±1.47a	42.10±1.85b	35.50±2.80b
7 DCLC 1.5	81.66 ± 1.66^{a}	70.26 ± 3.51^{a}	54.43 ± 1.58^{a}	53.26 ± 1.23^{a}	48.03 ± 1.88^{a}
7 DCLC 3.0	75.83 ± 2.00 b	76.08 ± 1.75^{a}	16.55±2.20b	7.26 ± 0.72^{c}	-
7 DCLC 5.0	71.66 ± 1.05 ^b	43.93 ± 3.28 ^b	5.31±1.89c	2.16±1.01°	-
P	*	*	*	*	*

a-c: Different superscripts within the same column demonstrate significant differences (P<0.05).

Table 2. Mean (±SEM) ram semen membrane integrity (HOS test, %) of short term preservation with 7-DCLC

Groups	0. hour	24. hour	48. hour	72. hour	96. hour
Control	66.36 ± 3.44^{a}	59.98±1.87b	54.09 ± 6.31^{a}	50.78 ± 2.63^{a}	38.58±4.69a
7 DCLC 1.5	60.94 ± 2.55^{a}	52.08 ± 3.06 ^b	47.76 ± 3.63^{a}	43.54 ± 4.25 ab	38.99 ± 5.17^{a}
7 DCLC 3.0	62.50 ± 5.08^{a}	51.72 ± 4.78^{b}	44.67 ± 4.26^{a}	33.65 ± 5.88 bc	25.12 ± 5.59^{ab}
7 DCLC 5.0	42.15±9.41 ^b	39.24 ± 2.14^{a}	26.12±4.15 ^b	23.20 ± 2.40^{b}	15.57±3.54b
P	*	*	*	*	*

a-c: Different superscripts within the same column demonstrate significant differences (P<0.05).

Table 3. Mean (±SEM) ram semen total abnormal spermatozoa rate (%) of short term preservation with 7-DCLC

Groups	0. hour	96. hour	
Control	12.81±0.90	17.58±1.28	
7 DCLC 1.5	10.60 ± 2.01	16.17±1.34	
7 DCLC 3.0	8.87 ± 1.31	16.88 ± 1.77	
7 DCLC 5.0	8.07 ± 1.09	19.67±1.38	
P	-	-	

^{-:} No significant difference (P > 0.05).

DISCUSSION

This study performed to evaluation the effect of short term storage with tris based extender supplementation with 7-DCLC. 7-DCLC 1.5 mg were improved to motility at started by 72. hour (53.26±1.23%) (P<0.05). As the same results were observed in 96. hour (P<0.05). Amorim et al. (2009) and Moraes et al. (2010) cryopreservation of bull semen with cholestryl conjugates and they were observed improved to motility compared to control group. Benhenia et al. (2018) was frozen ram semen

a-c:Aynı sütunda farklı harf taşıyan gruplar istatistiksel olarak farklıdır (P<0,05).

a-c: Aynı sütunda farklı harf taşıyan gruplar istatistiksel olarak farklıdır (P<0,05).

^{-:} Gruplar arasında istatistiki bir fark bulunmamaktadır (P>0,05).

with tris-egg volk extender containing with CLC (2 mg/120x106)+ Vit E combination. They were had a significant positive effect on progressive motility, total motility and membrane integrity. On the other hand, Oliveria et al. (2014) didn't find any significant differences between the CLC and control groups spermatozoa velocity after thawed the donkey semen. But, like present study, Inanc et al. (2017) preserved bull semen at +4 °C up to 72. hour with 7-DCLC and CLC. They were found 7-DCLC decreased the cell damage at the low temperature by the had improved to motility compared to control. Also, CLC 1.5 mg/120x106 was found to good option at +4 °C liquid storage in this study. These results showed that low level of 7- DCLC (1.5 mg/120x106) had increased membrane lipid protein structure. This could be caused increased osmotic changes into the cell membranes. Besides, the high level 7-DCLC group (5mg/120x106) decreased the motility at the all of the preservation time.

Membrane integrity in spermatozoa can be evaluated simply by the HOS test. (Revell and Mrode 1994). In present study, the highest dose (5 mg/120x106) 7-DCLC were decreased the membrane integrity compared to other groups. Partyka et al. (2018) were supported present study and highest level CLC (4 mg/120x106) had the lowest membrane integrity and motility in fresh and short term preservation chicken spermatozoa at +5 °C. Besides, 7-DCLC 3.0 mg membrane integrity 33.65± 5.88 % was lower than control at 72. hour (P<0.05). Inanc et al. (2018a) frozen-thawed ram semen and they found improvement to membrane integrity with 7-DCLC compared to control groups. Membrane integrity was increased with CLC supplementation in bull semen after freeze-thawing procedure (Purdy and Graham 2004). On the other hand, cholesterol modification in the cell membrane protects antioxidative enzymes and prevents spreading to reactive oxidative species (Lopez-Revuelta et al. 2007). Medeiros et al. (2002) observed that phospholipid / cholesterol rate is a good achive for evaluate the stability membrane and fluidity at lower temperature. All this information suggest that cholesterol, cholesterol conjugate and forms support to membrane integrity and support to our results.

In present study, there were no significant differences between the groups on abnormal spermatozoa rate (P>0.05) at the storage period. Oettle (1993) stated that low level morphological integrity was shown to be an important marker of infertility. CLC did not increase the total morphological integrity (Khan et al. 2017, Inanc et al. 2018b). This results were similar with current study findings. But there were same significant differences between the cholesterol conjugate and control groups after freze-thawed process (Moce et al. 2010). This differences could be

explain by different animal genotype and breed, different freezing-thawing procedure. Some researcher found high apoptotic level in the whole groups after cryopreservation procedure (Inoue et al. 1999, Inanc et al. 2018a). This could be occurred as a result of our freezing-thawing procedure. This apoptosis might be results higher abnormal spermatozoa rate compared to fresh or short term (liquid) storage conditions. By this way, short term storage should be used for higher fertility compared to freezing-thawing procedure.

In conclusion, tris/egg yolk extender which is supplementation 7-DCLC 1.5 mg/120x10⁶ was protected to ram semen motility and membrane integrity up to 96. hour at +4°C preservation. 7-DCLC 1.5 mg/120x10⁶ can be used in short-term artificial insemination applications in sheep breeding industry.

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RESEARCH ARTICLE

Evaluation of Computer Tomography Encountered Bone Proliferation in Dogs

Mehmet SAĞLAM^{1*}, Damla ÇAYIROĞLU¹, Hasan Onur ÇAYIROĞLU²

¹Ankara University, Faculty of Veterinary Medicine, Department of Surgery, Ankara-Turkey.

²Gaziemir, Veterinary Clinic, Izmir-Turkey.

ABSTRACT

In this study, the results of computed tomography were evaluated in 8 patients including 3 female and 5 male dogs with bone growth as a result of clinical and radiographic examinations in Surgery Department's clinic of Ankara University Veterinary Faculty, between the yaers of 2016-2017. Two dogs were under 1 year old and the others were between 1-4 years of age. When the cases were examined by computed tomography after clinical examination and radiography, chondromatous proliferation, avasculer necros, greenstick fracture and malunion after treatment complication including 4 femur, periostal proliferation and synostosis including 2 radius ulna, fibrochondroosteosarcom including a scapula and ankylosis after old bone fracture including a cubiti bone proliferation were detected.

Keywords: Bone, bone proliferation, computer tomography, dog.

Köpeklerde Karşılaşılan Kemik Üremelerinin Bilgisayarlı Tomografi ile Değerlendirilmesi

ÖZ

Bu çalışmada 2016-2017 yılları arasında Ankara Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne getirilen, klinik ve radyografik muayeneler sonucunda kemik üremeleri belirlenen 3 dişi ve 5 erkek köpek olmak üzere 8 olguda bilgisayarlı tomografi sonuçları değerlendirildi. Köpeklerden 2'sinin 1 yaşın altında, diğerlerinin 1-4 yaş aralığında olduğu belirlendi. Olgular bilgisayarlı tomografi ile incelendiğinde; kondromatöz üreme, avaskuler nekroz, yaş ağaç kırığı ve sağaltım komplikasyonu sonucu şekillenen malunion olmak üzere 4 femur, periostal üreme ve synostosis olmak üzere 2 radius-ulna, fibrokondroosteosarkom olmak üzere 1 scapula, eski kırık sonucu ankiloz olmak üzere 1 art. cubiti'de kemik üremesi saptandı.

Anahtar Kelimeler: Bilgisayarlı tomografi, kemik, kemik üremesi, köpek.

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ORCID ID; MS: 0000-0001-8934-8529, DÇ: 0000-0002-8899-4403, HOÇ: 0000-0002-5077-1060

*Corresponding author e-mail: msaglam@ankara.edu.tr

GİRİŞ

Canlı organizmadaki dokuların üzerinde bulunan mukavemet mekanik etkiler sonucunda dayanıklılığını kaybederek bölgedeki bütünlüğün bozulmasına neden olur. Bunun sonucunda bölgesel olarak kemikte kırık, çatlak gibi lezyonlar şekillenebilir. Bu lezyonlar hematojen yolla gelişen kemik yangılarının yanı sıra sivri cisim yaraları veya travma sonucunda da gelişebilir (Schwarz and Saunders 2011).

Kemik, oluşan lezyonlara kemik üremesi (osteogenez), kemik kaybı (osteoliz) ya da kemik kaybı ve kemik üremesi bir arada olmak üzere üç farklı şekilde yanıt verir (Dennis et al. 2001). Kemik üremelerinde, klinik olarak bölgede ağrılı şişkinlikler, ödem, apse ve fonksiyonel yönden yürüyüş bozuklukları ile karşılaşılır (Öztürk 2015).

Kemik kalınlaşmaları, periostun altında meydana gelen kemik üremesinden şekillenir. Trabekular kemik, primer veya metastatik neoplaziler osteomyelitis, fiziksel yaralanmalar, kemik kırıkları, kallus ve osteopetroz'da kemik kalınlaşmaları ile karşılaşılır (Houlton et al. 2006).

İskelet sistemi hastalıklarında ve değişimlerinde radyografik değerlendirme tanı için önemlidir (Öztürk 2015). Kemikler yumuşak dokuların aksine daha fazla X ışını tutarak (az geçirerek) açık renkli (radyoopak/hipersens) bir görünüme sahip olurlar. Tanı amaçlı alınan radyografiler yoğunluk değişimleri, boyut, şekil ve kontur değişimleri, kemik hattı ve periostal reaksiyonlar hakkında bilgi verir (Bumin 2015).

Sürekli periostal kemik üremeleri yeni bir kemiğin düzenli olarak oluşmasını sağlayan ve yavaş ilerleyen bir hastalık sürecidir. Bu süreç benign ve agresif olmayan lezyonlarda görülür. Radyografide radyoopaklık tek düzedir. Kesintili periostal reaksiyonlarda ise hızlı bir şekilde değişen lezyonlar korteks ve periostu bozarak onarıma başlar. Malign neoplazi ve agresif hastalık sürecinde karşılaşılır. Radyoopaklık değişkendir ve kısa bağlantısız bir görünüme sahiptir (Dennis et al. 2001).

Radyografinin aksine ileri görüntüleme tanı yöntemlerinden biri olan bilgisayarlı tomografi'de birden fazla görüntü alınabilmesi süperpozisyonu engellemiş olur. Doku kontrastlarında radyografiden daha duyarlıdır (Sanal 2013; Woertler 2003). Hasta hareket ettirilmeden bölgenin farklı yönlerde ve kalınlıklarda kesitsel (transversal, sagittal, dorsal ve frontal) görüntüler alınması yapıların üst üste gelerek meydana getirdiği artefaktları da önlemiş olur (Ünal 2008). Bilgisayarlı tomografide de radyografideki gibi X ışını kullanılması nedeniyle yorumlamaları benzerlik gösterir. Kemik dokuda radyografide değerlendirilemeyen dansite ölçümü, kortikal ve kansellöz kemik durumu, lezyonun yapısı, kalsifiye alanlar ve ossifikasyon daha kolay değerlendirilebilir (Ayvaz ve Aksoy 2006).

Bu çalışmada köpeklerde şekillenen kemik üremelerinin rutin klinik muayene ve radyografik değerlendirme sonrasında ileri görüntüleme tanı yöntemlerinden biri olan bilgisayarlı tomografi ile görüntülenerek ayırıcı tanının daha kolay olması, rutin kullanımın arttırılması ve benzer çalışmalar için katkıda bulunulması amaçlanmıştır.

MATERYAL ve **METOT**

Ankara Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne 2016-2017 yıllarında getirilen, rutin klinik ve radyografik muayeneleri sonucunda kemik üremesi belirlenen 8 köpek çalışma materyalini oluşturdu.

Anestezi protokolüne uygunluğu değerlendirilen hastaların kısa süreli anestezileri *Propofol* "200 mg/20 ml ampul" kullanımıyla sağlandı. Bunun için, premedikasyon uygulaması yapılmadan 6-8 mg/kg intravenöz yolla uygulandı ve 20-60 saniye içerisinde etkisini gösterirken, 20-30 dakika arasında anesteziden çıkış gözlendi.

Ankara Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'nde Radyoloji ünitesinde bulunan ileri görüntülü tanı yöntemlerinden biri olan bilgisayarlı tomografi ile uygun anestezi protokolünde anesteziye alınan hastaların ilgili bölgelerinin 1-3 mm kalınlığında transversal kesitler halinde görüntüleri alındı.

BULGULAR

Çalışma olgularına ait klinik veriler, belirlenen lezyonun lokalizasyonu ve tanısı tablo 1'de sunulmuştur.

Olgu no 1'de klinik muayenede sağ caput femoris'in fossa acetabuli'den sublukse olduğu ve sağ arka ekstremitenin sol arka ekstremiteve oranla olduğu belirlendi. Pelvis'in V/D ve coxafemoral eklemlerin de lateral pozisyonda radyografileri alındı. Fossa acetabuli ve caput femoris'de periostal üremeler görüldü. V/D pozisyonda coxafemoral eklemin bilgisayarlı tomografi ile alınan görüntülerinde radyografideki radyolusent görünümde olan periostal üremeler belirlendi. Bölgeden alınan biyopsi materyalinin histopatolojik değerlendirmeleri ile "kondromatöz üreme" tanısı konuldu.

Olgu no 2'nin klinik muayenesinde sol ön ekstremitede açık yara, bölgede ağrı ve duyarlılık belirlendi. Sol ön ekstremitenin A/P ve M/L alınan radyografilerinde radius-ulna'nın diyafizinden distaline doğru devam eden periostal üremeler

saptandı. Bilgisayarlı tomografi görüntülerinde periostal üremeler yer yer dağınık olarak radyoopak alanlar halinde gözlendi. Açık yara bölgesinden steril swap ile alınan örneğin mikrobiyoloji laboratuvarında osteomyelitis olmadığı lokalize bakteriyel üremeler olduğu bildirildi.

3'ün klinik muavenesinde Olgu no ekstremitelerde ağrı ve duyarlılık, sağ ekstremite kaslarında atrofi ile kalçada asimetri belirlendi. Ayrıca sağ coxafemoral eklemde ağrı duyumunun daha belirgin olduğu gözlendi. V/D pozisyonda pelvis ve M/L pozisyonda sol arka ekstremitenin alınan radyografilerinde, solda eski supracondyler femur kırığı ile taşkın kallus ve üremeleri ile karsılasıldı. Sol caput kemik femoris'de osteolitik ve güve yeniği görünümünde nekroze alanlar ile avaskuler nekroz tanısı konuldu. V/D pozisyonda transversal kesitler halinde alınan bilgisayarlı tomografi görüntülerinde fossa acetabuli ve caput femoris'de bölgesel olarak radyoopak alanlar halinde yaygın bir üreme alanı ile karsılasıldı. Olgu no 4'ün klinik muayenesinde sol art. cubiti'de asırı duyarlılık ile birlikte ağrı, yumuşak dokularda hafif derecede ödem, eklem hareketlerinin ekstensiyon ve fleksiyonda kısıtlılığı ile ankiloz şekillendiği belirlendi. M/L ve A/P pozisyonlarda sol cubiti'nin radyografisi alındı. Ulna'nın proc. anconeus'unda ve condylus humeri'de radyoopak halinde kısa periostal üremeler ile karşılaşıldı. Üremelerin proc. anconeus'da gözlenen kırık oluşumunun neden yorumlandı. D/V pozisyonda transversal kesitler halinde alınan bilgisayarlı tomografi görüntülerinde condylus humeri ve ulna'nın proc. anconeus'unda gözlenen periostal üreme radyoopak alanlar halinde görüntülendi.

Olgu no 5'in klinik muayenesinde palpasyonda sağ femur'da aşırı duyarlılıkla birlikte ağrılı bir şişkinlik belirlendi. Pelvis'in V/D, sağ femur'un M/L pozisyonda alınan radyografilerinde femur'da yas

ağaç kırığı belirlendi. Bölgesel olarak dağınık radyoopak alanlar halinde kallus oluşumları gözlendi. V/D pozisyonda alınan transversal bilgisayarlı tomografi görüntülerinde periostal üreme ve kallus radyoopak alanlar halinde görüldü.

Olgu no 6'nın klinik muayenesinde sol radiusulna'da ağrılı sert bir şişkinlik palpe edildi. Radiusulna'nın alınan A/P ve M/L radyografilerinde eski bir kırık ve taşkın kallus oluşumu belirlendi. Bölgenin bilgisayarlı tomografi görüntülerinde radyografide kesinleştirilemeyen synostosis tanısı konuldu.

Olgu no 7'nin klinik muayenesinde sağ scapula'da asırı duyarlılıkla birlikte oldukça büyük, sıcak ve ağrılı bir şişkinlik belirlenirken bölgede kas atrofisi gözlendi. L/L pozisyonda sağ ön ekstremite ve throax radyografileri alındı. Sağ ekstremitede agresif litik lezyonların şekillendiği ve bölgenin güneş patlaması görünümü ile birlikte dağınık üreme alanlarının bulunuşu nedeniyle malig bir kemik tümörü düşünüldü. Thorax'ın radyografisinde akciğerde metastatik alanlar da gözlendi. Bilgisayarlı tomografide bu dağınık üreme alanları radyoopak alanlar halinde görüntülendi. Alınan biyopsinin histopatolojik incelemeleri sonucunda fibrokondroosteosarkom tanısı konuldu.

Olgu no 8'in klinik muayenesinde sol femur'da gerçekleştirilen osteosentez sonrasında ekstremitenin kullanılamadığı ve nörolojik muayenede iatrojenik siyatik sinir hasarı sekillendiği tespit edildi. V/D ve M/L pozisyonda alınan grafilerde kırık hattında pin olduğu ancak kallus ve periostal üremelerin yanı sıra malunion şekillendiği gözlendi. Pinin ekstirpasyonu sonrasında alınan tomografi görüntülerinde bilgisavarlı iyileşmesinin tamamlanmadığı ve taşkın kallus oluşumu görüldü.

Table 1. Olgulara ait klinik veriler. **Table 1.** Clinical data's of cases.

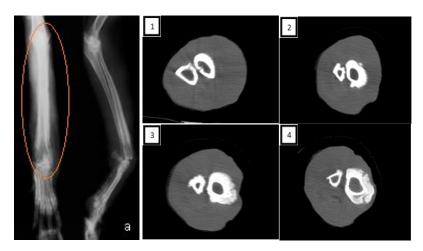
Olgu	Köpeğin			Lezyonun	
no	Irkı	Yaşı	Cinsiyeti	Lokalizasyonu	Tanı
1	Melez	2 yaş	8	Sağ femur	Kondromatöz üreme
2	Melez	3 yaş	3	Sol radius-ulna	Periostal üreme
3	Melez	8 ay	3	Sol femur	Avasküler nekroz
4	Melez	2 yaş	3	Sol art. cubiti	Ankiloz/eski kırık
5	Melez	8 ay	2	Sol femur	Yaş ağaç kırığı
6	Melez	2 yaş	3	Sol radius-ulna	Synostosis/taşkın kallus
7	Alman Çoban	1 yaş	2	Sol scapula	Fibrokonroosteosarkom
8	Melez	3 yaş	9	Sol femur	Kırık komplikasyonu/ taşkın kallus

♂: Erkek, ♀: Dişi



Şekil 1. Olgu no 1'in radyografi ve bilgisayarlı tomografi görüntüleri (a. Röntgen, b. ve c. Bilgisayarlı tomografi görüntüleri)

Figure 1. Radiography and computed tomography images of case no.1(a. Radiography, b. and c. Computed tomography images)



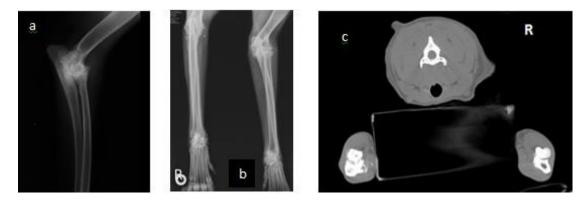
Şekil 2. Olgu 2'nin radyografi ve bilgisayarlı tomografi görüntüleri (a. Radyografi, 1.2.3. ve 4. Bilgisayarlı tomografi görüntüsü)

Figure 2. Radiography and computed tomography images of case no.2 (a. Radiography, 1.2.3. and 4. Computed tomography images)



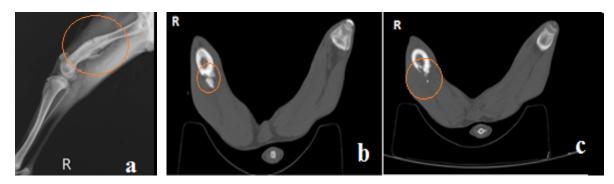
Şekil 3. Olgu 3'ün radyografi ve bilgisayarlı tomografi görüntüleri (a. Röntgen, b. ve c. Bilgisayarlı tomografi görüntüleri)

Figure 3. Radiography and computed tomography images of case no.3 (a. Radiography, b. and c. Computed tomography images)



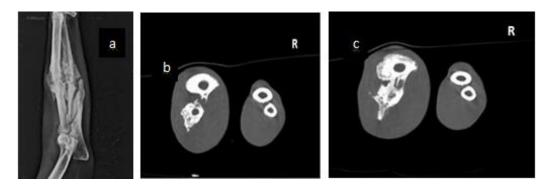
Şekil 4. Olgu no 4'ün radyografi ve bilgisayarlı tomografi görüntüleri (a. ve b. Radyografi, c. Bilgisayarlı tomografi görüntüleri)

Figure 4. Radiography and computed tomography images of case no.4 (a. and b. Radiography, c. Computed tomography images)



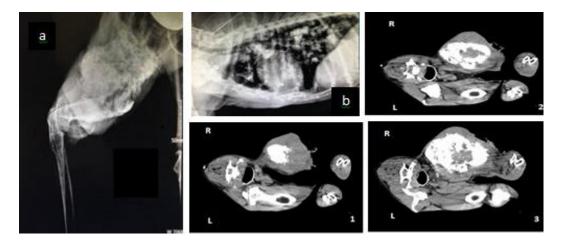
Şekil 5. Olgu no 5'in radyografi ve bilgisayarlı tomografi görüntüleri (a. Radyografi b. ve c. Bilgisayarlı tomografi görüntüleri)

Figure 5. Radiography and computed tomography images of case no.5 (a. Radiography, b. and c. Computed tomography images)



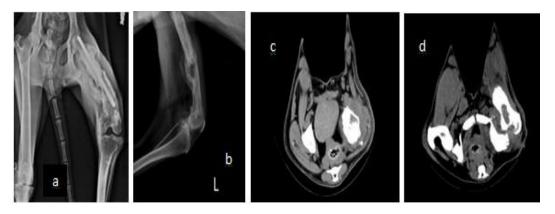
Şekil 6. Olgu no 6'nın radyografi ve bilgisayarlı tomografi görüntüleri (a. Radyografi, b. ve c. Bilgisayarlı tomografi görüntüleri)

Figure 6. Radiography and computed tomography images of case no.6 (a. Radiography, b. and c. Computed tomography images)



Şekil 7. Olgu no 7'nin radyografi ve bilgisayarlı tomografi görüntüleri (a. ve b. Radyografi, 1.2.3. Bilgisayarlı tomografi görüntüleri)

Figure 7. Radiography and computed tomography images of case no.7 (a. and b. Radiography, 1.2. and 3. Computed tomography images)



Şekil 8. Olgu no 8'in radyografi ve bilgisayarlı tomografi görüntüleri (a. ve b. Radyografi, c. ve d. Bilgisayarlı tomografi görüntüleri)

Figure 8. Radiography and computed tomography images of case no.8 (a. and b. Radiography, c. and d. Computed tomography images)

TARTIŞMA ve SONUÇ

Radyografi rutin olarak kullanılan kas ve iskelet sistemi hastalıklarında klinik muayeneyi destekleyen bir görüntülü tanı yöntemidir (Samsar ve Akın 2003). Radyografik görüntüler lezyonun sayısı, türü, lokalizasyonu ve yapısı hakkında bilgi verir (Houlton et al. 2006). Bilgisayarlı tomografinin kesitsel görüntüleme yapabilmesi, kas ve iskelet sistemi hastalıklarında tanı koyma kolaylığı sağlar (Lande et al. 2013). Çalışma olgularında klinik muayene sonrası rutin olarak radyografileri alındı.

İleri görüntülü tanı yöntemlerinden olan bilgisayarlı tomografi ile kesitsel olarak birçok görüntü alınabilmesi sonucunda süperpozisyonun önlenmesi hastalıkların tanısını yönünden önemlidir. Zaman yönünden hızlı ve kesit sayısı nedeniyle de kemik doku bütünlüğü hakkında daha ayrıntılı ve kesin bilgi verebilmektedir (Samsar ve Akın 2003). Çalışmada değerlendirilen olgularda

özellikle olgu no 6'nın kesin tanısı bilgisayarlı tomografi ile gerçekleştirilmiştir.

Radyografide opasite değişiklikleri kemik üremesi hakkında bilgi vermektedir. Çok yönlü X ışını kullanılması nedeniyle bilgisayarlı tomografi kırıkların tanısının konmasında konvansiyonel radyografiden daha etkilidir (Whatmough and Lamb 2006). Bilgisayarlı tomografide kortikal kemiğin daha iri ve yüksek dansite yapısına sahip olması, süngerimsi kemiğin trabekuler olarak görüntülenmesi kemik gösterir kompozisyonundaki farklılıkları lezyonlarda tanı kolaylığı sağlar (Cheon at al. 2018). Görüntülemede kemiğin intrameduller kanalı ve endosteal değişimleri net bir şekilde gözlenir. Bu değişimler kemik iyileşmesi ya da lezyon oluşumunda hastanın takibi sağlanır (Ketaki at al. 2012). Bilgisayarlı tomografi ile bu süreçte kesitsel görüntü alınmasıyla kemik iyileşmesinin daha ayrıntılı görüntülenmesini sağlar (Ünal 2008). Özellikle yapılan osteosentez gibi ortopedik operasyonlarda ve postoperatif dönemde kemik iyileşmesinin izlenmesini

kolaylaştırmaktadır (Houlton et al. 2006). Çalışmada olgu no 8'de kırık komplikasyonu değerlendirmesi radyografinin yanı sıra bilgisayarlı tomografinin kemikte kısa aralıklarla daha net bir görüntü sağladığı gözlenmistir.

Kemik lezyonlarında özellikle morfolojik değişimlerde düşük maliyet ve yüksek kullanılabilirlik açısından radyografi tercih edilirken geç evrede şekillenen deformasyonlar kolayca bilgisayarlı tomografi ile takip edilebilir. Yaygın olarak seyreden avaskuler nekroz ileri evrelere kadar asemptomatik seyredebilir. Bölgesel kan dolaşımının bozulması ve intraosseöz basıncın fazla olması bu süreci hızlandırır. Bu aşamalar, hastalığın radyolojik evreleri, prognozu ve tedavisi bilgisayarlı tomografi ile rutin olarak takip edilebilir (Manenti at al. 2015). Çalışmada olgu no 3'ün rutin kontrolleri ve bilgisayarlı tomografi ile görüntülenmesi sonucu avaskuler nekroz tanısı kondu.

Bu çalışmada, ileri tanı yöntemlerinden olan bilgisayarlı tomografinin veteriner klinik pratik yönünden rutin kullanıma olan katkıları ve önemi belirlendi. Değerlendirmede klinik muayene sonrasında alınan radyografilerin tanı konusunu açıklığa kavuşturduğu, ancak bilgisayarlı tomografi görüntülerinin kesitsel ve belirli aralıklarla olması komplike olgularda ayırıcı tanı için önemli olduğu gözlenmiştir.

Sonuç olarak, radyografi ile kesin tanının belirlenemediği kemik üremelerinde ileri görüntüleme yöntemlerinden olan bilgisayarlı tomografiden yararlanma olanakları varsa değerlendirilmesinin uygun olacağı kanısına varılmıştır.

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RESEARCH ARTICLE

Morphology and Volume Measurement of Bursa Fabricius by Stereology in Merlin (*Falco Columbarius*)

İsmail TÜRKMENOĞLU^{1*}, İbrahim DEMİRKAN², Aysun ÇEVİK DEMİRKAN¹, Murat Sırrı AKOSMAN¹, Mehmet Aydın AKALAN¹

¹Afyon Kocatepe University, Ahmet Necdet Sezer Campus, Faculty of Veterinary Medicine, Department of Anatomy, 03200, Afyonkarahısar ²Afyon Kocatepe University, Ahmet Necdet Sezer Campus, Faculty of Veterinary Medicine, Department of Surgery, 03200, Afyonkarahısar

ABSTRACT

Bursa fabricius (Bursa cloacalis or Bursa fabricii) is a lymphoepithelial organ of poultry and serves as the primary lymphoid organ. In addition to the development and differentiation of Bursa Fabricius B lymphocytes, immunoglobulin is also responsible for isotype change. Bursa fabricius is also seen as a secondary lymphoid organ due to the presence of a small T-lymphocyte cluster in the channel opening to cloaca, but early development of the organ is delayed. In this study, morphological and stereological examinations were performed on bursa fabricius obtained from 5 adult Merlin (Falco columbarius). Our morphological studies have shown that the bursa fabricius of merlin is located on the cloaca and that it is opened to proctodeum through a canal. We saw that it was oval shaped and had plums made by the mucosa layer in the lumen. The size of these structures was found to be $15,62 \pm 1,41$ mm, $10.14 \pm 0,66$ mm in the widest area and $0.98 \pm 0,07$ mm in the shortest area. The volume we calculated by the stereological methods was $0.190 \pm 0,008$ cm³. The morphological structure of bursa fabricius in merlin was determined and the volume was calculated by stereological method and it was thought that it might be the source of future studies in the species differentiation in poultry.

Keywords: bursa fabricius, Falco columbarius, merlin, morphology, stereology

Boz Doğanda (Falco Columbarius) Bursa Fabricius'un Morfolojisi ve Stereolojik Metod ile Hacminin Hesaplanması

ÖZ

Bursa fabricius (Bursa cloacalis veya Bursa fabricii) kanatlıların lenfoepitelial bir organı olup kanatlıların primer lenfoid organı organı olarak görev yapmaktadır. B lenfositlerinin gelişim ve farklılaşmasının yanında immunoglobulin izotip değişminden de sorumludur. Bursa fabricius cloaca'ya açılan kanalında küçük bir T lenfosit kümesi bulunduğundan dolayı aynı zamanda sekonder lenfoid organ olarak da görülmektedir ancak erken dönemde organın gelişimi gerilemektedir. Bu çalışmada 5 adet ergin bozdoğandan (*Falco columbarius*) elde edilen bursa fabricius'lar üzerinde morfolojik ve stereolojik incelemeler yapıldı. Morfolojik incelemeler sonucunda bozdoğan'larda bursa fabricius'un cloaca üzerinde bulunduğu, bir kanal vasıtasıyla proctodeum'a açıldığı, şeklinin oval olduğu ve lümeninde mukoza katmanı tarafından yapılmış plikalar bulunduğu gözlemlendi. Bu yapıların boyu 15,62 ± 1,41 mm, eni en uzun bölgede 10.14 ± 0,66 mm en kısa bölgede ise 0.98 ± 0,07 mm olarak saptandı. Stereolojik yöntemle yapılan hesaplamalarda hacmi 0.190 ± 0,008 cm³ olarak tespit edildi. Bozdoğanda bursa fabricius'un morfolojik yapısı ortaya konarak stereolojik metotla hacmi hesaplanmış olup, kanatlılarda tür ayrımında ileride yapılacak çalışmalara kaynak olabileceği düşünüldü.

Anahtar Kelimeler: bozdoğan, , bursa fabricius, Falco columbarius, morfoloji, stereoloji

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MSA: 0000-0001-6675-8840, MAA: 0000-0001-9924-2920 *Corresponding author e-mail: turkmen@aku.edu.tr

GİRİŞ

Doğangiller (Falconidae) familyasının en küçük kuş türü bozdoğandır. Bozdoğanlar Amerika, Avrupa ve Asya'nın kuzey bölgelerinde, Ortadoğu ve Orta Asya'da yaşarlar. Bu avcı kuş türü, 27-32 cm uzunluğundadır (Gooders 1995).

Bursa fabricius (Bursa cloacalis veya Bursa fabricii) kanatlıların lenfoepitelial bir organı olup ilk kez Hieronymus Fabricius tarafından açıklanmıştır. Bursa fabricius ve timus kanatlıların primer lenfoid organıdır. Bununla birlikte cloaca'nın dorsalinde yer almasından ötürü "cloacal bursa" olarak da adlandırılır (Glick 1979, Sayegh ve ark. 2000, Ratchlife 2002). Bursa fabricius cloaca'nın dorsaline kör bir kese şekillendirir ve aynı zamanda kısa bir kanal ile cloaca'nın proctodeum'una açılır (Hodges 1974, Glick 1979).

Bursa fabricius'un duvarı histolojik olarak tunica mucosa, tunica muscularis ve tunica serosa olmak üzere üç tabakadan oluşur. Tunica mucosa katmanı da lamina epitelyalis, lamina propria ve lamina submucosa katmanlarını içerir (Tanyolaç 1993, Hodges 1974, Hassa 1961, Karadağ ve ark. 2007). Tavuklarda oval bir yapıya sahip olan bursa fabricius'un tunica mucosa katmanı bursa lümenine doğru sayıları 12 ile 15 arası değişen kıvrımları yapar. Bu kıvrımların yüzeyleri follikülle alakalı epitelyum hücreleri, lenfositler, makrofajlar ve plazma hücrelerinden oluşan bursal follikülleri içerir (King ve McLelland 1984, Nickel ve ark. 1977, Glick 1979).

Bursa fabricius B lenfositlerinin gelişim ve farklılaşmasının yanında immunoglobulin izotip değişminden de sorumludur (Glick 1979, Schat ve Mayers 1991, Ratchlife 2002). Bursa fabricius epitelyumunda B hücre farklılaşma faktörleri de yer aldığından B hücre gelişiminde etkisi olduğu da bildirilmiştir (Olah ve Glick 1995, Glick 1991). Bunun yanında cloaca'ya açılan kanalında küçük bir T lenfosit kümesi bulunduğundan dolayı aynı zamanda sekonder lenfoid organ olarak da görülmektedir ancak erken dönemde organın gelişimi gerilemektedir (Sayegh ve ark. 2000, Pike ve ark. 2004, Ciriaco ve ark. 2003).

MATERYAL ve METOT

Bu araştırmada 2003 ile 2009 yılları arasında Afyon Kocatepe Üniversitesi Veteriner Fakültesi kliniklerine tedavi amacıyla getirilen, fakat kötü prognoz nedeniyle Cerrahi Anabilim Dalı tarafından ketamin (60 mg/kg) ve xylazine (6 mg/kg) kombinasyonu ile ötenazi edilerek %10'luk formaldehit solüsyonu içerisinde tespit işlemine tabi tutulmuş beş adet ergin bozdoğan kullanıldı. Kadavralar diseke edilerek bursa fabricius'lar

çıkarıldı ve Mitotoyo marka dijital kumpas ile ölçümler yapıldı. Elde edilen bulgular digital fotoğraf makinesi (Sony DSC F 717 Japon) ile fotoğraflandı. Anatomik terimlerin isimlendirmesinde Nomina Anatomica Avium (1993) referans alındı.

Morfolojik inceleme sonrasında materyaller 2 mm'lik esit aralıklarla dilimlendi (Sekil 1). Ardından histolojik doku takibi ile parafın bloklara gömülme islemlerine tabi tutuldu. Parafin bloklara gömülen örneklerden stereolojik inceleme amaçlı 5 µm kalınlığında seri kesitler alındı. Elde edilen kesitler lamlara alınarak Hematoksilen-Eozin boyama prosedürü ile boyandı ve entellan vasıtasıyla lamların üzerine lameller kapatıldı. Boyanan örneklerinden motorize tablaya (Lang MS 316) sahip Olympus MD2 ışık mikroskobuna entegre M-Shot MDX4 marka mikroskop kamerasında, M-Shot Digital Imaging System 9.3.3.1 ve Stereom I (Oğuz ve ark. 2007) yazılımları vasıtasıyla uzunluk ve hacim ölçümleri yapıldı.

Alan ölçümleri yaparken nokta uzaklığı 1 mm olan noktalı alan cetveli kullanıldı ve aşağıdaki formül kullanılarak bursa fabricius hacmi hesaplandı;

 $V = (t \cdot a/p \cdot \Sigma P) \text{ cm}^3$

t : kesit kalınlığı (~ 1.7 mm),

a/p : noktalar arası alan (1 mm x 1 mm),

 ΣP : Kesit yüzeyinde düğüme denk gelen

noktaların sayısı

BULGULAR

Bozdoğanda bursa fabricius'un cloaca ve üreterin dorsalinde bulunduğu tespit edildi. Oval şekilli ve bezsel bir yapıda olduğu gözlenen bursa fabricius'un üreterin cloaca'ya açıldığı bölüm olan urodeum'dan sonra kese şeklinde bir kanal vasıtasıvla proctodeum'a açıldığı tespit edildi. fabricius'un lümene bakan yüzünde mukoza tabakası üzerinde plikalar olduğu saptandı. Yapılan ölçümlerde bursa fabricius'un boyu 15,62 ± 1,41 mm, eni en uzun bölgede 10.14 ± 0,66 mm en kısa bölgede ise 0.98 ± 0.07 mm olarak tespit edildi.

Mikroskobik incelememizde bursa fabricius dokusunun içten dışa doğru tunica mucosa, tunica muscularis ve tunica serosa katmanlarına sahip olduğunu gözlemlendi (Şekil 2).

Tunica mucosa katmanı bir çok lenf follikülü içermekteydi (Şekil 3). Organın lümene bakan duvarında mukoza katmanının oluşturduğu birçok kıvrım olduğu saptandı. Plikaların iç yüzünde iki tip epitel hücre olduğu tespit edildi. Bunlar plikaların lümene bakan yüzlerini örten interfoliküler epitel (IFE) hücreleri ile her bir follikülün üzerini örtenleri

ise follikülle ilişkili epitel (FAE) hücreleriydi (Şekil 4). Lenf folikülleri incelendiğinde daha koyu görünen korteks ile daha açık renkte medulla olmak üzere iki kısımdan oluştuğu görüldü (Şekil 4). Korteks ve medulla'nın kortikomedullar sınır (corticomedullary border) vasıtasıyla birbirinden ayrıldığı tespit edildi (Şekil 4). Stereolojik olarak

yapılan ölçümler neticesinde 2 mm kesit aralığı ile alınan seri kesitler üzerinde noktalı alan cetveli yardımıyla yapılan ölçümlerde bozdoğanın bursa fabricius hacmi ortalama $0.190\pm0,008~\rm cm^3$ olarak hesaplandı.



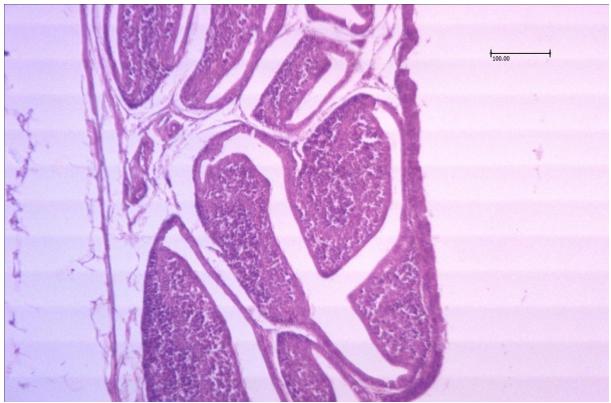
Şekil 1. Stereolojik inceleme amaçlı kesitleri alınmış bursa fabricius.

Figure 1. Bursa fabricius with cross sections for stereological examination.

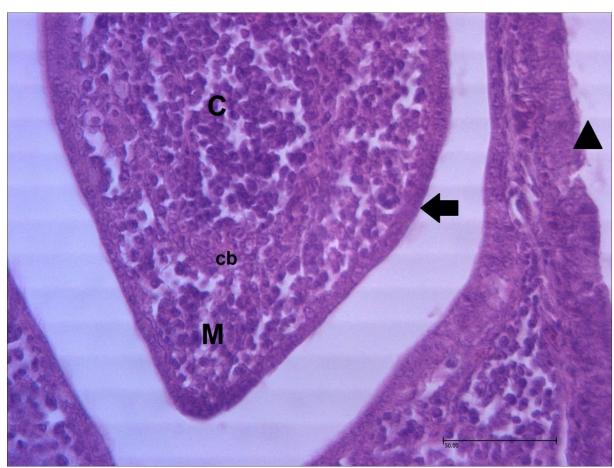


Şekil 2. Bozdoğan bursa fabricius dokusu (4X büyütme). M: tunica mucosa, *: tunica muscularis, S: tunica serosa.

Figure 2. Bursa fabricius texture of merlin (4X magnification) M: tunica mucosa, *: tunica muscularis, S: tunica serosa.



Şekil 3. Bozdoğan bursa fabricius dokusu ve lenf foliküllerinin genel görünümü (10X büyütme). **Figure 3**. General view of bursa fabricius tissue and lymph follicles of merlin (10X magnification).



Şekil 4. Lenfoid dokuda kortikomedullar sınır (cb), kortex (C), medulla (M), FAE (ok) ve IFE (okbaşı) (40X büyütme).

Figure 4. Corticomedullary border (cb), cortex (C), medulla (M) FAE (arrow) and IFE (arrow head) in the lymphoid tissue (40X magnification).

TARTIŞMA ve SONUÇ

Bursa fabricius kanatlı türlerinde genellikle benzer lokalizasyona sahiptir. Bu organ cloaca ile sacrum arasında yer almakta olup bursal kanal vasıtasıyla proctodeum'a açılmaktadır (Gülmez ve Aslan 1999, Nickel ve ark. 1977, Onyeanusi ve ark. 1993, Nagy ve Olah 2010, Glick ve Olah 1993). Bu yapının bozdoğanda da benzer lokalizasyonda olduğunu tespit edildi. Kanatlılarda bursa fabricius morfolojik olarak farklılıklar gösteriyor olmasına rağmen Nickel ve ark. (1977) bursa fabricius'un tüm kanatlı türlerinde yuvarlak ya da armut şeklinde olduğunu bildirmişlerdir. Öte yandan bazı araştırmacılar bursa fabricius'un tavuklarda yuvarlak ya da oval, kazlarda silindirik, ördekte uzamış sekum benzeri, hindide ise yine yuvarlak fakat kranial ucu sivri şekilde olduğunu da belirtmiştir (Karadağ-Sarı ve Kurtdede 2007). çalışmada bozdoğanlarda Yapılan bu fabricius'un tavuklarda bildirilenle (Nickel ve ark. 1977) benzer olarak oval şekilde olduğu tespit edildi. Kazlarda (Gülmez ve Aslan 1999), beç tavuklarında (Onyeanusi ve ark. 1993), tavuklarda (Davenport ve Allen, 1995, Olah ve ark. 1985) ve akbabada (Karadağ-Sarı ve ark. 2015) bursa fabricius'un lümene bakan iç yüzünde plikalar bulunduğu bildirilmiştir. Benzer şekilde yapılan bu çalışmada da bozdoğanlarda plikaların varlığı saptandı.

Çeşitli kanatlı türlerinde bursa fabricius üzerine gerek ışık mikroskobu gerekse elektron mikroskobu ile yapılan çalışmalarda bu yapının tunica serosa, tunica muscularis ve tunica mucosa olmak üzere üç katmanı olduğu ve mukoza katmanının birçok lenf folikülü içerdiği bildirilmiştir (Aughey ve Fyre, 2001, Lupetti ve ark. 1983, Sanchez-Refusta ve ark. 1996). Çeşitli hayvan türlerinde (Bockman ve Cooper 1973, Bockman ve ark. 1983, Davenport ve Allen 1995, Nickel ve ark. 1977, Olah ve Glick 1995, Saifuddin ve ark. 1988), güvercinde (Ciriaco ve ark. 1985), kazda (Gülmez ve Aslan 1999); ördekte (Scala ve ark. 1989), beç tavuğunda (Onyeanusi ve ark. 1993), tavukta (Romano ve ark. 1996) plikaların lümene bakan yüzlerini örten IFE hücrelerinden ve her bir folikülün üzerini örten FAE hücrelerinin varlığı bildirilmiştir. Bozdoğan bursa fabricius'unun histolojik yapısını incelediğimiz bu çalışmada da bahsi geçen hücre kısımlarının varlığı tespit edilmiştir. Bunun yanında Criaco ve ark. (2003) IFE hücrelerinin devamı niteliğinde epitelyum hücrelerinden olusan ve korteks ile medulla kısımlarını birbirinden ayıran bir kortikomedullar (corticomedullary border) olduğundan sınır bahsetmiş ancak Nagy ve ark. (2004) bıldıcınlarda bu sınırın olmadığını bildirmiştir. Yapılan çalışmada da doku örneklerinde kortikomedullar sınırın olduğu ve korteks ile medulla ayrımı tespit edildi.

Bursa fabricius hacminin hesaplanması ile ilgili yapılan deneysel bir çalışmada Aktaş ve ark. (2017)

10 haftalık kontrol grubu civcivlerde bursa fabricius hacmini ortalama 1.437 cm³ olarak bulmuştur. Bu çalışmada ise stereolojik olarak yaptığımız ölçümlerde 5 adet bozdoğanın bursa fabricius hacmini ortalama 0.190 ± 0.008 cm³ olarak tespit edildi.

Sonuç olarak bozdoğanlarda bursa fabricius'un lokalizasyonu, morfolojisi, histolojik yapısı ve hacmi ilk kez ortaya konmuştur. Çalışma bulgularının diğer kanatlı türleri üzerinde yapılacak çalışmalar için referans olabileceği düşünülmektedir.

TEŞEKKÜR

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RESEARCH ARTICLE

A Study on Gilthead Seabream (*Sparus aurata*) Fish Feed Replacement of Fishmeal at Different Rates Use of Canola Meal (*Brassica* spp.) on Growth Rate, Feed Utilisation and Digestibility

Adem KURTOĞLU¹, Ferhat ÇAĞILTAY*², İbrahim DİLER³, Mehmet SAĞLAM⁴

¹Mediterranean Fisheries Research, Production and Training Institute Directorate, Beymelek Central Unit, Demre/Antalya

²Istanbul University, Faculty of Water Sciences, Department of Aquaculture and Diseases, Laleli-Fatih/Istanbul

³Isparta Applied Sciences University, Faculty of Egirdir Fisheries, Department of Aquaculture, Isparta

⁴Ankara University, Faculty of Veterinary Medicine, Department of Clinical Sciences, Altındağ/Ankara

ABSTRACT

This study was conducted to identify optimum exchange ratio of canola meal in fish feeds instead of fishmeal, using %45 protein and %18 fat commercial control feeds and %10, %15, %20, %25 and %30 canola meal (KA) based feeds for sea breams with a starting weight of 49,86±0,009 gr by analyzing growth performance, food conversion ratio and digestibility. After 98 days of experiment, specific growth rate and relative weight gain although was statistically significantly different during 14-28 days (p<0,05), it was insignificant at the end of the trial. Food conversion ratio (FCR) was lowest for KA30 (4,54±0,1), and highest for KA10 (5,31±0,2) group, protein efficiency was highest in KA30 and lowest in KA10 group, productive protein value was highest in KA30 with 0,318±0,02 (p<0,05). Digestibility performance for crude protein and dry matter was the highest in the control group and lowest for KA10 group and for fats lowest was KA10 where highest was (p<0,05) KA30 with similar results compared to KA0 and KA15.

Keywords: Fish meal, growth performance, gilthead seabream, canola meal, digestibility, feed conversion

Çipura (*Sparus aurata*) Balık Yemlerine Balık Unu Yerine Farklı Oranlarda Kanola (*Brassica spp.*) Küspesi Kullanımının Büyüme, Yemden Yararlanma ve Sindirilebilirliliği Üzerine Bir Çalışma

ÖΖ

Bu çalışma, başlangıç ağırlığı 49,86±0,009 gr olan çipura balıklarında %45 protein ve %18 yağ içerikli kontrol yemi ile kontrol yemindeki balık unu yerine %10, %15, %20, %25 ve %30 oranlarında kanola (KA) küspesi ilaveli yemler kullanılmış ve balıkların büyüme performansı, yem değerlendirme oranı ve sindirilebilirlik düzeyi üzerine etkilerine göre kanola küspesinin optimum kullanım oranının belirlenmesi hedeflenmiştir. 98 günlük deneme sonunda gruplar arasında oransal canlı ağırlık artışı ve spesifik büyüme oranı 14-28. gün arası istatistiki olarak önemli (p<0,05) görülmesine rağmen, deneme sonunda önemsiz bulunmuştur (p>0,05). Yem değerlendirme oranı (YDO) en düşük KA30 grubunda (4,54±0,1), en yüksek ise KA10 grubunda (5,31±0,2), protein etkinlik oranı (PEO) en yüksek KA30 grubunda, en düşük KA10 grubunda, prodüktif protein değeri (PPD) KA30 grubunda (0,318±0,02) ile en yüksek değerde tespit edilmiştir. (p<0,05). Besin maddelerinin sindirilebilirlik performanslarının belirlenmesinde kuru madde ile ham proteinin sindirilebilirliği kontrol grubunda en yüksek seviyede bulunurken en düşük KA10 grubunda elde edilmiş, ham yağı en iyi sindirebilen KA30 grubu KA0 ve KA15 grubu ile benzerlik gösterirken diğer gruplardan farklı bulunmuştur (p<0,05).

Anahtar Kelimeler: Balık unu, büyüme performansı, çipura, kanola unu, sindirilebilirlik, yemden yararlanma

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ORCID ID; AK: 0000-0002-7615-7800, FC: 0000-0001-9866-083X, ID: 0000-0002-2182-2615, MS: 0000-0001-8934-8529

*Corresponding author e-mail: ferfez@istanbul.edu.tr

GİRİŞ

Balık yetiştiriciliğinde verimliliği ve maliyeti belirleyen en önemli etken yemdir. Balık yemleri tür ve büyüklüğüne bağlı olarak %25-55 arasında protein içermektedir. Balık unu, yüksek düzeyde protein içermesi, dengeli amino asit kompozisyonuna sahip olması ve balık tarafından lezzetli bulunması nedeniyle balık yemleri için vazgeçilmez bir protein kaynağıdır. Balık unu yalnızca balık yemlerinde değil kümes hayvanları, domuz ve kullanımı yasak olsa bile büyükbaş hayvan yemlerinde de az da olsa protein kaynağı olarak kullanılmaktadır (Doğan ve Bircan 2009, Yiğit ve ark., 2012).

Dünyadaki yem üreticileri balık ununa olan açığı kapatmak için bitkisel protein kaynaklarına (soya, ayçiçeği, mısır vb.) yönelmişlerdir (Yeşilayer ve ark., 2013). Alternatif protein kaynakları arayışında hayvansal kökenli proteinler (et unu, et-kemik unu, tavuk unu, tüy unu vb.), tek hücre proteinleri (mayalar, mantarlar, bakteriler, algler), ve bitkisel kökenli protein kaynakları ön plana cıkmaktadır.

vemlerinde kullanılan Balık bitkisel protein kaynaklarının başında soya, ayçiçeği tohumu, pamuk tohumu ve kolza küspeleri ile mısır gluten unu gelmektedir. Araştırıcılar tilapia yavrularında balık unu yerine soya ununu, sazan balıklarında protein kaynağı olarak ayçiçeği tohumu küspesini, çipura balıklarında soya ve kolza protein konsantrelerini, Nil tilapyası (Oreochromis niloticus) larvalarında dört bitkisel protein kaynağını soya unu, kanola unu, ayçiçeği unu ve pamuk ununun kullanım olanaklarını karşılaştırmalı olarak denemişlerdir (Kissil ve ark., 2000, De Souza ve ark., 2004, Uysal ve Bekcan 2006, Demir ve ark., 2010).

Bu çalışmada da, özellikle Akdeniz ülkelerinde yaygın olarak yetiştiriciliği yapılan çipura (*Sparus aurata*) balıklarının yemlerinde balık unu yerine besin maddeleri bakımından yaklaşık değer içeren bitkisel hammadde kaynaklarından kanola küspesinin kullanılabilirliğinin belirlenmesi ve büyüme, yemden yararlanma ve besin madde sindirilebilirliği üzerine etkilerinin araştırılması amaçlanmıştır.

MATERYAL ve **METOD**

İki aşamalı olarak planlanan çalışmanın besleme ve sindirim denemeleri, Akdeniz Su Ürünleri Araştırma Üretme ve Eğitim Enstitüsü Müdürlüğü (AKSAM) Beymelek Birimi Araştırma Ünitesi'nde gerçekleştirilmiştir. Besleme çalışmaları 98 gün olarak planlanmıştır.

Deneme materyali olarak, 9 aylık ve ortalama 50 gr ağırlığındaki çipura (*Sparus aurata*) balıkları kullanılmıştır. Aynı ağırlık grubuna ait balıkların tartımı tek tek yapılarak her bir deneme tankına tesadüfi parselleme yöntemi ile 35'şer adet stoklanmış ve toplam 630 adet balık kullanılmıştır.

Denemede kullanılan yem hammaddelerinin besin madde içerikleri AKSAM Kepez Birimindeki yem laboratuarında analiz edilerek bu bilgiler ışığında deneme gruplarının yem rasyonları hazırlanmıştır (Tablo.1). Belirlenen miktarlar doğrultusunda deneme yemleri %45 ham protein ve %18 yağ içerikli olarak hazırlanmıştır. 6 deneme yemine balık ununun yerine %0, %10, %15, %20, %25 ve %30'u oranında kanola küspesi ilave edilmiştir.

Deneme başında ve deneme süresince her 15 günlük ara ile balıkların canlı ağırlıkları 0,1 g. hassasiyetli bir terazi yardımı ile belirlenmiştir

Büyüme; oransal canlı ağırlık artışı ve spesifik büyüme oranı ile değerlendirilmiştir. Yemin değerlendirme performansı; yem değerlendirme oranı (YDO), protein etkinlik oranı (PEO) ve prodüktif protein değeri (PPD) ile belirlenirken, sindirilebilirlik kuru madde, protein ve yağ sindirim katsayıları ile değerlendirilmiştir.

Denemede elde edilen verilerin istatistiki değerlendirilmesinde varyans analizi (ANOVA) ile grup ortalamalarının karşılaştırılmasında Student's T yapılmıştır. amaçla testi Bu JMP paket programlarından yararlanılmış, istatistikî karşılaştırmalarda biyolojik araştırmalar için yaygın olarak kullanılan önem seviyesi (P=0,05) seçilmiştir.

BULGULAR

Balık unu proteininin %10, %15, %20, %25 ve %30'unu karşılayacak şekilde kanola küspesi kullanımının çipura büyümesine etkisi Tablo 2' de verilmiştir.

Canlı ağırlık artış ortalamaları için yapılan varyans analizi sonuçlarına göre; 14. ve 28. günlerde yapılan ölçümlerde gruplar arasında fark anlamlıdır (p<0,05). 42. günden itibaren 56., 70., 84. ve 98. günlerde yapılan ölçümlerin varyans analizi sonuçlarına göre ise gruplar arasında önemli bir farklılık görülmemiştir (p>0,05).

Spesifik Büyüme Oranı (SBO)

Balık unu proteininin yerine farklı oranlarda kanola küspesi proteini ilavesine ilişkin deneme gruplarının dönemlere göre spesifik büyüme oranları Tablo 3'de verilmiştir.

Muameleler arası dönemsel spesifik büyüme büyüme oranları, ilk iki hafta sonu hariç, hiçbir dönem istatistiksel olarak önemli olmamıştır (p>005). 14. günde spesifik büyümenin en yüksek olduğu KA10 grubu, KA0 ve KA15 grupları ile benzer, diğer

gruplardan önemli derecede daha yüksek bulunmuştur (p<0,05).

Yem Değerlendirme Parametreleri

Denemede kullanılan yemlerin yem değerlendirme oranı, protein etkinlik oranı ve prodüktif protein değeri Tablo 4'da verilmiştir.

Yem değerlendirme oranı (YDO)

Deneme sonu yem değerlendirme oranı incelendiğinde; verilen yemin en iyi değerlendirildiği grubun KA30 grubu yemi ile beslenen muamele grubu olduğu ve bu grubun KA0, KA20 ve KA25 grubu ile benzer iken KA10 ve KA15 grupları ile farklı olduğu belirlenmiştir (p<0,05).

Grupların protein etkinlik oranı karşılaştırıldığında, en yüksek protein etkinlik oranı KA30 grubunda $(0,52\pm0,01)$ bulunmuştur. Bu grubu KA25 $(0,5\pm0,02)$, KA0 $(0,49\pm0,05)$, KA20 $(0,48\pm0,03)$, KA15 $(0,47\pm0,03)$ ve KA10 $(0,44\pm0,02)$ takip etmiştir.

Protein etkinlik oranının en iyi olduğu KA30 grubu KA25, KA20, KA0 ve KA15, gruplarıyla benzer, KA10 grubu ile farklı bulunmuştur (P<0,05). En düşük protein etkinlik oranının bulunduğu KA10

grubu KA0, KA15 ve KA20 gruplarıyla benzer, diğer gruplardan farklı bulunmuştur (p<0,05).

Besin Maddelerinin Sindirilebilirliği

İki aşamalı olarak yürütülen denemenin ikinci aşamasında besin maddelerinin sindirilebilirlik katsayıları (kuru madde, ham protein ve ham yağ) belirlenmis ve Tablo 5'de verilmistir.

Çipura balık yemlerinde balık unu proteini yerine farklı oranlarda kanola küspesi proteini kullanımının deneme sonu itibari ile kuru madde sindirilebilirliği değerlendirilmiş ve en iyi sonucu %54,4±2,7 ile kontrol grubunda bulunmuş, bu grup KA15 ve KA20 grubu ile benzer diğer gruplar ile farklı bulunmuştur (p<0,05).

yemlerin Deneme sonunda ham proteinin sindirilebilirliği incelenmiş sindirilebilirlik ve değerlerinin %88,0 ile %95,7 arasında değiştiği, gruplar arası ilişkinin anlamlı olduğu belirlenmiştir. Yapılan istatistiksel analiz sonucunda en iyi protein sindirilebilirliği KA0 grubunda tespit edilmiş ve bu grubun diğer gruplardan farklı olduğu belirlenmiştir. Ayrıca en düşük protein sindirilebilirliğine sahip KA10 grubu diğer gruplardan farklı bulunmuştur.

Table 1. Deneme gruplarının rasyon yapısı **Table 1.** The ration structure of experimental groups

		45/18 Protein/ Yağ oranlı rasyon Kullanım Oranları (%)							
Hammaddeler	KA0	KA10	KA15	KA20	KA25	KA30			
Balık unu a	40	36	34	32	30	28			
Kanola küspesi unu ^b	0	8,68	13,02	17,37	21,71	26,05			
Soya küspesi unu ^b	24	24	24	24	24	24			
Buğday gluten unu b	5	5	5	5	5	5			
Mısır nişastası b	15	10,32	7,98	5,63	3,29	0,95			
Balık yağı ^b	13,5	13,5	13,5	13,5	13,5	13,5			
Vitamin ^c	1	1	1	1	1	1			
Mineral ^d	0,5	0,5	0,5	0,5	0,5	0,5			
C vitamini ^b	0,2	0,2	0,2	0,2	0,2	0,2			
Bağlayıcı ^b	0,4	0,4	0,4	0,4	0,4	0,4			
Antioksidant b	0,1	0,1	0,1	0,1	0,1	0,1			
Metiyonin ^b	0,1	0,1	0,1	0,1	0,1	0,1			
Lysin ^b	0,1	0,1	0,1	0,1	0,1	0,1			
Kolin	0,1	0,1	0,1	0,1	0,1	0,1			

a Kobyalar Grup (Trabzon), b Korkuteli Yem (Antalya)

c Vitamin karışımı: 1 kg.'da 18.000 IU A, 2.000 IU D3, 200IU E, 12 mg. K, 150 mg. C, 30 mg.B2, 20 mg.B1, 0.05 mg. B12, 20 mg. pyridoxine, 10 mg. pantotenik asit, 220 mg. niacin, 210 mg. inositol, 5 mg. folik asit, 0.5 mg. biotin, ve 2.000 mg. kolin bulunmaktadır.

d Mineral karışımı: 1 kg.' da 70 mg. çinko, 60 mg. mangenez, 60 mg. magnezyum, 4 mg. demir, 2 mg. bakır, 1.5 mg. iyot, 0.5 mg. kobalt, 0.05 mg. selenyum bulunmaktadır.

Tablo 2. Deneme gruplarının dönemlere göre canlı ağırlık ortalamaları

Table 2. The live weight averages of the experimental groups according to the periods

Dönemler			Dener	me Grupları (X	±SS)	·
(gün)	KA0	KA10	KA15	KA20	KA25	KA30
0	49,87±0,01	49,86±0,01	49,86±0.01	49,86±0.01	49,86±0.01	49,86±0.01
14	$53,00\pm0.69^{\mathrm{ab}}$	$53,16\pm0,28^{a}$	$52,99\pm0,46^{ab}$	52,09±0,07°	$52,46\pm0,09^{abc}$	$52,32\pm0,40^{bc}$
28	56,15±1,08a	$55,74\pm0,37^{ab}$	55,68±0,91 ^{ab}	54,71±0,66 ^b	$54,94\pm0,47^{ab}$	$55,04\pm0,90^{ab}$
42	59,66±1,35	$58,89\pm0,18$	$58,38\pm1,04$	57,88±0,57	58,13±0,36	58,48±1,63
56	63,11±0,63	62,31±0,97	62,31±0,80	62,31±0,91	62,31±0,79	62,31±2,03
70	68,90±0,56	67,19±1,59	67,28±1,32	66,42±1,22	66,90±1,53	$68,08\pm2,75$
84	72,69±1,19	71,15±1,52	70,12±2,32	68,43±1,65	69,82±1,72	69,78±4,49
98	79,21±2,25	77,29±2,26	75,84±2,84	76,46±2,66	77,15±1,99	$77,89\pm3,34$

Aynı satırda farklı harf olan ortalamalar arasındaki fark önemlidir (p<0,05)

 $\textbf{Tablo 3.} \ \ \text{Deneme gruplarının dönemlere göre canlı ağırlıkça spesifik büyüme oranları, } (X\pm SS)$

Table 3. Specific growth rates of live groups by periods in experimental groups, $(X \pm SS)$

Deneme Grupları		Dönemler (gün)									
Deneme Grupian	14	28	42	56	70	84	98				
KA0	$0,43\pm0,09^{abc}$	0,42±0,07ª	0,42±0,05ª	0,42±0,02ª	0,46±0,01ª	$0,44\pm0,02^{a}$	0,47±0,03a				
KA10	$0,45\pm0,04^{a}$	$0,39\pm0,02^{a}$	$0,39\pm0,01^{a}$	$0,397\pm0,03^{a}$	$0,42\pm0,03^{a}$	$0,42\pm0,03^{a}$	$0,44\pm0,03^{a}$				
KA15	$0,43\pm0,06^{ab}$	$0,39\pm0,06^{a}$	$0,37\pm0,04^{a}$	$0,41\pm0,02^{a}$	$0,42\pm0,03^{a}$	$0,40\pm0,04^{a}$	$0,42\pm0,04^{a}$				
KA20	$0,31\pm0,01^{d}$	$0,33\pm0,04^{a}$	$0,35\pm0,02^{a}$	$0,36\pm0,03^{a}$	$0,40\pm0,03^{a}$	$0,37\pm0,03^{a}$	$0,43\pm0,04^{a}$				
KA25	$0,36\pm0,01^{\text{bcd}}$	$0,34\pm0,03^{a}$	$0,36\pm0,01^{a}$	$0,38\pm0,02^{a}$	$0,41\pm0,03^{a}$	$0,40\pm0,03^{a}$	$0,44\pm0,03^{a}$				
KA30	$0,34\pm0,05^{cd}$	$0,35\pm0,06^{a}$	$0,37\pm0,07^{a}$	$0,40\pm0,06^{a}$	$0,44\pm0,06^{a}$	$0,39\pm0,08^{a}$	$0,45\pm0,04^{a}$				

Aynı satırda farklı harf olan ortalamalar arasındaki fark önemlidir (p<0,05)

Tablo 4. Deneme gruplarının yem performans parametreleri, (X±SS)

Table 4. Feed performance parameters of experimental groups, $(X \pm SS)$

Parametre	Deneme Grupları									
	KA0	KA10	KA15	KA20	KA25	KA30				
YDO	4,80±0,5ab	$5,31\pm0,2^a$	$5,27\pm0,3^{a}$	$5,08\pm0,3^{ab}$	4,60±0,2 ^b	4,54±0,1 ^b				
PEO	$0,49\pm0,05^{ab}$	$0,44\pm0,02^{b}$	0,47±0,03ab	$0,48\pm0,03^{ab}$	$0,51\pm0,02^{a}$	0,52±0,01 ^a				
PPD	$0,253\pm0,08^{abc}$	$0,317\pm0,06^{ab}$	0,196±0,04°	0,203±0,09bc	0,184±0,07°	$0,318\pm0,02^{a}$				

Aynı satırda farklı harf olan ortalamalar arasındaki fark önemlidir (p<0,05)

Tablo 5. Deneme sonu itibari ile besin maddelerinin sindirim oranları (%), $(X\pm SS)$ **Table 5.** At the end of the trial the digestibility rates of nutrients (%), $(X\pm SS)$

	Besin Maddeleri						
Deneme Grupları	Kuru Madde	Ham Protein	Ham Yağ				
KA0	54,4±2,7a	95,7±1,4 ^a	91,5±3,2 ^{ab}				
KA10	41,9±2,7 ^b	88,0±0,3 ^d	83,4±1,7 ^d				
KA15	53,0±4,1ª	91,2±0,2 ^{bc}	89,4±0,6ab				
KA20	$54,0\pm 4,0^a$	92,3±0,01 ^b	87,9±0,1 ^{bc}				
KA25	44,8±4,4 ^b	90,2±0,6°	85,0±0,7 ^{cd}				
KA30	45,8±2,3 ^b	89,9±0,4°	$92,9\pm0,1^{a}$				

Aynı satırda farklı harf olan ortalamalar arasındaki fark önemlidir (p<0,05)

TARTIŞMA

Bu çalışmada %45 ham protein ve %18 yağ içerecek şekilde balık ununa dayalı olarak hazırlanan kontrol yemi ve bu yemdeki balık unu proteininin %10, %15, %20, %25 ve %30'unu karşılayacak şekilde kanola küspesi proteini ilave edilerek hazırlanan deneme yemleriyle 98 gün süre ile beslenen çipura (*S. aurata*) balıklarının büyüme, yemden yararlanma, yem tüketimi, protein etkinlik oranı, prodüktif protein değeri ve yasama oranı incelenmistir.

Bir araştırıcı grubu, balık yemlerine kanola unu eklenmesinin genellikle düşük büyüme ile sonuçlandığını ve düşük büyümenin olası sebeplerini;

- 1. Düşük amino asit dengesi, tanen varlığı ve metabolik enerji içeriğinin düşük olması;
- 2. Yüksek lif içeriği, bağırsak içeriğinin geçiş süresini azaltabildiğini ve protein ve enerji sindirilebilirliğini düsürdüğünü,
- 3. Glukozinolatların seviyesinin yüksek olması olarak bildirmişlerdir (Webster ve ark., 1997).

Başka araştırmacılar ise, balık beslemede kanola (*Brassica sp.*) kullanımı ile yapmış oldukları çalışmada kanola küspesinin belli oranlara kadar balık yemlerinde olumsuz bir etkisi olmaksızın kullanılabileceğini belirtmişlerdir (Ölmez ve Aybal 2006).

Bununla birlikte, ayrıca aynalı sazan (*Cyprinus carpio*) yavrularının %22 oranına kadar kanola küspesi ile %14.30 soya küspesinin kombine olarak kullanılabileceğini, melek balığı (*P. scalare*) yavru yemlerinde ise, herhangi bir olumsuz etkisi olmaksızın balık unu proteininin %16'sı yerine kanola küspesi kullanılabileceğini, Japon levrek balığı (*L.l. japonicus*) yemlerinde büyümeyi etkilemeksizin %20'ye kadar kanola unu kullanılabileceğini bildirmiş olmalarına

rağmen, yapılan bu çalışmalarda en iyi büyümeyi kontrol gurubunda bulduklarını bildiren (Erdoğan ve Ölmez 2009, Cheng ve ark., 2010, Yiğit ve ark., 2013) ve bu bakımdan bizim çalışmamız ile benzer sonuç elde eden çalışmalar da mevcuttur.

Bu çalışmada çipura balıklarının verilen yemi en iyi değerlendiren grubun 4,54±0,1 ile KA30 grubu ile beslenen balıklarda olduğu belirlenmiştir. Yapılan analiz sonucu en ivi vem değerlendirme oranına sahip KA30 gurubu KA25, KA20 ve KA0 gurupları ile benzer (P>0,05), en düşük yem değerlendirme oranına sahip KA10 gurubu KA15, KA20 ve KA0 gurupları ile benzer diğer gruplardan bulunmuştur (P<0,05). Bu sonuçlar, 50 g ağırlığındaki çipura (Sparus aurata) balıklarında, melek balığı (Pterophyllum scalare) vavrularında, alabalıklarında (Oncorhynchus mykiss) ve kırmızı mercan balıklarında (Pagrus major) yapılan çalışmalar ile benzerlik göstermektedir (Kissil ve ark., 2000, Glencross ve ark., 2004, Erdoğan 2007, Drew ve ark.,

50 g ağırlığındaki çipura (*Sparus aurata*) yemlerinde balık unu proteini yerine kanola protein konsantresini denemişler ve 56 günlük deneme sonunda en iyi yem değerlendirme oranını 0,95 ile %60 kanola protein konsantresi içeren gurupta olduğunu ve bu gurubu 0,97 ile kontrol grubu, 0,98 ile %30 kanola protein konsantresi içeren gurubun takip ettiğini belirterek aralarındaki ilişkinin benzer olduğunu bulmuşlardır (Kissil ve ark., 2000). Bu bizim çalışmamız ile paralel sonuç elde etmislerdir.

Bu çalışmada deneme gruplarında protein etkinlik oranı hesaplanmış ve en yüksek protein etkinlik oranı KA30 grubunda (0,52±0,01) olarak bulunmuştur. Bulgularımıza paralel olarak; melek balıkları ile kanal yayın balıklarında yapılan bazı çalışmalarda da benzer sonuçlar elde etmişlerdir (Webster ve ark., 1997, Erdoğan 2007). Deneme gruplarının prodüktif

protein değeri karşılaştırıldığında, en yüksek prodüktif protein değeri KA30 grubunda (0,318±0,02) olarak bulunmuştur.

Yine, Kissil ve ark., (2000) 50 g ağırlığındaki çipura (S. aurata) balıklarında kanola protein konsantresi denenmiş ve %30 kanola protein konsantresi içerikli yem ile beslenen grubun prodüktif protein değerinin diğer gruplardan daha yüksek olduğunu bulmuşlardır. Denemede ham yağ sindirilebilirliği en yüksek KA30 muamele grubunda bulunmuş ve bu grubun kontrol grubu (%91,5) ve %15 (%89,4) kanola unu ihtiva eden gruplar ile benzer olduğu tespit edilmiştir. Bu bulgular melek balıklarında yapılmış olan deneme ile benzer, japon levrek balığı ve tilapya balıklarında yapılmış olunan denemeler ile farklı bulunmuştur. (Erdoğan 2007, Aybal 2007, Cheng ve ark., 2010).

SONUÇ

Yapılan bu çalışma sonucuna göre çipura balık balık unu proteini yerine küspesinin kullanımında büyüme parametreleri açısından herhangi bir olumsuz ve farklılık gözlenmemektedir. Buna ilaveten diğer besinsel parametreler açısından ise olumlu sonuçlar vermektedir. Ekonomik analiz açısından değerlendirildiğinde ise yoğun üretim koşullarında çipura balık yemlerine balık unu proteininin %30'u yerine kanola proteini kullanımı ile yaklaşık %20 oranında önemli bir ölçüde ekonomik fayda elde edilebileceği söylenebilir. Tüm bunların yanı sıra bizim çalışmamız ve daha önce yapılan benzer çalışmalar ortaya koymuştur ki kanola ununun balık yemlerinde alternatif hammadde olarak kullanımına ilişkin daha fazla AR-GE ve bilimsel çalışmalara ihtiyaç bulunmaktadır.

TEŞEKKÜR

Bu çalışma, aynı isimli yüksek lisans tezinin bir kısmından hazırlanmıştır.

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RESEARCH ARTICLE

Evaluation of Aflatoxin M₁ Presence in Raw Milk and Some Cheese Types Consumed in Kars

Aksem AKSOY*1, Çiğdem SEZER2

¹Kafkas University, Faculty of Engineering and Architecture, Department of Food Engineering, Kars ²Kafkas University, Faculty of Veterinary Medicine, Department of Food Safety and Public Health, Kars

ABSTRACT

This study was carried out to evaluate aflatoxin M₁ (AFM₁) contamination in the samples of raw cow milk, moldy chechil, kashar and gruyere cheese which are offered to consumers in Kars city of Turkey. For this purpose, a total of 200 samples, including 50 raw milk, 50 moldy chechil cheese, 50 kashar cheese and 50 gruyere cheese were examined. AFM₁ levels in the samples were determined by competitive ELISA method. Of the 50 raw cow milk samples analyzed, 22 (44%) were found to be below the AFM₁ detection limit (<5.00 ng / L) while 28 (56%) had AFM₁ at different levels. AFM₁ levels in the samples of moldy chechil cheese, kashar cheese and gruyere cheese were 42 (84%), 36 (72%), and 16 (32%), respectively, which were below the AFM₁ detection limit (<112.50 ng/kg) while 8 (16%), 18 (36%) and 34 (68%) of them had AFM₁ in terms of positive results. All samples of raw milk and cheese were found to be in compliance with the limits permitted in the Turkish Food Codex and the European Commission in terms of AFM₁. According to these results, it is pleasing for the public health that the level of AFM₁ in raw milk and cheese samples is below the legal limits. However, the levels of AFM₁ contamination in the samples suggested that it could be a potential risk in terms of public health and precaution is needed to be taken for the prevention of the contamination.

Keywords: AFM₁, Raw milk, Cheese, ELISA

Kars İlinde Tüketime Sunulan Çiğ Süt ve Bazı Peynir Çeşitlerinde Aflatoksin M₁ Varlığının Değerlendirilmesi

ÖΖ

Bu araştırma, Kars ilinde tüketime sunulan çiğ inek sütü, küflenmiş çeçil, kaşar ve gravyer peyniri örneklerinde aflatoksin M₁ (AFM₁) kontaminasyonunu değerlendirmek amacıyla yapılmıştır. Bu amaçla, 50 adet çiğ süt, 50 adet küflü çeçil peyniri, 50 adet kaşar peyniri ve 50 adet gravyer peyniri olmak üzere toplam 200 örnek incelenmiştir. Örneklerdeki AFM₁ düzeyi kompetitif ELISA yöntemiyle belirlenmiştir. Analize alınan 50 çiğ inek sütü örneğinin 22'sinde (%44) AFM₁ tespit limitinin (<5.00 ng/L) altında iken 28'inde ise (%56) farklı düzeylerde AFM₁ belirlenmiştir. Küflü çeçil peyniri, kaşar peyniri ve gravyer peyniri örneklerinin ise sırasıyla 42 (%84), 36 (%72) ve 16 (%32)'sında AFM₁ seviyesi tespit limitinin (<112.50 ng/kg) altında iken 8 (%16), 18 (%36) ve 34 (%68)'ünde ise AFM₁ yönünden pozitif sonuç elde edilmiştir. Çiğ süt ve peynir örneklerinin tamamının AFM₁ açısından Türk Gıda Kodeksi ve Avrupa Komisyonun'ca izin verilen limitlere uygun olduğu belirlenmiştir. Elde edilen sonuçlara göre çiğ süt ve peynir örneklerinde AFM₁ düzeyinin yasal limitlerin altında bulunması halk sağlığı açısından sevindiricidir. Ancak örneklerdeki AFM₁ kontaminasyon düzeyleri halk sağlığı açısından risk oluşturma potansiyelinin bulunduğunu ve kontaminasyonun önlenmesi için tedbir alınması gerekliliğini ortaya koymaktadır.

Anahtar Kelime: AFM₁, Çiğ süt, Peynir, ELISA

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ORCID ID; AA: 0000-0001-8523-1307, ÇS: 0000-0002-9722-3280

*Corresponding author e-mail: aksemaksoy@hotmail.com

GİRİŞ

Süt oldukça besleyici bir gıda olup büyüme, gelişme ve insan sağlığının sürdürülmesi için gerekli makro ve mikro besin öğelerinin önemli bir kaynağıdır. Ancak süt aynı zamanda hastalıklara neden olabilen gıda kontaminantı bir doğal kaynağı olabilmektedir. Süt ve süt ürünlerindeki aflatoksin M₁ (AFM₁) varlığının özellikle gelişmekte olan ülkeler başta olmak üzere dünya çapında önemli bir sorun oluşturmakta olduğu vurgulanmaktadır (Igbal ve ark. 2015). Aflatoksinler çoğunlukla A. flavus, A. parasiticus ve nadirende A. nominus tarafından üretilmekte olup bitkilerin ve bitkisel ürünlerin kontaminasyonuna neden olmaktadır. Aflatoksinler gıdalarda ve yemlerde bulunan toksik fungal metabolitlerdir. Ruminantlar laktasyon döneminde AFB₁ ile kontamine yemlerle beslendiklerinde, toksin sindirim sisteminde metabolize olarak sütte AFM₁ oluşumuna neden olmaktadır (Prandini ve ark. 2009). Ciddi karaciğer hasarı, tümör oluşumu, sistemin baskılanması, mutajenik, immun teratojenik ve karsinojenik etkilere vol acmaları nedeniyle insan ve hayvan sağlığı üzerinde son etkive derece olumsuz sahip bilesiklerdir (Campagnollo ve ark. 2016).

Süt ve süt ürünlerindeki AFM_1 pastörizasyon, sterilizasyon, fermantasyon, soğukta depolama, dondurma, konsantre etme veya kurutma gibi islemlerden çok düsük oranda etkilenmektedir (Ryser 2001). AFM₁'i kontrol etmek için, hayvansal kullanım amaçlı tarımsal ürünlerde küf gelişimini ve aflatoksin B₁ (AFB₁) önleyerek olusumunu süt sığırlarının beslenmelerinde AFB₁ kontaminasyonunu azaltmak gerekmektedir. Mısır ve mısır bazlı ürünler AFB₁ açısından en fazla kontaminasyona uğrayan yem maddelerindendir. Mısır silajı üretimi sırasında aflatoksin oluşumu çoğunlukla hasat zamanı, döllenme, sulama, haşere kontrolü, silaj nemi ve depolama uygulamaları gibi faktörlerden etkilenmektedir. Bu nedenle muhafaza nemi, çekirdek mekaniksel hasarı, tahıl temizleme uvgulamaları ve muhafaza sıcaklığının dikkatle kontrol edilmesi gerekmektedir (Prandini ve ark. 2009). Süt ve süt ürünlerinde AFM₁'in mevcudiyeti bu ürünleri daha çok tüketen yetiskinler ve özellikle çocuklar için önemlidir. Bebek ve çocuklar yetişkinlere göre mikotoksinlerin olumsuz etkilerine daha duyarlıdır (Prandini ve ark. 2009, Iqbal ve ark. 2015). AFB₁ ve bunun canlı organizmadaki metabolizma ürünü olan AFM1 insan sağlığı üzerindeki olumsuz etkileri nedeniyle bilim dünyasında güncelliğini korumaya devam etmektedir (Deligöz ve Bilge 2017).

Bu araştırmada Kars ilinde satışa sunulan çiğ süt, Kars çeçil peyniri, Kars kaşarı ve gravyer peynirinde AFM₁ düzeyinin belirlenmesi ve halk sağlığı için olası risklerinin değerlendirilmesi amaçlanmıştır.

MATERYAL ve METOD

Gıda Örnekleri

Bu çalışmada 50 adet çiğ süt, 50 adet Kars küflü çeçil peyniri, 50 adet Kars kaşar peyniri, 50 adet Kars gravyer peyniri olmak üzere toplam 200 örnek analiz edilmiştir. Gıda örnekleri 2018 Mayıs-Haziran aylarında Kars'ta bulunan 50 farklı satış noktasından rastgele temin edilmiştir. Steril numune poşetlerine (en az 200g/ml) alınan örnekler laboratuara getirilerek analiz gününe kadar -20°C'de muhafaza edilmiştir.

Süt örneklerinin Hazırlanması

Örnekler üretici firmanın talimatlarına göre hazırlanmıştır. Süt örnekleri yağın giderilmesi amacıyla soğutmalı santrifüjde 10°C'de, 3500 devirde, 10 dk santrifüj edilmiştir. Santrifüjleme işleminden sonra üst krema tabakası pastör pipeti yardımı ile tamamen uzaklaştırılmıştır. Analizde her bir kuyucuk için 100 µl yağsız süt kullanılmıştır.

Peynir Örneklerinin Hazırlanması

Dış yüzey (kabuk kısmı) olmaksızın peynir örnekleri öğütülmüştür. Homojenizatör'de 20 ml % 70 metanol ile 5 g öğütülmüş peynir homojenize edilmiştir. Numunenin tamamı 50 ml'lik bir tüpe aktarılarak 30 dk boyunca 50°C'de inkübe edilmiştir. İnkübasyon süresince 5 kez kuvvetlice çalkalama işlemi yapılmıştır. Yağın giderilmesi için süspansiyon 10°C'de, 3000 devirde, 10 dk santrifüj işlemine tabi tutulmuştur. 10 ml'lik bir santrifüj tüpüne 2 ml sulu fazdan (yağsız) aktarılarak üzerine 2 ml hekzan eklenerek 10 sn karıştırılmıştır. Faz ayrımı için 10°C'de, 3000 devirde, 10 dk santrifüj edilmiştir. Alt sulu fazın bir miktarı 1:5 oranında seyreltme çözeltisi ile seyreltilmiştir. Analizde her bir kuyucuk için 100 μl kullanılmıştır.

ELISA Testinin Uygulanması ve Sonuçların Değerlendirilmesi

Süt ve peynir örneklerinde AFM1 varlığı ve seviyesi kompetitif ELISA vöntemi ile Ridascreen® Aflatoxin M₁ test kiti (R1121) kullanılarak belirlenmiştir. Test için gerekli çözeltiler üretici firmanın önerileri doğrultusunda hazırlanmış ve tüm reaktiflerin ısısı oda sıcaklığına getirilmiştir. Standart solüsyonlar (0, 5, 10, 20, 40, 80 ng/L/ ng/kg) ile hazırlanan süt ve peynir örnekleri için yeterli sayıda kuyucuk, mikropleyte yerleştirilmiştir. Her bir kuyucuğa 100 µl antikor solüsyonu eklenerek hafifçe karıştırıldıktan sonra oda sıcaklığında 15 dakika boyunca inkübe edilmistir. Kuyucuklardaki S1V1 dökülerek tamamen uzaklaştırılmıştır. Kuyucuklara 250 µl yıkama çözeltisi eklenmiş ve aynı şekilde bu sıvı da uzaklaştırılmıştır. Yıkama işlemi iki kez daha tekrar edilmiştir. Numaralandırılmış kuyucuklara 100'er ul

standart solüsyonlardan ve hazırlanmış örneklerden eklendikten sonra mikropleyt hafifçe karıştırılarak oda sıcaklığında ve karanlıkta 30 dakika inkübe edilmiştir. Kuyucuklardaki sıvı dökülerek tamamen uzaklaştırılmıştır. Kuyucuklara 250 µl yıkama çözeltisi eklenmiş ve aynı şekilde kuyucuktan bu sıvı da uzaklaştırılmıştır. Yıkama işlemi iki kez tekrarlanmıştır. Her bir kuyucuğa 100 µl enzim konjugat eklenmis ve mikropleyt karıştırılarak oda sıcaklığında ve karanlıkta 15 dakika inkübe edilmiştir. Kuyucuklardaki sıvı tamamen uzaklaştırıldıktan sonra kuyucuklara 250 ul yıkama çözeltisi eklenmiş ve aynı şekilde kuyucuktan bu sıvı da uzaklaştırılmıştır. Yıkama islemi iki kez tekrarlanmıştır. Her bir kuyucuğa 100 ul substrat/kromojen cözeltisinden eklenerek mikropleyt hafifçe karıştırılmıştır. Oda ısısında ve dakika karanlıkta 15 bekletildikten reaksiyonun durdurulması amacıyla her kuyucuğa 100 µl stop solüsyonu eklenerek mikropleyt hafifce karıştırılmıştır. Absorbans değerleri ELISA okuyucuda (Mikropleyt okuyucu, BioTek ELx800) 450 nm de 10 dk içinde ölçülmüştür (Ridascreen R1121, 2017). Elde edilen sonuçların değerlendirilmesinde, üretici firmanın kit ile birlikte sağladığı Ridasoft Win PC Software kullanılmıştır.

BULGULAR

Bu çalışmada 50 adet çiğ süt, 50 adet küflü çeçil peynir, 50 adet kaşar peyniri ve 50 adet gravyer peyniri olmak üzere toplam 200 adet örnek incelenmiştir. Analize alınan 50 çiğ inek sütü örneğinin 22 adedinde (%44) AFM1 tespit limitinin (<5.00 ng/L) altında iken 28 adedinde (%56) farklı düzeylerde AFM₁ belirlenmiştir. Çiğ inek sütü örneklerinin tamamının Türk Gıda Kodeksi (Türk Gıda Kodeksi, 2011) ve Avrupa Komisyonun'ca (EC, 2006) süt için izin verilen limitlere (50 ng/L) uygun olduğu belirlenmiştir. Tablo 1'de çiğ süt örneklerinde AFM₁ varlığı ve dağılımı verilmiştir. Küflü cecil pevniri, kasar pevniri ve gravver pevniri örneklerinin ise sırasıyla 42 (%84), 36 (%72) ve 16 (%32) adedinde AFM₁ tespit limitinin (<112.50 ng/kg) altında iken, 8 (%16), 18 (%36) ve 34 (%68) adedinde AFM₁ pozitif sonuç elde edilmiştir. Peynir örneklerinin tamamının Türk Gıda Kodeksi ve Avrupa Komisyonun'ca izin verilen limitlere uvgun olduğu belirlenmiştir (Türk Gıda Kodeksi 2008, EC, 2006). Tablo 2'de peynir örneklerinde AFM₁ varlığı ve dağılımı verilmiştir.

Tablo 1. Süt örneklerinde AFM1 varlığı ve dağılımı

Table 1. The presence and distribution of AFM₁ in milk samples

örnek ek sayısı n		Örneklerin dağılımı n (%)		Syonu syonu ii örnek	Pozitif örneklerdeki AFM ₁ Konsantrasyonu (ng/L)		
Test edilen ör sayısı (n)	Pozitif örnek (%)	<5* ng/L	5-50 ng/L	Avrupa Komi ve Türk Gıda Kodeksi yasal limitlerini aşar sayısı >50 ng/L	ortalama± standart sapma	min-maks.	
50	28 (%56)	22 (%44)	28 (%56)	0	10.02±9.33	0-21.57	

^{*}Negatif örneklerin dağılımı, minimum tespit değeri altında belirlenen örneklerdeki veriler "0" olarak hesaplanmıştır.

Tablo 2. Peynir örneklerinde AFM₁ varlığı ve dağılımı

Table 2. The presence and distribution of AFM₁ in cheese samples

k sayısı 181 n (%)	sayısı n (%)	Örneklerin d	lağılımı n (%)		ronu yasal nek sayısı	čodeksi yasal örnek sayısı	Pozitif örneklerdeki AFM ₁ Konsantrasyonu(ng/kg)		
Peynir çeşidi	Test edilen örnek sayısı (n)	Pozitif örnek say	<112.50* ng/kg	112.50-150 ng/kg	151-250 ng/kg	Avrupa Komisyonu limitini aşan örnek s: >250 ng/kg	Türk Gıda Kodeksi yasal limitini aşan örnek sayısı >500 ng/kg	Ortalama± standart sapma	min-maks.
Küflü Çeçil Peyniri	50	8 (%16)	42 (%84)	1 (%2)	7 (%14)	0	0	30.45±72.04	0-230.10
Kaşar Peyniri	50	18 (%36)	36 (%72)	14 (%28)	4 (%8)	0	0	49.92±68.29	0-184.90
Gravyer Peyniri	50	34 (%68)	16 (%32)	14 (%28)	20 (%40)	0	0	107.86±78.79	0-216.45
Toplam	150	60 (%40)	94 (%62.7)	29 (%19.3)	31 (%20.7)	0	0	62.74±79.81	0-230.10

^{*}Negatif örneklerin dağılımı, minimum tespit değeri altında belirlenen örneklerdeki veriler "0" olarak hesaplanmıştır.

TARTIŞMA

Hayvanların yüksek oranda AFB₁ ile kontamine yem maddeleriyle beslenmesine bağlı olarak sütte önemli miktarda AFM₁ bulunabilmekte ve bu durum insan ve hayvan sağlığı açısından risk oluşturmaktadır (Igbal ve ark. 2015). Süt ve süt ürünlerinin üretimi Türkiye'de yaygın olup, özellikle Kars kaşarı ve gravyer peyniri tüketim açısından önemli bir potansiyele sahiptir. Bu çalışmada incelenen süt örneklerindeki AFM₁ seviyesi peynir örneklerine göre oldukça düşük bulunmuştur. AFM₁'in kazeine olan ilgisi nedeniyle peynirdeki AFM₁ konsantrasyonunun süte göre daha yüksek olduğu belirtilmektedir (Galvano ve ark. 1996, Bulca ve Bircan 2013). Bizim araştırma bulgularımızda bu görüşü desteklemektedir.

Bu çalışmada analize alınan çiğ süt örneklerinin %56'sı AFM₁ açısından pozitif bulunmuştur. Sütte AFM₁ varlığı ile ilgili çok farklı araştırma sonuçları bulunmaktadır. Rama ve ark. (2016) toplam 826 adet çiğ süt örneğinin 23 (%2.8)'ünün AFM1 açısından pozitif olduğunu rapor etmişlerdir. Bir diğer araştırmada De Roma ve ark. (2017) inek sütlerinin %12.3 AFM₁ ile kontamine olduğunu ifade etmiştir. Li ve ark. (2017) ise çiğ süt örneklerinde AFM₁ kontaminasyonunu %4.7 olarak bulduklarını bildirmişlerdir. Araştırmacıların bulguları bizim sonuçlarımıza göre düşüktür. Diğer taraftan bazı araştırmacılar ise çiğ sütte AFM1 kontaminasyonunun yüksek olduğunu rapor etmiştir. İşleyici ve ark. (2015), Hassan ve ark. (2018), Kireçci ve ark. (2007), Bahrami ve ark. (2016) ve Pei ve ark. (2009) çiğ süt örneklerinin sırasıyla %85, %85, %100, %84.3 ve %100 oranında AFM₁ ile kontamine olduğunu bildirmişlerdir.

Çalışmada incelenen çiğ süt örneklerin hiçbirinde Türk Gıda Kodeksi ve Avrupa Komisyonu tarafından verilen maksimum limitin (50 ng/L) üzerinde AFM₁ tespit edilmemistir. Rama ve ark. (2016) ve Hassan ve ark. (2018) bizim sonuçlarımızla benzer şekilde incelenen örneklerin hiçbirinde AFM1 seviyesinin Avrupa Birliği (AB) maksimum limitlerinin (50 ng/L) üzerinde olmadığını belirtmişlerdir. Diğer taraftan İşleyici ve ark. (2015) ile Kireçci ve ark. (2007) sırasıyla örneklerin %12 ve %90'nın Türk Gıda Kodeksine göre kabul edilebilir sınırların üzerinde olduğunu, Bahrami ve ark. (2016) süt örneklerinin %35.9'unun AB limitlerinin üzerinde AFM1 içerdiğini ifade etmiştir. De Roma ve ark. (2017) inceledikleri 416 inek sütü örneğinden sadece 1 (%0.24) örnekte Li ve ark. (2017) ise 5650 çiğ süt örneğinin 267 (%4.7)'sinde AFM₁ kontaminasyonu belirlediklerini ve sadece %1.1 ciğ süt örneğinin AB limitlerinin üzerinde AFM₁ icerdiğini ifade etmistir. Sonuclarımız bazı araştırmacıların bulgularından düşüktür. Bunun nedeni bölgenin mera açısından zenginliğine dolayısıyla sığır yetiştiriciliğinde ekstansif besiciliğin daha yaygın olmasına bağlanabilir. Bölgede bahar ve yaz aylarında hayvanlar yeşil otla beslenirken kış mevsiminde kuru otlarla beslenmektedir. Bununla birlikte hava şartlarının uygun olmadığı kurak geçen yaz mevsimlerinde veya uzun süren kış mevsimlerinde yeşil ot ve kuru otun az olması nedeniyle konsantre yemlerde kullanılabilmektedir. Diğer taraftan Pei ve ark. (2009) ise çiğ süt örneklerinin %100'ünün (n=12) AFM₁ ile kontamine olduğunu bildirmişlerdir. Yüksek konsantrasyon oranının büyük olasılıkla kentsel ve yarı kentsel alanlarda bulunan hayvanların uygun kaynaklandığını olmayan beslenmesinde ileri sürmüştür. Yurt ve Uluçay (2017), inceledikleri 25 adet çiğ inek sütü numunesinin tamamında (%100) AFM₁ tespit ettiklerini ve 20 örneğin (%80) Türk Gıda Kodeksi Gıda Maddelerindeki Bulaşanların Maksimum Limitleri Hakkında Tebliğ'inde belirtilen yasal limitin üzerinde olduğunu rapor etmişlerdir. Araştırmacıların bulguları bizim bulgularımıza göre oldukça yüksektir. Bunun nedeninin örneklerin toplandığı zaman ile ilgili olduğu düşünülmektedir. Keza arastırmacılar süt örneklerini hayvanlar meraya çıkmadan elde ettiklerini belirtmişlerdir. Çalışmamızda incelenen süt örnekleri hayvanlar meraya çıktıktan sonra temin edilmistir.

AFM₁ yaz aylarına göre kış aylarında daha yüksektir. Bunun nedeni yaz aylarında hayvanların merada yeşil ve taze otlarla beslenmesine bağlanmaktadır. Buna karşın kış mevsiminde taze ve yeşil yemlerin olmaması ve kıtlığı nedeniyle hayvanlar çoğunlukla mısır, buğday veya pamuk çekirdekleri gibi tahıl ürünlerine dayalı konsantre yemlerle beslenmektedir. Depo koşullarının uygun olmadığı durumlarda *Aspergillus* gibi toksijenik küfler üreyerek yüksek düzeyde mikotoksin oluşumuna ve aflatoksin üretimine neden olmaktadır. Bununla birlikte kış mevsiminde süt veriminin daha düşük olması nedeniyle AFM₁ ve diğer bileşenler sütte daha konsantre bulunmaktadır (Mulunda ve ark. 2013, Galvano ve ark. 1996, Kamkar 2014).

Çalışmamızda incelenen toplam 150 peynir örneğinin 60 (%40)'ında AFM1 pozitif pozitif bulunmuştur. Örneklere göre dağılımı incelendiğinde küflü çeçil peyniri, kaşar peynir ve gravyer peyniri örneklerinde sırasıyla, 8 (%16), 18 (%36) ve 34 (%68) olarak saptanmıştır. Türk Gıda Kodeks'inde çiğ süt, ısıl işlem görmüş süt, süt bazlı ürünlerin üretiminde kullanılan süt için AFM₁ maksimum limit değeri 50 ng/kg olarak belirtilmiştir. Süt ürünleri için maksimum limit değeri belirtilmemiştir (Türk Gıda Kodeksi, 2011). Türk Gıda Kodeksi (2008), peynirler için AFM₁ maksimum limit değeri 500 ng/kg olarak kabul etmektedir. Avrupa Komisyonu'nda ise bu değer 250 ng/kg olarak belirtilmistir. Buna göre incelediğimiz pevnir örneklerinin tamamının Türk Gıda Kodeksi ve Avrupa Komisyonu tarafından izin verilen yasal limitlere uygun olduğu belirlenmiştir (Türk Gıda

Kodeksi 2008, EC 2006). Sarımehmetoğlu ve ark. (2004) inceledikleri 400 adet peynir örneğinin 327 (81.75)'sinde AFM₁ tespit ettiklerini, 110 (%27.5) örneğin ise Türk Gıda Kodeksi yasal limitini aştığını rapor etmiştir. Kaşar peyniri örneklerinin ise % 85'inin AFM₁ ile kontamine olduğunu ve 34 (%34) örneğin yasal limitlerin üzerinde AFM1 içerdiğini ifade etmiştir. Benzer şekilde Hampikyan ve ark. (2010) inceledikleri toplam 80 adet peynir örneğinin 41 (%51.3)'inin AFM₁ pozitif olduğunu ve 8 (%10) örneğin Türk Gıda Kodeksi yasal limitinin üzerinde AFM₁ rapor etmistir. Erkan ve ark. (2018) 100 adet 100 (%100)'ünde örneğinin belirlediklerini ve örneklerin tamamında 100 (%100) AFM₁ konsantrasyonunun Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde bildirilen limit değerden yüksek olduğunu bildirmişlerdir. Atasever ve ark. (2010) toplam 304 peynir örneğinin 216 (%71.1)'sının AFM₁ açısından pozitif olduğunu ve sırasıyla 30 (%9.9) ve 63 (%20.7) örneğin Türk Gıda Kodeksi ve Avrupa Komisyonu'nca izin verilen yasal limitleri astığını bildirmişlerdir. Erkan ve ark. (2009) Diyarbakır örgü peynirinde AFM_1 varlığını belirledikleri araştırmanın sonucunda incelenen 90 adet pevnir örneğinin 42 (%46.67)'sinde AFM₁ tespit ettiklerini ve 13 (%14.44) örneğin Türk Gıda Kodeksi limitlerinin üstünde AFM_1 belirtmişlerdir. Kav ve ark. (2011) inceledikleri 127 beyaz-salamura Urfa peynirinin 36 (%28.3)'sında AFM₁ tespit ettiklerini ve 13 (%10.2) örneğin Türk Gıda Kodeksi yasal limitlerini aştığını bildirmişlerdir. Fallah ve ark. (2009) yaptıkları araştırmada İran'da pevnir örneklerinde AFM_1 üretilen varlığını araştırdıkları çalışmada toplam 220 peynir örneğinin 161 (%76.6)'inde AFM₁ belirlediklerini, krem peyniri örneklerinin %28.4'ünün ve beyaz peynir örneklerinin %19.1'inin Türkiye ve İsviçre yasal limitinin üzerinde olduğunu rapor etmiştir. Fallah (2010) yaptığı bir diğer araştırmada toplam 72 beyaz peynir örneğinin 59 (%81.9)'unun AFM₁ pozitif olduğunu ve 22 (%30.5)'sinin İran Standart ve Endüstrivel Araştırmalar Enstitüsü (ISIRI) limitinden yüksek olduğunu rapor etmiştir. Araştırmacıların bulguları bizim bulgularımızdan oldukça yüksektir. Bunun coğrafı farklılıklar, örneklerin temin edildiği mevsim, peynir çeşidi, peynir üretim teknolojisindeki farklılıklar, depolama koşulları, olgunlaşma süresi, peynire işlenecek sütün AFM1 ile kontaminasyon derecesi gibi birçok faktörden kaynaklanabileceği düşünülmektedir. Diğer taraftan Ardıç ve ark. (2008) inceledikleri 64 adet Urfa peyniri örneğinin sadece 4 (%6.25)'ünün AFM₁ pozitif olduğunu ve örneklerin hiçbirinin yasal limitleri aşmadığını ifade etmiştir. Dinçel ve ark. (2012) Kars yöresine ait 20 adet kaşar peyniri örneğinin hiçbirinin tespit edilebilir düzeyde AFM₁ icermediğini ifade etmistir. Bir diğer araştırmada 9 adet Konya küflü peyniri, 1 adet Kars kaşarı, 1 adet deri tulum peyniri olmak üzere toplam 11 adet örneğin 10'unda tespit edilebilir düzeyde

AFB₁ ve AFM₁ olmadığı rapor edilmiştir (Güley ve ark, 2013). Araştırmacıların bulguları bizim bulgularımızla kısmen benzerlik göstermektedir.

Yukarıda bahsedilen araștırma bulguları incelendiğinde peynirlerle ilgili çok farklı araştırma sonuçları bulunmaktadır. AFM1'in baskın bir şekilde kazein ile ilgili olduğu bilinmektedir. Bu nedenle peynir pıhtısında AFM1 konsantrasyonu peynir altı suyuna göre daha yüksektir. AFM₁'in kazeine olan ilgisi, peynir yapımı sırasında AFM₁ için bir zenginleştirme faktörü olarak ifade edilmektedir. Yapılan çalışmalar AFM₁ konsantrasyonunun birçok yumuşak peynirde yaklaşık 3 kat, sert peynirlerde ise yaklaşık 5 kat sütten daha fazla olduğunu göstermiştir. Bazı çalışmalar, peynirin olgunlaşmasının ve kazeinin proteolizinin, doğal olarak kontamine edilmiş sütten AFM₁'in geri kazanımını arttırdığını göstermiştir. Proteolizin, AFM1 oluşumuna neden olan kazein ilişkili moleküller üzerindeki hidrofobik bölgeleri etkilevebildiği belirtilmiştir (Prandini ve ark. 2009). Toksini ekstrakte etme tekniği, analiz yöntemi, türü, konsantrasyonu, peynir yapımında kullanılan sütün kalitesi ve sonuçların değerlendirilme yönteminin de belirlenen AFM1 konsantrasyonunu etkilediği yapılan arastırmalar sonucunda gösterilmistir (Bulca ve Bircan 2013).

SONUÇ

Bu araştırma sonucunda çiğ süt, çeçil peyniri, kaşar ve gravyer peyniri örneklerindeki AFM1 seviyesinin Avrupa Komisyonu ve Türk Gıda Kodeksi limitlerine uygun olduğu ve halk sağlığı için risk oluşturmadığı tespit edilmiştir. Ancak incelenen örneklerde farklı konsantrasvonlarda AFM_1 tespit edilmistir. Aflatoksinler, insan sağlığı özellikle çocuklar üzerinde etkilerinin bulunması, sterilizasyon pastörizasyon işlemlerine dayanıklı olmaları nedeniyle sürekli izlenmesi gereken önemli bileşiklerdir. Çiftlikten sofraya kadar her aşamada gerekli koruyucu önlemlerin alınması gereklidir. Özellikle süt ineği yetistiriciliği yapan kişiler ile süt ve süt ürünleri üreticilerinin aflatoksinlerin neden olduğu potansiyel sağlık problemleri ve sonuçları konusunda bilgilendirilmelerine yönelik eğitim programlarına ağırlık verilmelidir. Süt ve süt ürünlerinde AFM₁ kontaminasyonunu minimize etmek için hayvan yemlerinin belirli periyotlarla AFB1 açısından kalite kontrolünün yapılması, modern üretim tekniklerinin yaygınlaştırılması, süt hayvanlarının beslenmesinde kullanılan yem maddelerinin depolanma koşullarının ivileştirilmesi önem arz etmektedir. İyi tarım uygulamalarının hayata geçirilmesi, üretici ve tüketici bilincinin arttırılması ile halk sağlığı korunacaktır.

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RESEARCH ARTICLE

Effect of Sex on Some Biochemical and Hematological Parameters in Healthy Boer x Hair Goat Crossbreed

Özgür Yaşar ÇELİK*1, Kıvanç İRAK2, Gülşah AKGÜL1

¹Siirt University, Faculty of Veterinary Medicine, Department of Internal Medicine, Siirt-TURKEY

²Siirt University, Faculty of Veterinary Medicine, Department of Biochemistry, Siirt-TURKEY

ABSTRACT

The aim of this study was to determine some hematological and biochemical parameters in male and female Boer x hair goat crossbreed (n=34). Blood samples were collected from the jugular vein in sample tubes with and without anticoagulants. A Mindray BC2800 fully-automated device was used to measure the WBC, RBC, Hgb, Hct, MCV, and MCH values and ADVIA 1800 brand auto-analyzer was used to obtain Na, K, Cl, Mg, Ca, Crea, Tp, Alb, Gluo, Chol, Trig, AST, ALT, and ALP values. WBC (p<0.01), RBC (p<0.05), HCT (p<0.01) and MCV (p<0.05) levels were found to be statistically higher in female specimens, while HGB and MCH levels were found to be statistically identical for both sexes. Meanwhile, males had statistically higher levels of K (p<0.001) and Mg (p<0.01) levels compared to females. Crea (p<0.001), Tp (p<0.05), Alb (p<0.05), Gluo (p<0.001) levels were also statistically higher in males, while Chol level was statistically higher in females (p<0.001). No statistically significant variation was detected between the Trig values of the sexes. ALT, ALP activities of males were found to be statistically higher in males than in females (p<0.05). The results of the study revealed that some hematological and biochemical parameters of Boer x hair goat crossbreeds are affected by sex.

Keywords: Boer x Hair goat crossbreed, Biochemical and Hematological Parameters, Sex.

Sağlıklı Boer x Kıl Keçisi Melez Irklarında Cinsiyetin Bazı Hematolojik ve Biyokimyasal Parametreler Üzerine Etkisi

ÖZ

Bu çalışmada, erkek ve dişi Boer x Kıl keçisi melezlerinde bazı hematolojik ve biyokimyasal parametrelerin belirlenmesi amaçlandı. Hayvanların vena jugularisinden antikoagulanlı ve antikoagulansız tüplere kan örnekleri alındı. WBC, RBC, Hgb, Hct, MCV, MCH değerleri Mindray BC2800 tam otomatik kan sayım cihazında, Na, K, CI, Mg, Ca, Crea,Tp, Alb, Gluo, Chol, Trig, AST, ALT ve ALP analizleri ADVIA 1800 marka otoanalizörde yapıldı. WBC (p<0.01), RBC (p<0.05), HCT (p<0.01) ve MCV (p<0.05) düzeylerinin dişilerde erkeklere göre istatistiksel olarak daha yüksek olduğu, HGB, MCH düzeyleri karşılaştırıldığında ise istatistiksel olarak iki cinsiyet arasında fark olmadığı belirlendi. K (p<0.001) ve Mg (p<0.01) seviyeleri erkeklerde dişilere göre daha yüksek tespit edildi. Erkeklerde Crea (p<0.001), Tp (p<0.05), Alb (p<0.05), Gluo (P<0.001), düzeyleri dişilere göre yüksek, Chol seviyesi ise dişilerde erkeklerden istatistiksel olarak yüksek bulundu (p<0.001). Cinsiyetler arasında Trig değerleri bakımından fark tespit edilmedi. ALT (p<0.05), ALP (p<0.05) aktiviteleri karşılaştırıldığında erkek hayvanların dişilere göre daha yüksek değerlere sahip olduğu belirlendi. Sonuç olarak, Boer x Kıl keçisi melezlerine ait bazı hematolojik ve biyokimyasal parametrelerin cinsiyetten etkilendiği, bazılarında ise cinsiyet faktörünün etkili olmadığı belirlendi.

Anahtar Kelimeler: Boer x Kıl Keçisi, Biyokimyasal ve Hematolojik parametreler, Cinsiyet.

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ORCID ID; ÖYC: 0000-0001-6365-2688, KI: 0000-0001-9765-0330, GA: 0000-0003-4804-6502

*Corresponding author e-mail: oyc@siirt.edu.tr

INTRODUCTION

Sheep and goat farming have an important place in Turkey's economy. According to the June 2017 data of the Turkey Statistics Institute (TÜİK), 11 million goats exist in Turkey, 464 thousand of which are positioned in the city of Siirt (Anonymous 2018). While numerous native goat breeds are being raised in different places of Turkey based on the particular location's geographical configuration and climatic conditions, the studies are also being carried on to obtain highyield crossbreed breeds. Approximately 98% of goats in Turkey are hair goats (Anonymous 2018). In addition to being a combined-product breed, the hair goats have high adaptability to insufficient care and nutrition, and to severe environmental conditions; thus they are bred in all regions of Turkey (Bolacali et al. 2017b).

The Boer goat breed was developed in South Africa and was then brought forth to Europe (Casey and Van Niekerk 1988). The most important characteristics of Boer goats are their high genetic potential, extraordinary resistance against diseases, and their ability to adapt to arid climatic and harsh conditions (Malan 2000). Boer goats were imported by numerous countries as a means to increase their overall meat production levels. Boer goats often have been crossbred with Spanish, Ankara, and Nubian goat breeds (Malan 2000; Urge et al. 2004). There were also studies to crossbreed hair and Boer goats in Turkey (Bolacali et al. 2017a; Bolacali et al. 2017b). The determination of the reference values for hematological and biochemical parameters of healthy animals offers valuable reference data for the veterinarian, and are influential in early diagnosis, etiology, and clinical outcome of diseases. Numerous variables like nutrition, stress, temperature, climate conditions, diseases, amount of muscular activity, age, sex, and breed may actively influence the blood parameter levels (Awah and Nottidge 1998; Gündüz 2000; Haliloğlu and Cinar 2004).

Determination of reference values of blood parameters for various breeds is also important. Due to this fact, many studies in the literature inspect biochemical and hematological parameter levels for numerous goat breeds cultivated at various locations all around the world (Rastogi and Singh 1990; Mbassa and Poulsen 1993; Kumar *et al.* 1997; Azab and Abdel-Maksoud 1999; Njidda *et al.* 2013). While there are some studies conducted in Turkey about the determination of various hematological and biochemical parameters in Kilis (Iriadam 2004), Siirt hair (Tanrıtanır *et al.* 2010), and Ankara goats (Aşkın 2013), no such studies exist for Boer x hair goat crossbreeds. The aim of

this study was to investigate some hematological and biochemical blood parameters in male and female Boer x hair goat crossbreeds.

MATERIALS and METHODS

Animal Material

All applicable international, national, and/or institutional guidelines for animal testing, welfare, animal care and use of animals were taken into account and followed by the authors. The material of the study consisted of 17 male (min: 621, max: 687, mean: 663.71 days old), and 17 nonpregnant female (min: 616, max: 689, mean: 663.94 days old) a total of 34 goats raised in Siirt province. All animals were raised under similar condition through intensive *ad-libitum* breeding in a goat farm. The female goats allocated in the study were never been pregnant before.

Hematological and Biochemical Analyzes

Blood samples were collected from jugular veins of animals in the morning into the sample tubes both with and without an anticoagulant. A fullyautomated blood panel device (Mindray BC2800, China) was used to measure white blood cell (WBC), red blood cell (RBC), hemoglobin (Hbg), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) values in the sample tubes with an anticoagulant. Meanwhile, the samples in the tubes without an anticoagulant were kept in room temperature for 30 minutes, followed by a 10-minute centrifugation in 3000 rpm, after which the serum were transferred into Eppendorf tubes. The sera in these tubes were analyzed using an auto-analyzer (Siemens, ADVIA 1800, Germany) to determine the sodium (Na), potassium (K), chloride (CI), magnesium (Mg), calcium (Ca), Creatinine (Crea), total protein (Tp), albumin (Alb), glucose (Gluo), cholesterol (Chol), triglyceride (Trig), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) levels.

Statistical Analysis

The obtained data were analyzed using the SPSS 22 package software. Independent Sample T-test was used to determine the statistical variance between male and female group. Results are given as mean \pm SEM (Standard Error Mean). Differences were considered significant when p values were less than 0.05.

RESULTS

Table 1 displays the results of tested hematological parameters for male and female Boer x hair goat crossbreds. WBC (p<0.01), RBC (p<0.05), Hct (P<0.01), and MCT (P<0.05) levels of female

specimens were found to be statistically higher compared to males', while Hgb and MCH levels were found to be not statistically different between gender.

Table 2 displays the results of biochemical parameters in male and female Boer x hair goat crossbreds. While K (p<0.001) and Mg (p<0.01) levels of male specimens were found to be statistically higher compared to females, no statistical difference was detected in the Na, Cl, Ca levels between the sexes. Males had statistically higher Crea (p<0.001), Tp (p<0.05), Alb (p<0.05), Gluo (p<0.001) levels compared to females, while females had statistically higher Chol (p<0.001) level compared to males. No statistical difference was detected in the Trig levels between the sexes. Finally, the ALT (p<0.05), and ALP (p<0.05) levels of the male specimens were found to be significantly higher compared to females. No statistically significant variation was detected in AST values.

DISCUSSION

It was reported that hematological and biochemical parameters of animals may vary based on factors like breed, age, and sex (Iriadam 2004; Njidda et al. 2013; Simsek et al. 2015). Determination of the reference levels for these parameters for various animal species or breeds present important guidelines for veterinary practitioners (Aşkın 2013). For goats, the WBC level was reported to be between 4000-13000 µL (Schalm et al. 1975; Kramer 2000), and the findings of the present study are higher than these values. WBC levels were determined for Kano Brown goats as 18.3 ± 0.65 (x109/L) for males and 20.3 ± 1.33 (x109/L) for females, while in Borno White goats it was determined as 13.3±0.6 (x109/L) for males and 33.4 ± 0.4 (x109/L) for females. The relatively high WBC level for the goats was interpreted as a welldeveloped immune system (Njidda et al. 2013). In our study, the WBC level findings are in line with the findings of (Niidda et al. 2013), and was determined as (25.28±1.68) in females, higher than that of males (18.23±1.1). It is possible to evaluate the relatively high WBC levels in Boer x Hair goat crossbreeds as a characteristic property for the breed.

RBC levels of Boer x hair goat crossbreeds in the present study were found to be lower in males (p<0.05) compared to females, and that this difference between sexes is thought to be a characteristic of the breed. Similarly, the RBC levels of male Ankara goats were found to be lower than females (p<0.05) (Aşkın 2013).

While in this study the hematocrit levels for male and female specimens were within the reference limits of (%22–38) specified by Schalm et al. (1975), the females' hematocrit value was higher than the males', and the difference was found to be statistically significant (p<0.01). High hematocrit value indicates either an increase in the RBC count in the circulation, or the decrease in the plasma volume (Askın 2013). The increase of hematocrit percentage in the present study is in accordance with the increase in RBC count. In Borno White goats (Njidda et al. 2013) and in healthy Ankara goats (Aşkın 2013), the hematocrit percentages of females were found to be higher than the females', paralleling the findings of our study. In mature (3+ vears of age) Kilis goats, the high hematocrit values (% 37.60±1.90) were interpreted as a characteristic of the breed (Iriadam 2004). Various researchers have reported the hemoglobin values, and particularly hematocrit values, are affected by the altitude of the animals, and their nutrition (Egbe-Nwiyi et al. 2000; Adejumo 2004; Addass et al. 2010; Isidahomen et al. 2010).

Schalm *et al.* (1975) reported on the MCH and Hgb reference values for goats as 5.2-8.0 pg and 8-12 g/dL, respectively. In the present study, the MCH and Hgb levels of male specimens were found as 7,59±0,82 pg and 11,36±1,35 g/dL respectively, and the females' MCH and Hgb levels were determined as 6,94±0,11 pg and 11,22±0,31 g/dL respectively. All of these results are within the reported reference values and no statistically significant variation between sexes was detected for these parameters. This result is in line with the results of the study of Elitok (2012), who reported no statistical difference in terms of MCH and Hgb levels between male and female Saneen goats older than 8 months.

The reference value for MCV for goats is reported as 16-25 g/dL (Schalm et al. 1975). In the present study, the level of MCV in male goats was found to be lower $(15,61\pm0,34)$ than the reported reference limits, while it was found to be within the reference limits (17,01±0,44) for female goats. Adult Ankara female goats were reported to have statistically higher MCV values by Aşkın (2013), while Njidda et al. (2013) reported that in Borno White goats (adult males and young females) had statistically higher MCV values compared to their counterparts.

Biochemical parameters help veterinary practitioners to evaluate the health condition and metabolic activities of the animals. Factors like sex and age may influence the physiologic levels of the blood parameters (Yigit *et al.* 2002; Bozdoğan *et al.* 2003; Cenesiz *et al.* 2011). The reference values for Na, Cl, and Ca levels of goats have been reported

as 142-166 mmol/L, 98-110 mmol/L, and 8,9-11,7 mg/dL respectively (Kaneko *et al.* 1997; Karagül *et al.* 2000). In the present study, the levels of Na, Cl, and Ca of the specimens were found to be perfectly within the reference values given in the literature, and no significant differences were detected between the sexes in this regard.

Serum K levels of males were reported to be statistically higher than those of females in Kano Brown, Borno white, and Sokoto red goats by Njidda et al. (2013), and for Ankara goats by Aşkın (2013). The findings of the present study are concordant to the findings of the studies of Njidda et al. (2013) and of Aşkın (2013). Serum K levels were higher in male Boer x Hair goat crossbreeds compared to females', which could be surmised as a racial characteristic. A literature survey has revealed that only a few studies had inspected Mg levels in goats. The mean Mg levels of specimens determined in the present study were lower than the reference values of 2,8 -3,6 mg/dL reported by Karagül et al. (2000). Male specimens having lower Mg values compared to females, however, they are in accordance with the findings of Aşkın (2013). The serum creatinin levels are affected by factors like the muscle mass, glomerular filtration, and tubular reabsorption (Guignard and Drukker 1999; Otukesh et al. 2012). In the present study, the comparison of male and female specimens reveals a similar relative height of male creatinin levels compared to females, which was found to be statistically significant (p<0.001), in line with other studies in literature (Aşkın 2013; Njidda et al. 2013). It is possible to interpret the lower amounts of creatinin level in females to the lower muscle mass, which results in lower secretion of creatinin (Alex and Laverne 1983).

Kaneko *et al.* (1997) and Karagül *et al.* (2000) have reported the total protein and albumin values as 6-7 mg/dL and 2,7 – 3,9 mg/dL respectively. The findings of the present study are within these reference values, and both parameters were found to be statistically higher in males compared to females (p<0.05). The literature studies also report that Pasmina (Sharma *et al.* 1990) and Raini (Sakha *et al.* 2009) goats' total protein levels vary between sex and are lower in adult females compared to adult males.

Kaneko et al. (1997), Karagül et al. (2000) and Mert (1996) have reported a glucose level varying

between 50 and 75 mg/dL for goats. In the present study, the females' blood glucose levels (52,82±2,51 mg/dL) were within the reference limits given in the literature, while males' (101,06±6,29 mg/dl) were higher. The statistically relevant (p<0.001) difference between the glucose levels of sexes might be related to the metabolism differences.

Blood cholesterol level reference limits for goats have been reported as 55 – 200 mg/dL in the literature (Karagül *et al.* 2000). In the present study, the female specimens' cholesterol levels were within the reference values, while males were lower (p<0.001). Female Saneen (Elitok 2012) and Kano brown and Sokoto red goats (Njidda *et al.* 2013) were also reported in the literature to have higher cholesterol levels compared to males. Alex and Laverne (1983) have reported that cholesterol levels are influenced by breed, sex, and age.

Elitok (2012) reports that female Saanen goats older than 8 months raised in Afyonkarahisar have higher triglyceride levels compared to males and that the triglyceride level variation between the sexes is statistically insignificant (p>0.05). The findings of the present study are in accordance with the findings of Elitok (2012).

AST is present in numerous tissues of the body but is mostly concentrated in skeletal muscles, cardiac muscles, and in the liver, and its amount can be used as an indicator of tissue damage. Liver damage increases the ALT activity levels. On the other hand, ALP is present in the liver, bones, kidneys, intestines, and placenta, in the form of isoenzyme groups. It is important to determine the cause of the ALP increase for accurate diagnosis. ALP is known as the most sensitive indicator for cholestasis (Mert 1996). In our study, both sexes had lower AST (167-513 U/L) and ALT (24-83 U/L) activities compared to the values reported in the study of Mert (1996), while the ALP activity levels (93-387 U/L) were within the reference values.

As a result, it was determined that some hematological and biochemical parameters were affected by gender in Boer x Hair goat crossbreeds. The results of this study will hopefully provide useful quide for further studies that will be conducted on Boer x Hair Goat crossbreeds.

Table 1. Results of hematological parameters in male and female Boer x hair goat crossbreds

Parameters	Gender	Mean± SEM	P Values	
WBC (x10 ⁹ /L)	Male	18.23±1.1	**	
WDC (XIO-/L)	Female	25.28 ± 1.68	sissis.	
RBC (x10 ¹² /L)	Male	16.12 ± 0.31	*	
	Female	17.60 ± 0.50		
LICT (0/)	Male	25.13 ± 0.76	**	
HCT (%)	Female	28.99 ± 0.87		
MCII ()	Male	7.59 ± 0.82	NIC	
MCH (pg)	Female	6.94 ± 0.11	NS	
LICD / /II)	Male	11.36 ± 1.35	NIC	
HGB (g/dL)	Female	11.22 ± 0.31	NS	
MCV (A)	Male	15.61 ± 0.34	*	
MCV (fL)	Female	17.01 ± 0.44	Α	

NS: non-significant (p>0.05), *: p<0.05, **: p<0.01

Table 2. The results of biochemical parameters in male and female Boer x hair goat crossbreds

Parameters	Gender	Mean± SEM	P Values
	MINERALS	3	
NI. (1/I)	Male	144.24±0.64	NIC
Na (mmol/L)	Female	142.29 ± 1.75	NS
CI (1/I)	Male	103.47 ± 0.80	NIC
CI (mmol/L)	Female	100.65 ± 2.04	NS
$C \left(\right) \left(\right) $	Male	8.12 ± 0.09	NIC
Ca (mg\dL)	Female	8.43 ± 0.37	NS
17 (1/1)	Male	6.28 ± 0.19	***
K (mmol/L)	Female	4.18 ± 0.19	***
M () II)	Male	2.45 ± 0.08	**
$Mg (mg \dL)$	Female	2.22 ± 0.03	**
	METABOLIT	ES	
C / /II)	Male	0.88±0.04	***
Crea (mg/dL)	Female	0.57 ± 0.03	<i>ተተተ</i>
Tp (g/dL)	Male	7.22 ± 0.12	*
	Female	6.34 ± 0.33	Ψ
A 11 / \ 1T \	Male	3.46 ± 0.04	*
Alb (g \dL)	Female	2.98 ± 0.18	Ψ
C1 () II)	Male	101.06±6.29	***
Gluo (mg\dL)	Female	52.82±2.51	<i>ተተተ</i>
C1 1 () II)	Male	46.76±2.66	***
Chol (mg\dL)	Female	66.18 ± 4.05	<i>ተተተ</i>
/T': / / IT)	Male	19.59±1.95	NIC
Trig (mg/dL)	Female	26.24 ± 2.86	NS
	ENZYMES		
ACT (II) I)	Male	69.29±2.10	NIC
AST (U L)	Female	66.59 ± 3.98	NS
A T 'T' /T I\ T \	Male	20.71 ± 0.56	*
$ALT (U \setminus L)$	Female	18.35 ± 1.00	τ
AID (II) I)	Male	185.35±12.82	*
$ALP (U \backslash L)$	Female	139.47±14.16	*

NS: non-significant (p>0.05), *: p<0.05, **: p<0.01, ***: p<0.001

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RESEARCH ARTICLE

Effect of Non-Genetic Factors on The Reproductive Performance and Milk Yield Characteristics of Hair Goats

Memiş BOLACALI*1, Yahya ÖZTÜRK2, Orhan YILMAZ3, Mürsel KÜÇÜK3, Mehmet Akif KARSLI4

¹Siirt University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Siirt, TURKEY

²Mehmet Akif Ersoy University, Burdur Food, Agriculture and Livestock Vocational School, Program of Food Technolog, Burdur, TURKEY

³Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Van, TURKEY

⁴Kırıkkale Üniversity, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, Kırıkkale, TÜRKEY

ABSTRACT

The aim of this study was to determine the potential effect of non-genetic factors on certain production characteristics of Hair goats raised under semi-intensive management and dry- sub humid conditions, and to investigate phenotypic correlations between these traits. A generalized linear model was used to investigate the effect of non-genetic factors on gestation length (GL), number of services per conception (NSPC), lactation length (LL), lactation milk yield (LMY) and daily milk yield (DMY), while the Chi-square method was used to evaluate other fertility parameters. The pregnancy rate, birth rate and single-birth rate in the Traditional Group were higher, while the infertility rate and NSPC were lower, than those in the Artificial Group (P<0.001). Furthermore, it was determined that LL, LMY and DMY increased (P<0.001), the NSPC decreased (P<0.001), and birth and twinning rates fluctuated (P<0.01) as age increased. The best fertility and milk yield characteristics were in the ≥5 year-old-goats and live weights of 45.0-49.9 kg. In addition, it was determined that kid yield was increased in the goats synchronized with intravaginal sponges impregnated with fluorogestone acetate and in those bred via the traditional method.

Key words: artificial insemination, estrus synchronization, milk yield, reproductive performance.

Kıl Keçilerinin Döl ve Süt Verimi Özellikleri Üzerine Genetik Olmayan Faktörlerin Etkisi

ÖΖ

Bu çalışmanın amacı, yarı entansif ve kurak az nemli iklim koşullarında yetiştirilen Kıl keçilerinin döl verimi ve süt verimi üzerine genetik olmayan faktörlerin etkisini belirlemek ve bu faktörler arasındaki fenotipik korelasyonu araştırmaktır. Gebelik süresi (GL), gebelik başına düşen tohumlama sayısı (NSPC), laktasyon uzunluğu (LL), laskyason süt verimi (LMY) ve günlük süt verimi (DMY) üzerine genetik olmayan faktörlerin değerlendirilmesinde Genear linear model, diğer döl verim parametrelerinin değerlendirilmesinde ise Chi-square metodu kullanılmıştır. Tabii tohumlama yapılan gruptaki keçilerin gebelik oranı, doğu m oranı ve tek doğum oranı suni tohumlama uygulananlardan daha yüksek iken, NSPC sayısı daha düşüktür. Yaş arttıkça LL, LMY ve DMY artmış (P<0.001), NSPC azalmış (P<0.001), tek ve ikiz doğum oranları ise dalgalanma göstermiştir (P<0.01). En iyi döl verimi ve süt verimi nin 5 ve daha yukarı yaşlı ve 45.0-49.9 kg canlı ağırlığındaki keçilerde olduğu belirlenmiştir. Fluorogestone acetate içeren intravajinal sünger ile senkronizasyon ile tabii tohumlama uygulaması nın keçilerde oğlak verimini arttırdığı sonucuna varılmıştır.

Anahtar Kelimeler: suni tohumlama, östrüs senkronizasyonu, süt verimi, döl verimi.

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ORCID ID; MB: 0000-0002-4196-2359, YÖ: 0000-0003-0376-0868, OY: 0000-0002-6261-5196,

MK: 0000-0002-0544-444X, MAK: 0000-0002-3081-9450

*Corresponding author e-mail: bolacali@gmail.com

INTRODUCTION

Goats have had important roles throughout the history of Anatolian civilizations, and have been bred for over 1000 years. Goat breeding is widespread in Turkey due to the geographiceconomic conditions of the country, the historical accumulation of agricultural experience, and the traditions and customs of the Turkish people. Despite this, the number of goats has decreased by 53% to 10.3 million in the last 5 decades (TUIK, 2017). Among the possible reasons for this decrease are the low yield and income obtained from goat breeding and the lack of sustainable breeding programs and animal husbandry policies. Although Hair goat is generally a low-yield breed in terms of fertility characteristics, milk yield, meat yield and hair yield, it is one of the five indigenous goat breeds of Turkey that has adapted resistance to harsh environmental factors, such as diseases, poor nutrition conditions, and extreme climatic conditions. Hair goat breeding is performed depending on the season and geographical region semi-intensive under and/or extensive management conditions. The population of Hair goats in Turkey is reported to be 10.1 million, accounting for approximately 97% of the total goat population, with the Hair goats commonly raised in the Eastern Anatolian region (TUIK, 2017).

The milk yield and reproductive performance of goats has a major impact on the profitability of goat farming. In assessing milk yield, lactation length and lactation milk yield are typically used; in assessing fertility characteristics, the pregnancy, birth, infertility, mortality, abortion, single kidding and twinning rates, along with litter size and kid yields, are used as key parameters. These parameters are considered to be particularly important when regarding economical goat breeding, milk production, goat meat production, goat production and kid sales (Akçapınar and Özbeyaz 1999, Atay and Gokdal 2016).

Both genetic and non-genetic factors affect the milk yield and reproductive parameters of goats. In addition to animal genetics, the effects of nongenetic factors on milk yield and reproductive performance, and the importance of optimum environmental conditions in enhancing goat productivity, have attracted increasing attention in recent years (Rhone et al. 2013, Atay and Gokdal 2016, Keskin et al. 2017). Non-genetic factors include feeding (nutrition), housing, rearing systems and management conditions, climate, (including temperature), kidding year, synchronization type, insemination methods, birth type, body weight, number of pregnancies, diseases, LL, and number of lactations (Bolacalı and Küçük 2012, Furstoss et al. 2015).

Knowing the effects of non-genetic parameters on yield properties can provide explanations for variations in milk yield and fertility characteristics, and aids in the assessment of yield parameters when designing prospective breeding programs (Haldar et al. 2014). As a result of these optimized programs, goat productivity may be increased as a direct outcome, while supply of the daily protein needs of individuals living in rural areas and improvement in life standard may be observed as an indirect outcome.

Atay and Gokdal (2016) identified that Hair goats birthing multiple kids at 6 years of age gave the highest milk vield. Meanwhile, Yotov et al. (2016a) studies reported in their that applying synchronization increased the kid yield in goats. However, there is a lack of data on the effects of non-genetic factors on the yield parameters of Hair goats in the Eastern Anatolian region, which is classified to have a dry-sub humid (C1) climate according to the Thornthwaite Classification. Therefore, the aims of this study were (i) to determine the effects of non-genetic factors on certain production characteristics of Hair goats raised in semi-intensive management systems under dry sub-humid conditions; (ii) to investigate phenotypic correlations between milk yield and fertility traits; and (iii) to present data that may be of benefit to goat producers in the region.

MATERIALS and METHODS

All animal protocols were carried out in accordance with the Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes (EUD, 2010). This research was conducted according to the Yuzuncu Yil University Animal Researches Local Ethic Committee on animal use (protocol / decision number 2015/07).

To include flock diversity in our analysis, this research was conducted in two flocks (1st flock located at latitude 38°34'33.0"N and longitude 43°17'15.1"E; 2nd flock located at latitude 38°05'29.1"N and longitude 43°06'46.6"E) from August 2011 to April 2014 in Van Province (altitude, 1600 m) in the Eastern Anatolian region of Turkey. The climate in Van Province is classified as dry-sub humid (C1) according to the Thornthwaite climate grading system (Eken et al. 2008, Şensoy et al. 2012), and rainfall averages at 528.4 mm per annuum (TSMS, 2017).

In this study, the lactation records of 194 Hair goats and reproductive data on 252 Hair goats aged 2, 3, 4, and ≥5 years were collected. The live weight and age of the Hair goat dams were recorded at the beginning of synchronization as fertility

parameters, and at kidding as milk yield parameters. Different performance characteristics of the goats were determined under semi-intensive conditions, with the goats given approximately 0.50 kg/head of concentrated feed per day in addition to pasture in the evenings during the experimental period. The nutritional composition of concentrated feed and its estimated nutrient supply is presented in Table 1. The concentrate diets formulated to appropriate the nutrient requirements specified by the National Research Council (NRC, 2007). The kids were suckled by their dams for 60 days after birth.

Goats are seasonally polyestrous animals with peak sexual activity occurring in the fall when day length is decreasing. In the Eastern Anatolian region where the study was conducted, goats typically start their cycle between September and November and give birth from February to April.

The goats were grouped into Sponge and Spontaneous Groups according to estrus synchronization, and into Artificial and Traditional Groups according to the insemination method.

Estrus synchronization in does was performed at the beginning of normal breeding seasons (October and November), and was carried out for the Sponge group using intravaginal progestogenimpregnated sponges containing 20 mg doses of fluorogestone acetate (FGA, Chronogest-CR, Intervet, Turkey), inserted for 11 days. The goats also received an intramuscular injection of 50 mg cloprostenol (Estrumate, Intervet, Turkey) and 400 equine chorionic gonadotropin (Chronogest/PMSG, Intervet, Turkey) 48 hours before sponge withdrawal. The Spontaneous Group did not undergo estrus synchronization; instead, the spontaneous estrus principle was adopted.

Estrus detection in does during the normal breeding seasons of the research period was based on the assistance of a teaser buck, and was checked twice daily at approximately 12-hour intervals. Subsequently, according to insemination type, goats in the Artificial Group were inseminated with frozen-thawed buck semen containing 75 × 106 sperm/0.50 ml per straw. The does were first inseminated between 18 and 24 hours after detection of standing estrus symptoms, and the second artificial insemination (AI) administered between 36 and 48 hours after estrus detection. The AI was administered by means of the transcervical intrauterine insemination method, using an insemination pipette and a speculum with a light source. The semen was deposited as deeply as possible in the genital tract. Meanwhile, the goats in the Traditional Group were hand-mated via a traditional system using 1 buck (3-5 years old) per 30 does (Akçapınar and Özbeyaz 1999).

Blood samples were taken from the jugular vein in 10 ml vacuum tubes (venoject) 21 days after mating or insemination to test for pregnancy. Serum was recovered by centrifugation and analyzed for progesterone concentration using a commercially available ELISA kit. A serum progesterone level of greater than 1.5 ng/ml was taken as evidence of pregnancy (Boscos et al., 2003; Islam et al., 2014), In this research, the data regarding fertility performance were calculated according to the methods of Akçapınar and Özbeyaz (1999) and Cinar et al. 2017 as follows:

Pregnancy rate: (number of pregnant does / total number of does during mating season) × 100 Infertility rate: (number of does not pregnant / total number of does during mating season) × 100 Birth rate: (number of does giving birth / total number of does during mating season) × 100 Abortion rate: (number of does experiencing abortion / number of pregnant does) × 100 Single kidding rate: (number of does with single kid / number of does giving birth) \times 100 Twinning rate: (number of does with twin kids / number of does giving birth) × 100 Litter size: (number of kids born / number of does giving birth) \times 100 Kid yield: (number of kids born / total number of does during mating season) × 100

Milk vield controls were collected every month starting 10 days after kidding and were continued until daily milk production was <50 ml. These were collected twice a day in the morning and evening by hand milking. The kids were separated from their mothers approximately 12 hours before collection of the milk controls. The LMY of each goat was estimated with the Fleischmann method according to the ICAR procedure (ICAR, 2016). Fertility parameters (except for GL and NSCP) were analyzed by the Chi-square test, and phenotypic correlations among fertility (GL and NSPC) and milk vield (LL, LMY and DMY) characteristics were calculated in SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). The data points bearing different letters are significantly different at P<0.05.

A general linear model in the SAS statistical program (SAS, 2002) was used to determine the effects of flock diversity, kidding year, estrus type, insemination type, birth type, kidding age and doe live weight on fertility (GL and NSCP) and milk yield traits. The Duncan's multiple-range test was used for multiple comparisons of the subgroups. The following model was used to evaluate factors affecting fertility and milk yield traits: Yijklmno = μ + Fi + Yj + Sk + Il + Bm + An + Wo +

eijklmnop, where μ = the mean milk yield of the Hair goat population, Fi = the effect of flock diversity (i = 1, 2), Yj = the effect of kidding year (j = 2012, 2013, 2014), Sk = the effect of estrus type (k = spontaneous, sponge), Il = the effect of insemination type (l = artificial, traditional), Bm = the effect of birth type (m = single, multiple), An = the effect of age at kidding (n = 2, 3, 4, \geq 5), Wo = the effect of doe live weight (o = 25.0-29.9, 30.0-34.9,, \geq 50.0 kg).

RESULTS and DISCUSSION

The fertility parameters of the Hair goats are listed in Tables 2, 3 and 4. The pregnancy rate, infertility rate, birth rate, abortion rate, single birth rate, twinning rate, litter size, kid yield, GL and NSPC were 82.94%, 17.06%, 76.97%, 7.18%, 60.71%, 16.27%, 1.23, 94.84, 148.7 days, and 1.65, respectively. Regarding the duration of pregnancy, previous results most similar to those recorded presently were documented by Greyling (2000) in Boer goat to be 148.2 days. In this study, the pregnancy rates of the Spontaneous and Traditional Groups were found to be lower than recorded in Hair goats synchronization during breeding season (Şimşek et al. 2006, Toplu and Altinel 2008), while litter size and kid yield were higher than those recorded previously (Sengonca et al. 2003, Toplu and Altinel 2008). Additionally, the number of services per conception value of the Traditional Group was found to be similar to that reported by Faruque et al. (2010) for Black Bengal goats bred in semiintensive conditions.

In terms of flock diversity, it was determined that the pregnancy rate, birth rate and twinning rate of the 1st flock were higher than those of the 2nd flock (P<0.01, P<0.01 and P<0.05, respectively), while the infertility rate of the 1st flock was lower (P<0.01; Table 2). No statistically significant difference between the flocks regarding NSPC was observed (Table 4), which complies with the results reported by Mellado et al. (2008). This variation in fertility parameters due to the diversity of the flocks may have stemmed from differences in the breeders and bioregion (Hoque et al. 2002).

It was observed in the study that fertility performance increased over the subsequent years (Table 2). Most studies (Sengonca et al. 2003, Gül et al. 2016) agree that the variation among yearly results when evaluating the influence of year on fertility traits may be due to differences in total rainfall, which can affect the quantity and quality of foraging, as well as yearly management changes. Thus, variations in the weather, nutrition and farm management from year to year may be responsible

for the altered yearly fertility traits in the present study.

The pregnancy rate in the goats synchronized with fluorogestone acetate was 76.43%, which was similar to those reported by Yotov et al. (2016b). More notably, this synchronized pregnancy rate was lower than those observed by Ritar and Solomon (1982) and Susilawati et al. (2014), and higher than those reported in other studies (Arrebola et al. 2012, Yotov et al. 2016a). This variation in pregnancy rate in synchronized goats may be due to interactions between the synchronization method, management strategies, and climatic conditions in the region where the goats were raised. Regarding estrus synchronization among the different estrus-type groups, it was determined that the pregnancy rate, birth rate and single-birth rate in the Spontaneous Group were higher than those in the Sponge Group (P<0.01), while the NSPC of the Spontaneous Group was lower (P<0.05). Therefore, synchronization was determined to decrease the pregnancy rate in this study, which does not comply with the results reported by Yotov et al. (2016b). However, although Sponge application generally decreased fertility performance, it notably increased the twinning rate and kid yield, which are important criteria for goat breeding.

It was determined that the pregnancy rate in the Artificial Group (subjected to transcervical intrauterine insemination application) was 66.67%. This result is similar to that reported by Yotov et al. (2016b), higher than those reported by Arrebola et al. (2012), and lower than that reported by Bhattacharyya et al. (2002). This variation in the pregnancy rate of artificially inseminated goats might have stemmed from differences in the AI method used and in the number of inseminations administered to each animal. In terms of the insemination type, it was determined that the pregnancy rate, birth rate, and single birth rate in the Traditional Group were higher those in the Artificial Group (P<0.001), while the infertility rate and NSPC of the Traditional Group were lower (P<0.001). This reduction in the fertility characteristics of the Artificial Group may have been due to the AI method and/or the frozenthawed buck semen used.

As the age of the Hair goats increased (2 to \geq 5 years old), the birth rate increased (P<0.01), the twinning rate increased (P<0.01), and the NSPC decreased (P<0.001), and the best fertility performance was observed in \geq 5-year-old goats. Interestingly, all of the 2-year-old goats produced single births. The result that the 5 year-old-goats had the highest pregnancy rate is consistent with that reported by Bolacalı and Küçük (2012); and the increase in litter size with age is consistent with

the result reported by Rhone et al. (2013). Synman (2010) documented that the fertility traits of goats increased until the age of 7 and started to decrease thereafter. Conversely, the highest litter size was observed in 3-year-old goats by Hoque et al. (2002), and in ≥4-year-old goats by Ibnelbachyr et al. (2014). Unlike these results, Dadi et al. (2008) found that litter size was not affected by age.

In this study, it was determined that the pregnancy rate (P<0.01) and birth rate (P<0.001) increased from 25.0-29.9 kg to 45.0-49.9 kg doe live weight, and that twinning rate (P<0.05) increased from 25.0-29.9 kg to 40.0-44.9 kg doe live weight, all of which started to decrease at subsequent higher weights. Meanwhile, the infertility rate (P<0.01) and NSPC (P<0.05) decreased from 25.0-29.9 kg to 45.0-49.9 kg doe live weight, after which the parameters started to increase. Hoque et al. (2002) reported that litter size increased until 19-20 kg doe live weight and decreased at subsequent higher weights.

The effects of flock diversity, year, estrus type, insemination type, birth type, doe age, and doe live weight on the milk yield characteristics of the Hair goats are shown in Table 5. The LL, LMY, and daily milk yield (DMY) were found to be 170.93 days, 145.58 kg, and 0.835 kg, respectively. The LL measured in the present study was found to be similar to that reported by Simsek et al. (2006); longer than that reported by Olfaz et al. (2011); and shorter than those reported by Toplu and Altinel (2008) and Atay and Gokdal (2016). Although the LMY and DMY were similar to those reported by Şimşek et al. (2006), they were higher than those reported for Hair goats in previous studies (Toplu and Altinel 2008, Olfaz et al. 2011, Atay and Gokdal 2016). This variation may be due to differences in the management and climatic conditions.

In the present study, it was also determined that the LMY and DMY increased over the subsequent years, 2012 data vs. 2013/14 data (P<0.05). The increases in the LMY and DMY over subsequent years were expected, and reflect the regional effects on grazing incurred from the variability in climatic conditions and flock composition, and from fluctuations in the availability of nutrients over time (Ishag et al. 2012), which are beyond the influence of management alterations (Lôbo et al. 2017).

This study found that does that nursed multiple kids produced more milk (higher LMY) than those with single kids (P<0.05). Does birthing multiple kids produce more milk due to greater hormonal stimuli deriving from the multiple fetuses, as indicated from measurements of the levels of the hormones placental lactogen, progesterone and prolactin during gestation, which are mammary gland stimulants (Lôbo et al. 2017). In addition, there is a greater tendency for does rearing multiple kids to produce more milk due to the suckling reflex and the physiological mechanism during pregnancy that prepares the udder to produce more milk for does carrying multiple fetuses (Idowu and Adewumi 2017).

With increasing age of doe (2 to ≥5 years old), the LL, LMY and DMY significantly increased (P<0.001), and the highest LL, LMY and DMY were detected in the goats that were ≥5 years old. These results are consistent with those reported by Toplu and Altinel (2008). Similarly, Atay and Gokdal (2016) found that 6-year-old goats gave the highest milk yield. Conversely, the highest milk yield performance was reported by Sam et al. (2017) in 3-year-old goats, by Ibnelbachyr et al. (2015) in 3-4-year-old goats, and by Keskin et al. (2017) in ≥4 year-old-goats. The milk yield of animals increases with age due to age-dependent increases in hormonal levels, metabolic activity, secretory cells and nutrient intake, all of which are used in milk synthesis. Additionally, the significant effect of age on milk yield in the present study suggests that milk production tends to increase with age, possibly due to the accumulation of mammary alveoli from previous lactation periods, until this process is interrupted by further advances in age (Idowu and Adewumi 2017).

It was also determined that the live weight of does had significant effects on LL, LMY and DMY (P<0.05); there were increases in the milk yield performances as the doe live weights increased, and the best performances were observed in goats between 45.0-49.9 kg live weight.

Furthermore, as presented in Table 6, litter size was identified to be positively correlated with LL, LMY and DMY (P<0.05). This association between litter size and milk yield may be due to the stimulus provided by suckling, which increases milk production.

Table 1. Ingredient composition and analyzed content of nutrients in the feed used in the study (2012, 2013, 2014).

Ingredients (%)	2012	2013	2014	Nutritional content, DM basis (%)	2012	2013	2014
Yellow corn	50.00	50.00	38.40	Dry matter	89.62	89.48	89.51
Barley	17.00	15.00	33.00	Metabolic energy			
Wheat		5.00		kcal/kg	2699	2701	2693
Vetch seed	10.00	5.00		MJ/kg	11.30	11.31	11.27
Vegetable oil			0.50	Crude protein	16.20	16.11	16.18
Soybean meal (44% CP)	8.00	10.00	14.00	Crude fat	2.71	2.74	3.01
Sunflower meal (28% HP)	12.20	12.20	10.50	Crude fibre	6.06	5.91	5.99
Limestone	1.75	1.75	2.00	Crude ash	5.16	5.15	6.16
Salt	0.50	0.50	0.80	Calcium	0.92	0.92	1.02
DCP	0.25	0.25	0.50	P	0.40	0.40	0.49
Vit. min. prem.*	0.30	0.30	0.30	Na	0.22	0.22	0.34

^{*}Supplied per kilogram of diet: 3.000 mg Vitamin D3, 15.000 mg Vitamin A, 30 mg Vitamin E, 5 mg Vitamin B1, 0.3 mg Selenium, 50 mg Manganese, 0.15 mg Cobalt, 50 mg Ferrum, 300 mg Niacin, 0.8 Iodine, 10 mg Copper.

Table 2. Effect of flock diversity, year, estrus type and insemination type on reproductive traits in Hair goats.

traits in Hair	0							
Variation	•	Infertility	Birth		Single birth	_		Kid
Sources	rate	rate	rate	rate	rate	rate	size	yield
Flock								
1	90.68	9.32	86.44	4.67	74.51	25.49	1.16	79.85
2	76.12	23.88	68.66	9.80	83.70	16.30	1.29	111.86
Chi-square	9.397	9.397	11.200	1.166	1.269	5.412	0.303	3.467
P	0.002	0.002	0.001	0.209	0.159	0.015	0.582	0.063
Year								
2012	71.70 ^b	27.36 ^a	63.21 ^b	11.84	85.07	14.93 ^b	1.15	72.64
2013	91.07^{a}	8.93^{b}	87.50 ^a	5.88	81.63	18.37^{ab}	1.18	103.57
2014	91.11 ^a	10.00^{b}	86.67 ^a	3.66	71.79	28.21 ^a	1.33	115.56
Chi-square	14.194	14.194	19.611	2.358	4.922	8.052	0.494	5.301
P	0.001	0.001	0.0001	0.308	0.085	0.018	0.781	0.071
Estrus type								
Sponge	76.43	23.57	69.29	9.35	75.26	24.74	1.18	101.79
Spontaneous	91.07	8.93	86.61	4.90	82.47	17.53	1.29	89.29
Chi-square	8.421	8.421	10.536	0.391	9.703	0.062	0.227	0.523
P	0.004	0.004	0.001	0.532	0.002	0.804	0.634	0.470
Insemination	ı type							
Artificial	66.67	33.33	63.41	4.88	75.64	24.36	1.29	82.11
Traditional	98.45	1.55	89.92	8.66	81.03	18.97	1.19	106.98
Chi-square	42.726	42.726	24.970	2.258	16.368	0.031	0.186	2.121
P	0.000	0.000	0.000	0.133	0.000	0.861	0.666	0.145
Overall	82.94	17.06	76.97	7.18	60.71	16.27	1.23	94.84

P: Statistical significance value,

^{a, b, c}: Means with different superscripts in the same column significantly differ (P<0.05).

Table 3. Effect of doe age and live weight on reproductive traits in Hair goats.

Variation	Pregnancy			*	Single Birth		Litter	Kid
Sources	rate	rate	rate	rate	rate	rate	size	yield
Age of doe								
2	73.68	26.32	68.42 ^{ab}	7.14	100.00	0.00^{b}	1.00	68.42
3	75.36	24.64	63.77^{b}	15.38	81.82	18.18^{ab}	1.18	75.36
4	82.61	17.39	73.91 ^{ab}	7.89	88.24	11.76 ^b	1.12	82.61
≥5	88.98	11.02	87.29 ^a	2.86	71.84	28.16^{a}	1.32	115.25
Chi-square	6.998	6.998	14.904	6.417	3.172	12.713	0.777	5.210
P	0.072	0.072	0.002	0.093	0.366	0.005	0.855	0.157
Live weight	of doe							_
25.0-29.9	62.50 ^b	37.50 ^a	46.88 ^b	25.00	100.00	0.00^{b}	1.00	46.88
30.0-34.9	75.00^{ab}	25.00^{a}	69.44 ^{ab}	7.41	92.00	8.00^{a}	1.08	75.00
35.0-39.9	80.95^{ab}	19.05^{a}	73.81 ^a	8.82	77.42	22.58^{a}	1.23	90.48
40.0-44.9	89.58 ^a	10.42^{ab}	87.50^{a}	2.33	71.43	28.57^{a}	1.29	112.50
45.0-49.9	91.18 ^a	8.82^{b}	88.24 ^a	3.23	73.33	26.67^{a}	1.33	117.65
≥50.0	90.00^{a}	10.00^{ab}	85.00^{a}	5.56	76.47	23.53^{a}	1.27	108.33
Chi-square	16.408	16.408	25.367	7.396	3.700	13.870	0.737	8.336
P	0.006	0.006	0.000	0.193	0.593	0.016	0.981	0.139
Overall	82.94	17.06	76.97	7.18	60.71	16.27	1.23	94.84

P: Statistical significance value.

Table 4. Effect of flock diversity, year, estrus type, insemination type, birth type, doe age, and doe live weight on gestation length and number of services per conception in Hair goats.

Variation Sources	n	GL (day)	NSPC
Flock		NS	NS
1	102	148.4±0.38	1.67±0.13
2	92	149.0±0.36	1.70 ± 0.12
Year		NS	NS
2012	67	148.8 ± 0.42	1.76±0.14
2013	49	148.0 ± 0.43	1.69 ± 0.15
2014	78	149.4 ± 0.40	1.62 ± 0.15
Estrus type		NS	*
Sponge	97	148.7 ± 0.34	1.80 ± 0.12
Spontaneous	97	148.8 ± 0.40	1.58 ± 0.14
Insemination type		NS	***
Artificial	78	148.6±0.38	2.20±0.13
Traditional	116	148.8 ± 0.37	1.18±0.13
Birth type		NS	NS
Single	153	148.8 ± 0.25	1.66±0.18
Multiple	41	147.7 ± 0.53	1.72±0.09
Age of doe		NS	*
2	13	149.1±0.78	1.74 ± 0.27^{a}
3	44	148.5 ± 0.45	1.74 ± 0.21^{a}
4	34	149.0 ± 0.60	1.69 ± 0.16^{ab}
≥5	103	148.3 ± 0.30	1.58 ± 0.11^{b}
Live weight of doe		NS	*
25.0-29.9	15	147.9 ± 0.67	1.80 ± 0.23^{a}
30.0-34.9	25	148.6 ± 0.56	1.75 ± 0.18^{ab}
35.0-39.9	31	148.3±0.54	1.70 ± 0.19^{bc}
40.0-44.9	42	148.7 ± 0.53	1.63 ± 0.17^{cd}
45.0-49.9	51	149.1 ± 0.52	1.55 ± 0.19^{d}
≥50.0	30	149.8±0.48	1.70 ± 0.18^{bc}
Overall	194	148.7±0.19	1.65±0.08

^{a, b}: Means with different superscripts in the same column significantly differ (P<0.05).

NS: Not significant (P>0.05); *: P<0.05; **: P<0.01; ***: P<0.001.

a, b, c, d: Means with different superscripts in the same column significantly differ (P<0.05).

GL: Gestation length; NSPC: Number of services per conception.

Values (except overall) represent the least square mean \pm standard error.

Table 5. Effects of flock diversity, year, estrus type, insemination type, birth type, doe age,

and doe live weight on milk yield characteristics in Hair goats.

Variation Sources	n	LL	LMY	DMY
Flock		NS	NS	NS
1	102	161.42±4.35	131.18±6.40	0.797±0.028
2	92	158.66±3.99	127.40±5.88	0.790 ± 0.026
Year		NS	*	*
2012	67	156.54±4.35	121.54±6.40 ^b	0.755±0.028 ^b
2013	49	159.56±4.70	126.89 ± 6.92^{b}	0.784 ± 0.030^{b}
2014	78	164.02±4.95	139.44 ± 7.29^{a}	0.841 ± 0.032^{a}
Estrus type		NS	NS	NS
Sponge	97	160.06±3.96	126.25±5.83	0.778±0.026
Spontaneous	97	160.02±4.46	132.33 ± 6.57	0.808 ± 0.029
Insemination type		NS	NS	NS
Artificial	78	161.63±4.18	129.92±6.15	0.794±0.027
Traditional	116	158.45±4.26	128.66 ± 6.28	0.792 ± 0.027
Birth type		NS	*	NS
Single	153	156.34±5.87	124.25±8.65	0.788±0.038
Multiple	41	161.74±3.02	133.33±4.45	0.809 ± 0.019
Age of doe		***	***	***
2	13	142.92±9.48°	102.97±13.96°	0.714±0.061 ^b
3	44	145.64 ± 4.73^{c}	109.79±6.97°	0.734 ± 0.031^{b}
4	34	168.20 ± 6.41^{b}	140.73 ± 9.43^{b}	0.843 ± 0.041^a
≥5	103	183.40±3.15 ^a	163.66 ± 4.64^{a}	0.882 ± 0.020^a
Live weight of doe		*	*	*
25.0-29.9	13	149.34±5.56 ^{bc}	121.13±8.18 ^{cd}	0.778 ± 0.036^{bcd}
30.0-34.9	26	152.43±5.82°	119.67±8.58 ^d	0.766 ± 0.038^{cd}
35.0-39.9	29	160.48 ± 7.33^{bc}	114.09±10.79 ^d	0.721 ± 0.047^d
40.0-44.9	41	165.05±5.84 ^a	135.40±8.60 ^{bc}	0.806 ± 0.038^{ab}
45.0-49.9	53	169.73±5.23 ^a	148.27±7.69 ^a	0.862 ± 0.034^{a}
≥50.0	32	163.21±6.02 ^{ab}	137.18±8.86 ^{ab}	0.828 ± 0.039^{abc}
	194	170.93±2.51	145.58±3.80	0.835±0.014

NS: Not significant (P>0.05); *: P<0.05; ***: P<0.001.

Table 6. Phenotypic correlations among reproductive traits and milk yield characteristics of Hair goats.

	LMY	LL	DMY	GL	NSPC
LL	0.864**				
DMY	0.903**	0.587^{**}			
GL	0.070	0.104	0.020		
NSPC	-0.062	-0.019	-0.080	0.080	
Litter Size	0.211^{*}	0.187^{*}	0.176^{*}	0.045	0.090

^{*:} P<0.05; ***: P<0.001; LL: lactation length; LMY: lactation milk yield; DMY: daily milk yield; GL: gestation length; NSPC: number of services per conception.

CONCLUSION

In this study, it was determined that the majority of fertility parameters in Hair goats were influenced by flock diversity, year, estrus synchronization type, insemination type, doe age and doe live weight. It was also determined that milk yield parameters were influenced by estrus synchronization type, birth type, and doe age and live weight. The highest performances in the Hair goats regarding fertility

^{a, b, c, d}: Means with different superscripts in the same column significantly differ (P<0.05).

Values (except overall) represent the least square mean \pm standard error.

LL: Lactation length, LMY: Lactation milk yield, DMY: Daily milk yield

traits and milk yield were determined in 5-year-old goats, and in goats between 45.0-49.9 kg live weight. In addition, it was observed that kid yield was increased by synchronization with the fluorogestone acetate intravaginal sponge and in goats bred via the traditional method, and a positive correlation was identified between litter size and milk yield parameters. The results further indicated that increased performance can be minimizing attained by the effects environmental factors. While environmental effects be eliminated completely, cannot precautions, such as providing better nutrition regimes during extreme climatic conditions, providing green feeds and better management in farms, and providing multivitamin and mineral supplements during pregnancy, may contribute to better performance.

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RESEARCH ARTICLE

Effects of Ice Cream Produced with Lemon, Mandarin, and Orange Peel Essential Oils on Some Physicochemical, Microbiological and Sensorial Properties

Oktay TOMAR 1*, Gökhan AKARCA1

¹Engineering Faculty, Food Engineering Department, Afyon Kocatepe University, Afyonkarahisar 03200, Turkey

ABSTRACT

In this study, some physicochemical, microbiological and sensorial properties of ice cream produced with essential oils obtained from lemon (L), mandarin (M), and orange (P) peels at different ratios (0.1%, 0.3%, and 0.5%) were investigated. The highest amount of dry matter was determined in sample P3 which contains 0.5% lemon peel essential oil as 39.36%. The pH and % acidity values of the samples were found to vary between 6.36-6.45 and 0.22-0.27, respectively. At the end of the study, the lowest total aerobic mesophilic bacteria (3.80 log CFU/g), psychrophilic bacteria (4.01 log CFU/g), yeast and mold (3.71 log CFU/g) and *Pseudomonas* spp. (2.04 log CFU/g) counts were determined in ice cream sample P3. The total coliform group bacteria, *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* were not detected in any of the ice cream samples. As a result of the sensory evaluation, it was determined that the most admired sample was P3 and the least preferred sample was the control sample.

Keywords: Essential Oil, Ice Cream, Lemon, Mandarin, Orange

Limon, Mandalina ve Portakal Kabuk Esansiyel Yağlarıyla Üretilen Dondurmaların Bazı Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikleri Üzerine Etkileri

ÖZ

Bu çalışmada farklı oranlarda (%0.1, %0.3, %0.5) limon (L), mandalina (M) ve portakal (P) kabuklarından elde edilen esansiyel yağlarla üretilen dondurmaların bazı fizikokimyasal, mikrobiyolojik ve duyusal özellikleri araştırılmıştır. En yüksek kuru madde miktarı 39.36% ile %0,5 oranında limon kabuk esansiyel yağı içeren P3 örneğinde saptanmıştır. Örneklerin pH ve % asitlik değerlerinin ise sırasıyla; 6.36-6.45 ve 0.22-0.27 arasında değiştiği belirlenmiştir. Araştırma sonucunda en düşük toplam aerobik mezofilik bakteri (3.80 log kob/g), psikrofilik bakteri (4.01 log kob/g) maya küf (3.71 log kob/g) ve *Pseudomonas* spp. (2.04 log kob/g) sayılarıyla P3 dondurma örneğinde tespit edilmiştir. Dondurma örneklerinin hiçbirinde toplam koliform grup bakteri, *Salmonella* spp., *Staphylococus aureus, Escherichia coli ve Listeria monocytogenes* türü bakteri gelişimi tespit edilmemiştir. Duyusal değerlendirme sonucunda en çok beğenilen örnek P3 olmuşken, kontrol örneği ise en az tercih edilen örnek olduğu tespit edilmiştir.

Anahtar Kelimeler: Dondurma, Esansiyel Yağ, Limon, Mandalina, Portakal

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ORCID ID; OT: 0000-0001-5761-7157, GA: 0000-0002-5055-2722

*Corresponding author e-mail: oktomar@aku.edu.tr

INTRODUCTION

Ice cream which is claimed to be made by the Chinese 3000 years ago for the first time (Tekinşen 2000) is one of the oldest dairy products preferred all over the world (Manoharan and Ramasamy 2013). Nowadays, its sales at the global level increase nearly by 5% every year and have exceeded 73.8 billion dollars (Goff and Hartel 2013). Although its definition varies among the countries, ice cream is expressed as a milk product which is produced by cooling and mixing and transforming into pasty (Clark 2012). In addition to milk obtained from different dairy animals (Marshall et al. 2012) ice cream may contain many more components as well as sugar, oil, stabilizer and emulsifier in the mixture (Corvitto 2011).

Ice cream is a food product with an extremely rich nutritional value. The nutritional value of ice cream directly depends on the nutritional values of the components that make up ice cream. Ice cream also has all nutritional elements owned by milk. Furthermore, ice cream contains 3-4 times more oil and more proteins by 12% - 16% compared to milk. In addition to these, it is a richer product than milk due to the addition of additives such as fruits, nuts, and eggs (Arbuckle 2011).

Citrus fruits are the plants that belong to the Rutaceae family and are grown in the Mediterranean countries such as Italy, Spain, Turkey and Egypt (Di Vaio 2010). Citrus fruits are composed of many species, mainly sweet orange (Citrus aurantium L. subsp. dulcis), sour orange (Citrus aurantium L. subsp. amara), lemon (Citrus limon L.), mandarin (Citrus reticulata L.) and grapefruit (Citrus paradisi L.) (Bampidis and Robinson 2006). Citrus fruits take an important place in human nutrition since they have high vitamin and mineral content. Furthermore, citrus fruits and their process wastes are also used in various industrial areas (Baratta et al. 1998).

Citrus peels are frequently used in various fields in the industry due to their high essential oil content (Bampidis and Robinson 2006). In general, citrus essential oils are rich in monoterpenic hydrocarbons (at ratios reaching 97.3g /100 g) and basically contain d-limonene (IARC 1993). As a result of these studies, it has been revealed that these oils have antioxidant, antibacterial, antimicrobial and antiparasitic activity (Singh et al. 2010).

Lemon (Citrus limon) has many important natural chemical components including citric acid, ascorbic acid, minerals, flavonoids, and essential oils. Lemon leaves are used in folk medicine for the treatment of obesity, diabetes, blood lipid lowering, cardiovascular diseases, brain disorders and certain types of cancer (Lidianne et al. 2011). The essential oils extracted

from lemon peel are commonly used in perfume, cosmetics and food industries (Aloisi et al. 2002). Mandarin (*Citrus reticulata* L.) peel essential oils are predominantly composed of hydrocarbons. Limonene is the main component (52.2 - 96.2%), and Υ-terpinene (tr-36.7%), α-Pinene (0.1-2.1%), linalool (0.1-2.5%), myrcene (1.3- 1.8%) and sabinene (0.1-1.3%) are other components (Lota et al. 2000). Mandarin essential oils are frequently used among people due to their relaxing, antiseptic and antifungal properties (Azadi et al. 2012).

Orange (Citrus aurantium L.subsp. dulcis) essential oils mainly contain β -pinene, limonene, trans- β -ocimene, linalool and α -terpineol (Sarrou et al. 2013). Orange blossom and blossom juice are used as tranquilizers and diuretic agents in folk medicine (Azadi et al. 2012).

In this study, it was aimed to investigate the effects of essential oils obtained from lemon, orange and mandarin peels on physical, chemical, microbiological and sensorial quality properties of the products obtained by the use of them in ice cream production.

MATERIALS and METHODS

Hydrodistillation

After fresh lemon (Citrus limon L.) and mandarin (Citrus reticulata L.) and orange (Citrus aurantium L.subsp. dulcis) peels were crushed in a blender (Waring 8010S/G, USA), 200 g was taken and subjected to hydrodistillation for 3 hours up to the point when the oil in matrix was depleted using a Clevenger type apparatus in accordance with the European Pharmacopoeia Specification (Anonymous 1996). The essential oils collected were dried on anhydrous sodium sulfate and stored at 4 °C until use.

Production of ice cream

The production of ice cream was performed by modifying the formulation indicated by Sağdıç et al. (2012). 7 L of cow's milk (4% fat) was used in ice cream production. At first, the fat content of ice cream was standardized to 9% using milk cream (60% fat) (400 mL). Then, the mixture was subjected to heat treatment at a moderate temperature. At the same time, milk powder (650 g), salep (30 g), emulsifier (20 g) (commercial mixture of mono- and diglycerides), stabilizer (gelatine) (8 g) and sugar (1500 g) were combined and mixed continuously and then slowly added to the mixture at 45-55 °C. After the mixture was pasteurized at 85 °C for 1 min, it was cooled to 43 °C. The mix was rested for 4-5 h until the temperature decreased to 4 °C. The mix ready for processing was divided into 10 equal parts under aseptic conditions. The samples were formed by adding lemon, mandarin, and orange peel essential oils at different ratios to the mixes divided (Table 1).

Then, the mixes were frozen by mixing at -5 °C in the ice cream freezer (CRM,-GEL 25C, Italy). 250 g of each mixture was taken into sterile glass containers and hardened at -24 °C. Then, the samples were stored at -18 °C until their analyses were completed.

Physicochemical Analyses

The dry matter, oil and % acidity values of the ice cream produced by adding different citrus essential oils were calculated according to AOAC (2016). The pH values of the samples were measured using the Ohaus (ST 5000) device.

Microbiological Analyses

10 g of ice cream samples were taken into a stomacher bag under sterile conditions, 90 ml of sterile ringer solution (1:9 w/v) was added, and it was homogenized in the stomacher (Lab-Blender 400, London, UK) for 3 minutes. 1 ml of this sterilized mixture was taken and added to the tube containing 9 ml of sterile ringer solution and mixed with vortex (IKA MS3, Germany). Thus, 10-2 dilution was prepared. The process was maintained in the same way, and serial dilutions were prepared until series 10-6 (Anonymous 2001).

Ice cream samples were subjected to total aerobic mesophilic bacteria (ISO 2013), psychrophilic bacteria (ISO 2013), yeast and mold (ISO 2008), total coliform (ISO 1991a), *Pseudomonas* spp. (ISO 2010), *Salmonella* spp. (ISO 2017a), *Staphylococcus aureus* (ISO 1991b), *Escherichia coli* (ISO 2001, ISO 2015), and *Listeria monocytogenes* (ISO 2017b, ISO 2017c) count analyses.

Sensory Analyses

In the sensory evaluation of the ice cream samples, the scorecards created by modifying the sensory test parameters indicated by Akarca et al. (2016) were used, and the differences were determined by the scoring system on a scale. Sensory panel was carried out by twenty trained panelist that were from the Food Engineering Department of Afyon Kocatepe University. Sample were evaluated for, color and appearance, texture and consistence, taste and odor and general appreciation with a hedonic scale between 1-9 as follows: 1-3 (not acceptable), 4-5 (fairly acceptable), 6-7 (good aceptable), 8-9 (very good) (Önoğur and Elmacı 2012, Anonymous 2012).

Statistical Analysis

The statistical analysis of the results was performed using the SPSS statistical package program (SPSS Inc., USA). Data obtained from the study were analyzed by one-way analysis of variance in order to test for significant differences between treatments. Significance of differences was defined as P < 0.05. When we observed any differences among the groups, Duncan test was also applied in order to determine the significance levels.

RESULTS and DISCUSSION

Physicochemical Analyses

The physicochemical analysis results of ice cream samples are presented in Table 2. In our ice cream samples, the highest dry matter value was determined by 39.36% in the sample with 0.5% lemon peel addition in its production, and the lowest dry matter value was determined in the control sample by 38.17% (P > 0.05).

Similarly to the results of our study, Macit et al. (2017) produced ice cream samples using some spice essential oils and reported that they determined the highest dry matter value in the samples with 0.4% lemon peel addition in their production by 39.81% and the lowest dry matter value in the control sample by 39.21%.

In the study, the pH values of the samples were found to vary between 6.36 and 6.45, and the lowest pH value was determined in the sample with 0.5% lemon peel essential oil addition in its production, while the highest pH value was determined in the control sample. It was determined that the difference between the samples was not statistically significant (P > 0.05). Pater et al. (2004) indicated that the pH values of 50 samples taken from ice cream offered for sale in Elazığ varied between 6.05 and 7.41.

The % acidity values of the samples were determined to vary between 0.22 and 0.27. It was determined that the sample with 0.5% lemon essential oil addition in its production had the lowest degree of acidity (P > 0.05). Öksüztepe et al. (2005) reported that they determined that the % acidity values of 50 samples taken from fruit and flavored ice cream in Elazığ varied between 0.12 and 0.48.

The pH and % acidity values obtained in other relevant studies are different from the results of this study. It is thought that the difference is caused by the substances used in the formulation of the mixture applied in ice cream production and the usage rates. It was determined that the amounts of oil of the ice cream samples produced by adding different citrus peel essential oils were very close to each other (14.24% - 14.26%). Similarly to the results of our study, Macit et al. (2017) reported that oil ratios varied between 14.10% and 14.50% in ice cream samples produced by adding lemon essential oil at two different ratios, and they stated that the essential oils added had no effect on the oil ratio of ice cream.

Microbiological Analyses

In our study, it was determined that the highest total aerobic bacteria (Figure 1) and psychrophilic bacteria counts (Figure 2) were 5.12 and 4.94 log CFU/g, respectively, in the control sample (P < 0.05) and that the lowest total aerobic bacteria and psychrophilic

bacteria counts were 3.80 and 4.01 log CFU/g, respectively, in the samples with 5% lemon peel essential oil addition in their production (P < 0.05). In their study, Akarca and Kuyucuoğlu (2009) reported that the total aerobic bacteria counts varied between 2.99 and 6.17 log CFU/g in unpacked ice cream samples sold across Afyonkarahisar province. Similarly, Bostan and Akın (2002) investigated the microbiological qualities of industrial ice creams in their study, and they determined that the total aerobic bacteria counts of the samples varied between 2.00 and 4.26 log CFU/g.

Çubukçı (2016) reported that the psychrophilic counts of ice cream sold in Erzurum market varied between 1.5 and 7.26 log CFU/g, while Mukan and Evliya (2002) determined that the psychrophilic bacteria counts of plain-vanilla ice-cream sold in Adana market varied between 0 and 4.46 log CFU/g. The results determined by the researchers are similar to our results.

It was determined that the minimum and maximum yeast and mold counts of ice cream samples produced by adding two different citrus peel oils were 3.71 and $4.59 \log \text{CFU/g}$, respectively (P < 0.05) (Figure 3).

Korel et al. (2005) stated that the yeast and mold counts in unpacked ice cream samples sold in Manisa province were between <1 and 4.48 log CFU/g. Çınar (2010) reported that the yeast and mold counts in ice cream samples offered for sale in Tekirdağ province were between <1 and 4.62 log CFU/g.

In our ice cream samples, it was determined that the lowest *Pseudomoas* spp. count was 2.04 log CFU/g in the sample with 0.5% lemon peel essential oil in its production and that the highest count was 3.68 log CFU/g in the control sample (P < 0.05) (Figure 4).

Erol et al. (1998) determined that *Pseudomonas* spp. counts in ice cream samples sold in various pastry shops in Ankara varied between < 2.30 and 4.90 log CFU/g. The values obtained by the researchers are higher compared to the results of our study. The difference between them is thought to be due to the failure to adequately comply with the hygiene and sanitation rules in the production, storage, and sale of ice cream.

In our study, the total coliform group bacteria, Salmonella spp., Staphylococcus aureus, Escherichia coli, and Listeria monocytogenes type bacterial growth was not detected in any of the ice cream samples. As a result of the microbiological analyses, it was determined that all our ice cream samples were in conformity with all criteria specified in the Turkish Food Codex, Communique on microbiological criteria, Food safety criteria (Anonymous 2011).

In their study, Keskin et al. (2007) determined that the total coliform count in ice cream samples was between 2.48 and 5.68 log CFU/g. Coşkun (2005) stated that the presence of coliform group bacteria was determined in all plain ice cream samples sold in the provincial borders of Tekirdağ and that their count varied between 1.47 and 4.38 log CFU/g.

Erol et al. (1998) reported that they determined the development of *Escherichia coli* and *Salmonella* spp. in 2% of 100 ice cream samples offered for sale in pastry shops operating in the provincial borders of Ankara. Çubukçı (2016) reported that the presence of *Escherichia coli* and *Listeria monocytogenes* type bacteria was not detected in any of a total of 75 samples taken from ice cream offered for sale in Erzurum market.

In their study, Yücel et al. (2000) reported that *Staphylococcus aureus* counts in 30 ice cream samples offered for sale in Ankara varied between 2 and 3.48 log CFU/g. Uraz et al. (2001) carried out a study on 39 ice cream samples offered for sale in pastry shops located in different districts of Ankara province, and they reported that *Staphylococcus aureus* counts varied between 2.48 and 4.30 log CFU/g.

The total coliform group bacteria, Salmonella spp., Staphylococcus aureus, and Escherichia coli type bacteria counts obtained from similar studies by the researchers are higher than the values obtained in our study (Yücel et al. 2000, Uraz et al. 2001). It is thought that the differences were caused by the microbial quality of raw materials, auxiliaries, and additives used in the production of ice cream, and by the contaminations due to the failure to pay adequate attention to hygiene and sanitation rules in production, packaging and storage conditions.

According to the results of the microbiological analysis of ice cream samples produced by the addition of two different citrus peel essential oils, it was determined that the sample produced by adding 5% lemon essential oil had the lowest total aerobic mesophilic bacteria, psychrophilic bacteria, and yeast and mold counts. According to the analysis results, it was determined that the microorganism counts of the samples decreased as the amount of essential oil added increased.

It was indicated that the composition of lemon essential oils contained Limonene (61.68%), Neral (21.66%), β -Pinene (10.23%), Υ -Terpinene (6.42%) (Ammada et al. 2018). The antibacterial and antifungal effect of lemon essential oils in samples is thought to be caused especially by limonene, β -Pinene, and Υ -Terpinene.

The sensory analysis results of ten different ice cream samples are presented in Table 3. Panelists gave the

highest score in terms of color and appearance to the control sample by 8.66. This sample was followed by the sample with 1% lemon peel essential oil addition in production by 8.21 points. The lowest score, 7.23, was given to the sample with 5% mandarin peel oil addition in production. The increase in the amount of essential oil used in production had a negative effect on color and appearance.

In the sensory analyses conducted, in the evaluation of the structure and consistency, the panelists gave all the samples the values that were very close to each other. It was determined that the essential oils added at different ratios and types in production had no negative effect on the structure and consistency of the ice cream.

In the evaluation made in terms of taste and smell, the product with 5% lemon peel essential oil addition

in its production had the highest score by 8.93. This product was followed by the sample with 2% lemon peel essential oil addition in production with a score of 8.76 and the sample with 1% lemon peel essential oil addition in production with a score of 8.27. The samples with citrus essential oils addition were scored higher than the control sample by the panelists participating in the analysis. Similarly, the samples with lemon peel essential oil addition had higher taste and smell scores than the samples with orange and mandarin peel essential oil addition.

In terms of general admiration, the sample with 5% lemon peel essential oil addition had the highest score of 8.85 among ten different ice cream samples. It was determined that the control sample was the least admired sample by the panelists with a general admiration score of 7.56.

Table 1. Essential oils and ratios

Samples	Added essential oils and ratios	
K	Control Sample	
L1	% 0.1 Lemon peel essential oil	
L2	% 0.3 Lemon peel essential oil	
L3	% 0.5 Lemon peel essential oil	
M1	% 0.1 Tangerine peel essential oil	
M 2	% 0.3 Tangerine peel essential oil	
M3	% 0.5 Tangerine peel essential oil	
P1	% 0.1 Orange peel essential oil	
P2	% 0.2 Orange peel essential oil	
P3	% 0.5 Orange peel essential oil	

Table 2. Physicochemical Analysis Results of Ice Cream Samples

Samples	Dry Matter (%)	pН	Acidity (%)	Fat (%)
K	38.17	6.45	0.27	14.26
L1	38.41	6.41	0.25	14.25
L2	38.86	6.39	0.23	14.25
L3	39.24	6.36	0.22	14.24
M1	38.39	6.43	0.26	14.26
M2	38.88	6.39	0.25	14.25
M3	39.33	6.38	0.24	14.25
P1	38.40	6.43	0.26	14.26
P2	38.84	6.38	0.25	14.25
P3	39.36	6.37	0.23	14.24

Table 3. Sensory evaluation results of ice cream samples

Samples	Color and Appearance	Structure and Consistency	Taste and Smell	General Appreciation
K	8,66a	8,85a	7,63d	7,56e
L1	8,08b	8,93a	8,27b	8,42b
L2	7,65c	8,88a	8,76a	8,56ab
L3	7,49d	8,95a	8,93a	8 , 85a
M1	8.16b	8.78a	7.80c	7.58e
M2	7.46d	8.80a	7.95c	7.85d
M3	7.23e	8.89a	8.12b	8.04c
P1	8,21b	8,77a	8,06b	8,27b
P2	7,76c	8,82a	8,24b	8,35b
P3	7,32de	8,91a	8,82a	8,42b

Means within a column with different letters are significantly different (P < 0.05).

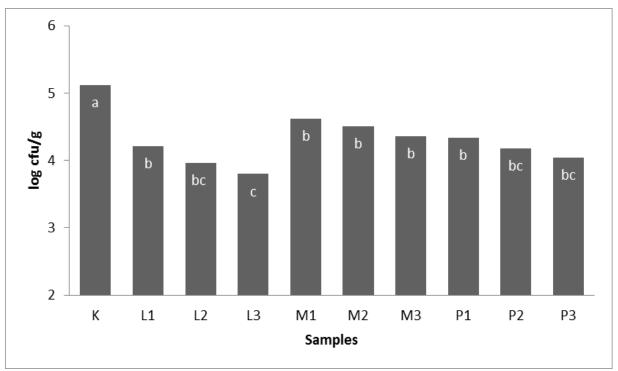


Figure 1. Total aerobic mesophilic bacteria counts of ice cream samples (Log CFU /g). Means within a factor with different letters are significantly different (P < 0.05).

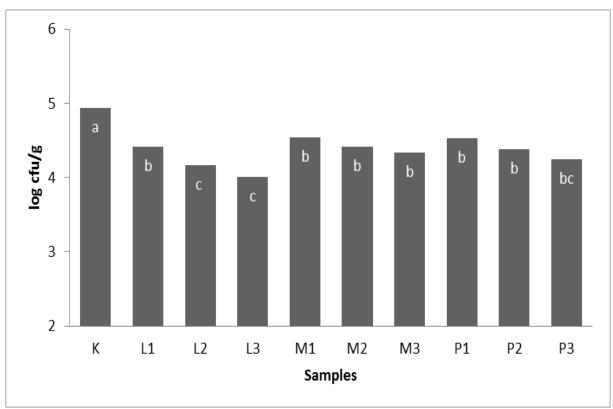


Figure2. Psychophilic bacteria counts of ice cream samples (Log CFU/g). Means within a factor with different letters are significantly different (P < 0.05).

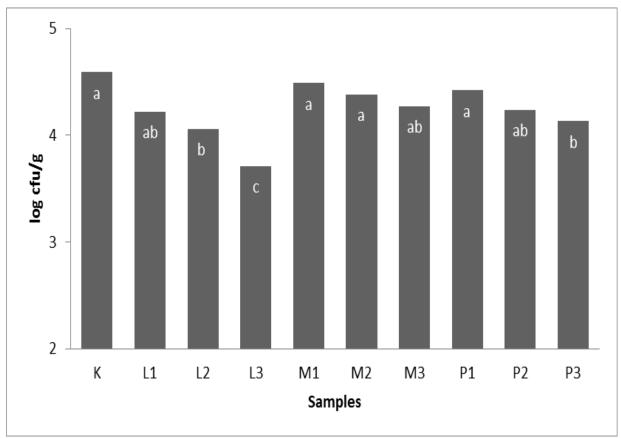


Figure 3. Yeast / mold numbers of ice cream samples (Log CFU/g). Means within a factor with different letters are significantly different (P < 0.05).

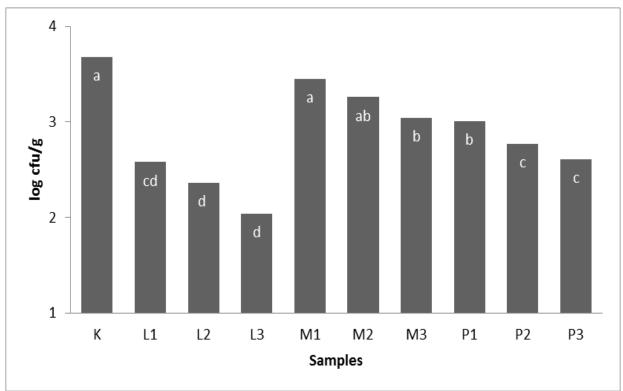


Figure 4. *Pseudomonas* spp. number of ice cream samples (Log CFU/g). Means within a factor with different letters are significantly different (P < 0.05).

CONCLUSION

Lemon, mandarin and orange peels are the most important residues of many processes, especially fruit juice. Although the essential oils obtained from these

residues are today utilized in many different areas such as cosmetics, pharmaceuticals, and chemistry, they are not much preferred in the food industry. The data obtained as a result of the study reveal that these essential oils have high antibacterial and antifungal properties. Furthermore, it has been determined that when they are added to a product which is largely consumed throughout the world such as ice cream, they do not impair the sensorial properties, on the contrary, they improve them. Along with the studies to be carried out, the usability of these oils in other fields of the food industry, especially in other dairy products, can be proven.

Nowadays, the products produced by using chemical additives have become less preferred by consumers with each passing day. Therefore, food manufacturers have been obliged to use more natural additives. It is obvious that lemon and orange peel essential oils can be used in food industry as antimicrobial preservatives and flavor enhancers.

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RESEARCH ARTICLE

Levels of Serum 25 (OH) D3 in Naturally Infected Lambs With *Giardia* duodenalis

Güzin ÇAMKERTEN*1 Hasan ERDOĞAN2, Deniz ALIÇ URAL3, İlker ÇAMKERTEN4, Songül ERDOĞAN2, Kerem URAL2

¹Aksaray University, Vocational School of Technical Sciences, Department of Veterinary Medicine, Aksaray, Turkey

²Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine, Aksaray, Turkey

³Adnan Menderes University, Veterinary Faculty Animal Farm, Aksaray, Turkey

⁴Aksaray University, Faculty of Veterinary Medicine, Department of Internal Medicine, Aksaray, Turkey

ABSTRACT

Giardia duodenalis is a binuclear flagellated protozoan that can infect birds, reptiles and many mammals. The pathogen is one of the most important causes of diarrhea in humans and farm animals. The aim of this study was to determine the changes in serum 25-hydroxyvitamin D3 levels in naturally infected lambs with Giardia duodenalis. The animal material of the study was used Sakız sheep which had naturally infected with Giardia duodenalis between 15-35 days of age from the Aydın region (n = 30), healthy lambs in the same age range (n = 15) and both sexes. Sterile stool samples with the method of rectal touching of fecal samples from infected and healthy animals, blood samples were obtained in a total of 5 ml of v.jugularis according to the technique in order to determine serum 25-hydroxyvitamin D3 levels. Giardia screening was performed by direct microscopic examination of stool samples. 25-OH-D levels were measured with Savant brand fluorescent immunoassay device from the obtained serum samples. Mann-Whitney U test was used in statistical analysis. Statistically significant changes were observed in 25-hydroxyvitamin D3 levels of healthy and natural infected animals (p <0.05).

Keywords: Giardiasis, Lamb, 25-hydroxy vitamin D3

Giardia duodenalis ile Doğal Enfekte Kuzularda Serum 25 (OH) D3 Seviyeleri

ÖZ

Giardia duodenalis kuşlar, reptiller ve bir çok memeli hayvanı enfekte edebilen binükleuslu flagellalı bir protozoondur. Söz konusu patojen insanlarda ve çiftlik hayvanlarında başta olmak üzere şekillenen ishalin en önemli nedenleri arasında yer almaktadır. Çalışmada Giardia duodenalis ile doğal enfekte kuzularda serum 25-hidroksi vitamin D3 seviyelerindeki değişimlerin belirlenmesi amaçlanmıştır. Araştırmanın hayvan materyalini Aydın bölgesinde bulunan yaşları 15-35 günlük arasında her iki cinsiyetten sakız ırkı Giardia duodenalis ile doğal enfekte (n=30), benzer yaş aralığındaki aynı ırktan (n=15) ve her iki cinsiyetten sağlıklı kuzular oluşturdu. Enfekte ve sağlıklı hayvanlardan dışkı örnekleri rektal tuşe yöntemi ile steril dışkı örnekleri, serum 25-hidroksi vitamin D3 seviyelerinin belirlenmesi amacı ile de tekniğine uygun olarak v.jugularisden toplamda 5 ml olacak şekilde kan örnekleri alındı. Dışkı örneklerinden direkt mikroskobik bakı yöntemi ile giardia taraması yapıldı. Elde edilen serum örneklerinden Savant marka flöresan immunoassay cihazı ile 25-OH-D seviyeleri ölçüldü. İstatistiksel analizde Mann-Whitney U testinden yararlanıldı. Sağlıklı ve doğal enfekte hayvanlara ait 25-hidroksi vitamin D3 seviyeleri arasında istatistiksel (p<0,05) anlamlı değişimlerin olduğu belirlendi.

Anahtar Kelimeler: Giardiasis, kuzu, 25-hidroksi vitamin D3

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ORCID ID; GÇ: 0000-0003-2732-9490, HE: 0000-0001-8109-8537, DAU: 0000-0002-2659-3495, İÇ: 0000-0002-6952-7703,

SE: 0000-0002-7833-5519, KU: 0000-0003-1867-7143 *Corresponding author e-mail: oguzalperen@hotmail.com

GİRİŞ

Giardia duodenalis kuşlar, reptiller ve bir çok memeli hayvanı enfekte edebilen binükleuslu flagellalı bir protozoondur. Söz konusu patojen insanlarda ve çiftlik hayvanlarında başta olmak üzere şekillenen ishalin en önemli nedenleri arasında yer almaktadır (Aytuğ ve ark. 1990). Özellikle dışkı ile yüksek miktarda kist saçılımına bağlı olarak gerek genç hayvanların gerekse de çevrenin önemli ölçüde kontamine olmasına neden olmaktadır (Gökçe ve ark. 2010). Özellikle çiftliklerde çalışan insanlar göz önüne alındığında hastalığın zoonotik önemi de göz ardı edilemez ölçüde olduğu görülmektedir (Radostits ve ark. 2007). Giardiasis' in sağaltımında dimetridazole, albendazol, nitazoxanide ve metronidazol gibi ajanların kullanımı beşeri ve veteriner hekimlikte bilinmektedir (Aytuğ ve ark. 1990, Baljer ve Wieler 1989, Hall ve ark. 1996, Bilal 2005).

Giardiosis'e bağlı hastalıkların şekillendiği vakalarda etkenin özellikle bağırsak lümenine zarar verdiği bu sebeple bozulan bağırsak lümeni mimarisinin malabsorbsiyon gibi olumsuz sonucların şekillenmesine neden olduğu bilinmektedir (Lunn ve Northrop 1992, Read ve ark. 2002). Hastalığa bağlı olarak gelişen villöz atrofi ve mukozal hasar ile giardianın neden olduğu sekrotorik değişimlere bağlı olarak glukoz, sodyum gibi önemli moleküllerin emiliminde eksiklikleri beraberinde getirmekte ve sıvı kavıplarına bağlı olarak da hastaların genel durumlarında önemli derecede bozulmalar görülmektedir 2008). Farklı (Buret helmint enfestasyonlarına bağlı ishal şekillenen çocuklarda yapılan bir araştırmada Giardiasis ile enfekte olan çocukların vitamin D düzeylerinde kontrol grubuna göre istatistiksel anlamlı farklılıkların olmadığı ancak söz konusu değisimler ele alındığında çocukların bir örnek yaş gruplarında bulunmaması ve coğrafi ve beslenme açısından farklılıklarına bağlı olarak söz konusu durumun şekillendiği bildirilmektedir (Voloc 2015). Okul yaşındaki çocuklarda ise Giardia lamblia infeksivonlarının vitamin Α emiliminde karaciğerdeki vitamin A depolarında da azalmalara neden olduğu ortaya konulmaktadır (Astiazaran-Garcia ve ark. 2010). Söz konusu çalışmada Giardia duodenalis ile doğal enfekte kuzularda serum 25hidroksi vitamin D₃ seviyelerindeki değişimlerin belirlenmesi amaçlanmıştır.

MATERYAL YÖNTEM

Hayvan materyali ve örneklerin alınması

Araştırmanın hayvan materyalini Aydın bölgesinde bulunan yaşları 15-35 günlük arasında her iki cinsiyetten sakız ırkı *Giardia duodenalis* ile doğal enfekte (n=30), komşu işletmede yer alan benzer yaş aralığındaki aynı ırktan (n=15) ve her iki cinsiyetten anamnez ve klinik bulgular ışığında sağlıklı olduğu belirlenen kuzulardan secildi. Enfekte ve sağlıklı

hayvanlardan dışkı örnekleri rektal tuşe yöntemi ile steril dışkı kaplarının içerisine, serum 25-hidroksi vitamin D₃ seviyelerinin belirlenmesi amacı ile de tekniğine uygun olarak *v.jugularis* aracılığı ile toplamda 5 ml olacak şekilde kan örnekleri serum tüplerine alındı.

Dışkı analizleri ve serum 25-hidroksi vitamin D₃ seviyelerinin ölçümü

Hasta ve sağlıklı hayvanlardan toplanan dışkı örneklerinin giardia enfeksiyonunun varlığının belirlenmesinde direkt mikroskobik bakı yöntemi kullanılmıştır.

Kan örnekleri alım işleminin hemen akabinde laboratuvara taşınarak 15 dakika 3000 dv/dk olacak şekilde santrifüj edildikten sonra serum örnekleri ependorflara aktarıldı. Elde edilen serum örnekleri Savant marka flöresan immunoassay cihazı yardımı ile söz konusu cihazın 25-OH-D₃ test kitleri kullanılarak (Florasan immuno kromatografi, Beijing Savant Biotechnology Co., Ltd.) üretici fırmanın belirttiği şekilde ölçümleri gerçekleştirildi.

İstatistiksel analiz

Araştırma sonucunda sağlıklı ve *Giardia duodenalis* ile doğal enfekte kuzuların serum 25-hidroksi vitamin D₃ seviyeleri tanımlayıcı istatistikleri gerçekleştirildikten sonra ortalama ve standart hata olacak şekilde belirtildi. Hasta ve sağlıklı kuzuların ortalama değerlerinin karşılaştırılmasında Mann-Whitney U testinden yararlanılarak p<0.05 değeri istatistiksel anlamlı kabul edildi. Tüm istatistiksel analizlerde SPSS 22.0 programı kullanıldı.

BULGULAR

Klinik olarak ishal semptomu gösteren tüm kuzuların dışkı analizleri sonucunda *Giardia duedenalis* ile enfekte olduğu belirlendi. Örnekleme işleminin ardından hastalara yapılan ve etkinliği kuzularda Giardia enfeksiyonlarında ortaya konulan (Ural ve ark. 2014) seknidazol kullanılarak tedavi başarılı bir şekilde sağlandı. Sağlıklı ve doğal enfekte hayvanlara ait 25-hidroksi vitamin D₃ seviyeleri arasında istatistiksel anlamlı değişimlerin olduğu belirlendi (Tablo 1).

Tablo 1. Sağlıklı ve doğal enfekte hayvanlara ait 25-hidroksi vitamin D₃ seviyeleri

Table 1. 25-hydroxy vitamin D3 levels of healthy and natural infected animals

	X ± SD	P değeri
Sağlıklı kontrol (n=15)	54.72 ± 17,40	p<0.05
Giardia duedenalis (n=30)	20.64 ± 5.94	p <0.03

TARTIŞMA

Giardia duodenalis hayvan ve insanlar arasında çapraz

enfeksiyona yol açabilir. Bu bağlamda evcil ve çiftlik

hayvanlarının dışkı ve su kaynaklı bulaş temelinden ziyade insanları enfekte edebilme potansiyelinin de bulunduğu bilinmektedir (Gaydos 1998, Robertson 2009). Özellikle koyun çiftliklerinin su kaynaklarını enfekte etmesine bağlı olarak giardiosis salgınları tehlikesinin açısından potansiyel bulunduğu bilinmekte ve insanlarda görülen enfeksiyonlar görüşü acısından rezervuar olabilecekleri bulunmaktadır (Geurden ve ark. 2011, Robertson 2009). Bu sebeple hastalığın subklinik yada klinik olarak seyir ettiği hemen tüm hayvanlarda bulaş kaynağının ortadan kaldırılması amacı ile hastaların sağaltımlarının yapılması önerilmektedir (Geurden ve ark. 2011). Çalışmamızda yer alan ve Giardia duodenalis ile doğal enfekte kuzuların tamamı seknidazol 10 mg/kg dozunda iki doz olacak şekilde sağaltılmış ve olası klinik iyileşmenin yanında hastaların insan sağlığı açısından da rezervuar teşkil etmemesi sağlanmıştır. Giardia duodenalis ile birlikte sekillenen ishalin aralıklı ve mükoz bir karakterde olduğu bu durumun malbabsorbsiyonu da tetiklediği villöz atrofinin de işin içerisine karışması ile hastalardaki klinik tablonun ağırlaştığı bildirilmektedir (Lunn ve Northrop 1992, Read ve ark. 2002, Ruest ve ark. 1997). Söz konusu bu patolojik değişimlerin özellikle genç hayvanlarda canlı ağırlık artışında meydana gelen azalmalar ve vemden vararlanma oranının düsmesi ile ekonomik yansımalarının da olduğu görülmektedir (Olson ve ark. 1995, Sweeney ve ark. 2010). Gastrointestinal bariyerin hastalıklara bağlı olarak bozulması ve enflamatorik özellikle insanlardaki hastalığında olduğu gibi değişmesinin Vitamin D seviyelerinde azalmalara neden olduğunu (Dignass 2001), vitamin D vetmezliği olusabilecek yaşayan coğrafyalarda insanların Vitamin seviyelerindeki azlığa paralel olarak enflamatorik bağırsak hastalığına yatkınlığının arttığını bildiren araştırmalar mevcuttur (Lim ve ark. 2005, Loftus 2004). Giardia enfeksiyonlarının benzer şekilde vitamin B12 seviyelerinde de azalmalara neden olduğu Giardiosis geciren hastalarda Vitamin düzeylerinin sağlıklı insanlara göre de düşük olduğu bildirilmektedir (Cordingley ve Crawford 1986). Çalışmamızda Giardia duedenalis ile doğal enfekte olan hayvanlarda 25-hidroksi vitamin D₃ seviyelerinin sağlıklı hayvanlara göre istatistiksel anlamlı düşük olduğu belirlendi. Benzer şekilde akut ishalli çocuklarda vitamin A, D ve çinko seviyelerinin azaldığı araştırma (Talachian ve ark. 2015) ile benzerlik göstermektedir. Özellikle gastrointestinal mukozal bariverin sağlamlığının kontrol altında tutulması için önemli bir role sahip olan vitamin D seviyelerinin geçirgenliği bozulmuş olan bağırsak segmentlerinde de onarici etkilerinin bulunduğu bilinmektedir (Kong ve ark. 2008). Söz konusu araştırmada vitamin D ve giardiasis arasındaki ilişkinin

tam olarak belirlenebilmesi için vitamin D seviyelerinin takibinin ishal oluşmadan (mukozal bariyer hasarı) önce oluştuğunda ishal süresince ve sağaltım sonrası gibi zamanlarda da gerçekleştirilmesi gerektiği ve araştırmanın bu yönü ile kısıtlandığı kanaatındeyiz. Beşerideki araştırmalarda göz önüne alındığında özellikle çocuklarda ishal klinik semptomu ve ishale neden olan etiyolojik nedenlerin ayrımlarının da yapıldığı ve vitamin D seviyelerinin ölçüldüğü araştırmaların sınırlı sayıda olduğu görülmektedir.

Vitamin D seviyelerindeki değişimlerin birçok hastalık ile ilişkisinin olabileceği özellikle de enflamasyonla ilişkili olduğu söylenmektedir. Vitamin periferal reseptörlerinin kandaki enflamatorik hücrelerde de bulunması bu ilişkiyi doğrulamaktadır. Özellikle T hücrelerinin cevabının azalma eğilimine girmesi Vitamin D seviyelerindeki azalmalar ile ilişkilendirilmekte ve T hücre yanıtını doğrudan etkilediği ifade edilmektedir (Nicholson ve ark. 2012, Ulitsky ve ark. 2011). Söz konusu etkiyi vitamin D'nin interferon gama ve interlökin-2 salınımını baskılayarak hücrelerinin çoğalmasını durdurarak gerçekleştirdiği bilinmektedir (Lim ve ark. 2005, Nerich ve ark. 2011). Vitamin D seviyelerinde gelen değişimlere bağlı meydana olarak proinflamatuar sitokinlerin sentezindeki azalmaların vitamin D ile enfeksiyonlar arasındaki ilişkiyi açıklar niteliktedir (Hassan ve ark. 2013). Çalışmamızda bulunan Giardia dudeonalis ile enfekte hayvanların serum vitamin D seviyelerinin sağlıklı hayvanlara göre düsüklüğü göz önüne alındığında söz konusu enfeksiyonun oluştuğu inflamatuvar cevap üzerinden vitamin D seviyelerinde azalmaların olabileceği söylenebilir.

Araştırmanın sonucunda *Giardia duedenalis* ile doğal enfekte kuzularda vitamin D seviyelerindeki azalmaların intestinal mukozal bariyerdeki hasardan etkilenebileceği düşünüldü. *Giardia duedenalis* ile enfekte olan hayvanların değerlendirildiği longitidunel araştırmalar ile Vitamin D ile hastalık arasındaki ilişkinin ortaya konulabileceği daha fazla araştırma yapılması gerekmektedir.

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RESEARCH ARTICLE

Evaluation of Adding Erythritol to Farrell Medium for Primary Isolation of the *Brucella melitensis* Strains

Mustafa Sencer KARAGÜL*

Kartepe Vocational School of Equine Science, Kocaeli University, TR-41380 Kocaeli-TURKEY

ABSTRACT

This study aims to evaluate the addition of erythritol to Farrell medium (FM) for the primary isolation of *B.melitensis* strains. Selective media, particularly FM, is commonly used for *Brucella* isolation. Isolation is the gold standard for the diagnosis of brucellosis. The isolation success of selective media depends on their ingredients. Isolation rate may decrease particularly in contaminated samples; therefore, the addition of components like erythritol that augments the growth of *Brucella* species on media can support isolation. In this study, organ samples associated with 14 small ruminant abortion cases caused by *B.melitensis* strains were utilized. Organ suspensions were inoculated to FM and Farrel medium with erythritol (F+ER) simultaneously. The media's isolation and inhibition performance was observed during incubation. The addition of erythritol was evaluated based on whether it provides earlier isolation with the help of increasing the colony growth rate and colony size. According to the findings, F+ER is more advantageous for 12 samples among 14 through isolation as it enables faster colony growth and larger colonies. The results confirm the recommendation of adding erythritol particularly in the samples with inadequate contaminant inhibition. Therefore, high isolation performance can be achieved through erythritol addition to the selective media like FM.

Keywords: Brucella melitensis, erythritol, Farrell, isolation, selective medium.

Brusella melitensis Şuşlarının İlk İzolasyonu İçin Farrell Besiyerine Eritritol İlavesinin Değerlendirilmesi

ÖZ

Bu çalışmanın amacı, *B.melitensis* şuşlarının ilk izolasyonu için selektif Farrel besiyerine (FB) eritritol ilavesini değerlendirmektir. Selektif besiyerleri ve özellikle FB, *Brusella* (B.) etkenlerinin izolasyonunda yaygın olarak kullanılmaktadır. Bruselloz teşhisinde altın standart bakteri izolasyonudur. Selektif besiyerlerinin izolasyon başarısı içeriklerine bağlıdır. Özellikle kontamine örneklerde izolasyon yüzdesi düşebilmektedir. Bu bakımdan *Brusella* türlerinin besiyerlerinde üremelerini provoke edecek eritritol gibi bileşenlerin selektif besiyerlerine ilavesi izolasyona fayda sağlayabilir. Çalışmada *Brusella melitensis* şuşlarından kaynaklı 14 küçük ruminant atık vakasına ait iç organ numuneleri kullanılmıştır. Örneklerden hazırlanan suspansiyonlar eş zamanlı olarak FB ve eritritol ilaveli Farrel besiyerine (F+ER) inokule edilmiştir. İnkubasyon boyunca besiyerleri gözlemlenmiş ve besiyerlerinin gösterdiği izolasyon ve inhibisyon performansları takip edilmiştir. Özellikle eritriol ilavesi, koloni gelişim hızı ve koloni çapı büyüklüğü sağlayarak daha erken izolasyon imkanı verebilmesi açısından karşılaştırılmıştır. 14 numune arasında 12'si için F+ER besiyerinde koloni gelişim hızı veya koloni çapı büyüklüğüne göre izolasyon için avantaj elde edilmiştir. Sonuçlar selektif besiyerine eritritol ilavesinin tavsiye edilmesini özellikle kontaminant inhibisyonun yetersiz kaldığı numuneler için desteklemektedir. Bu nedenle çalışmada olduğu gibi eritritolun, FB gibi selektif besiyeri formulasyonlarına dahil edilmesi ile yüksek izolasyon başarısının elde edilmesi mümkün görünmektedir. **Anahtar Kelimeler:** *Brusella melitensis*, eritritol, Farrell, izolasyon, selektif besiyeri.

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ORCID ID; MSK: 0000-0001-7215-5229

*Corresponding author e-mail: msencerk@hotmail.com

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INTRODUCTION

Brucellosis, caused by Brucella spp., is one of the most common zoonotic diseases (Songer and Post 2012). According to the World Health Organization (WHO), brucellosis is a worldwide zoonotic infection which causes important problems of health by risking public health and of economy through loss in husbandry (Godfroid et al 2005, Yumuk and O'Callaghan 2012). The roots of this disease might be traced in the 5th plague of Egypt around 1600 BC (Pappas et al. 2006, Seleem et al. 2010). In addition, in the WHO laboratory biosecurity manual, it is revealed that Brucella organisms belong to risk group 3 microorganisms (Perez-Sancho et al. 2015, WHO 2004). According to the WHO, all around the world, there are 500,000 brucellosis cases reported annually (Doganay and Aygen 2003, Perez-Sancho 2015). As Brucella species can be transmitted through aerosol way, it is also classified as a potential bioteror agent (Godfroid et al 2005, Songer and Post 2012). Bacteria isolation is considered to be a gold standard for the diagnosis of brucellosis (OIE 2009). However, no method other than classical bacteriology has been recommended until now, which is sufficiently sensitive for all kinds of biological samples (Garin-Bastuji 2006). For brucellosis eradication programs, test&slaughter and vaccination are important implementations (Her et al. 2010). What is more, the investigation of the epidemiological source of the disease is equally important (Krstevski 2015). The source and the spread of the infection can be determined with the help of isolation and identification of the etiological agent, which is important for this investigation. Therefore, as the diagnostic material includes plenty of fast growing organisms, it is necessary to use a selective medium for Brucella spp. isolation (Marin et al. 1996a, Stack et al. 2002). There are different types of selective media which include different basal media, antibiotic mixture, and concentration (Hornsby et al. 2000). According to Marin et al. (1996b) and Vicente et al. (2014), each medium has a specific impact on the species of Brucella, its biovars and contaminants because of the differences in media. After the first selective medium was developed, the ecological range of Brucella genus has expanded as a result of the identification of new species and strains in different hosts (Godfroid et al. 2005, Pappas 2010, Yumuk and O'Callaghan 2012). In order to address the problems associated with the transmission of the disease, it is important to isolate and identify the etiological agent (Her et al. 2010). Thus, the selective media having an important role in isolation are fundamental for bacteriological isolation as a gold standard as well. The World Organization for Animal Heath (OIE), in its Chapter of Cattle Brucellosis, also suggests using two different media at the same time in order to sensitivity of isolation (Marin et al. 1996b, OIE 2012),

one of which is FM (Karagul and Ikiz 2017, OIE 2012). In the same way, in their study, Nardi Junior et al. (2015) compared and contrasted *Brucella* agar, Farrell, and CITA media and, despite the similarities between their isolation numbers, FM was found to be the best regarding the inhibition of the contaminants, and it is also considered to be the best selective medium for microbiological diagnosis. De Miguel et al. (2011) defines FM as the most common selective medium for the bacterial diagnosis of brucellosis as it can inhibit most of the contaminants.

In animals, abortion is an expected complication of brucellosis in that organisms tend to be settled in the animals' placenta, where erythritol, a growth stimulant of B. abortus, exists (Corbel 2006). Erythritol, which is a four carbon atom sugar alcohol (polyol), is found in some fruits in small amounts, and it is also found in high concentration in foetal tissues of ruminants (Garcia Lobo and Sangari Garcia 2005). As erythritol is absent in humans, it is believed to make human placenta more resistant against brucellar placentitis. (Madkour 2001). Among all the bacterial species, members of Brucella genus are the ones that are most closely related to erythritol. The Brucellae can not only utilize erythritol, but they prefer to use it instead of other sugars. A recent report illustrated that the erythritol concentration in foetal sheep blood was 60 times higher than the one in the maternal blood (Garcia Lobo and Sangari Garcia 2005).

This study aims to evaluate the benefits of adding erythritol to FM, which is commonly used for *Brucella* isolation. It is particularly aimed to elaborate on whether the addition of erythritol leads to an earlier isolation by the help of faster growth rate and larger colony size.

MATERIAL and **METHOD**

Organ samples associated with 14 small ruminant abortion cases caused by B.melitensis strains were utilized in this study. The number of samples belonging to sheep or goat abortions is 10 and 4, respectively. The media included in this study are FM, F+ER and Tryptic Soy Agar (TSA) as a non-selective medium. The content of FM (Farrell 1974), includes Brucella Medium Base with Calf Sera (BMB-CS), (25,000 IU/liter), Polymyxin (5,000 Bacitracin IU/liter), Nalidixic acid (5mg/liter), Natamycin (50 mg/liter, Vancomycin (20mg/liter). F+ER also contains erythritol (1gr/liter). For this study, the basal medium needed for the selective media was prepared and sterilized through autoclaving (MVR-121°C ± 3°C, 15-20 minutes). Erythritol solution composed of 10 gr of erythritol and 10 ml distilled water was sterilized by filtration. Antibiotics, sterile new born calf sera and erythritol (1ml) were added to the media at about 56 °C depending upon their contents (Alton

et al. 1988). Solidified media were incubated at 37 °C for 48 h for sterility control (ISO/TS 2009).

The suspensions obtained from organ samples were prepared in a 1/10 dilution with phosphate buffered saline in a biosafety cabinet (Vicente et al. 2014). Organ suspensions were inoculated to media and incubated in 37°C, 5-10% CO₂ condition for 5-8 days. At the end of the incubation period, Brucella strains isolated were identified through a conventional biotyping method. Biotyping was carried out according to CO₂ requirement, H₂S production, growth in media which contain thionin (20µg/ml), basic fuchsin (20µg/ml), safranine (100µg/ml), penicillin (5 IU/ml), streptomycin (2,5µg/ml), and erythritol (1mg/ml) sensitivity, lysis with Tibilisi and R/C phages and agglutination with monospecific A and M antisera (Alton et al. 1988).

In addition, the growth level of contaminant microorganisms was observed. The inhibition ability of the selective media against contaminant microorganisms was categorized as total inhibition (TI) or partial inhibition (PI) with regards to contaminant growth diffuseness by counting colonyforming units (CFUs) (ISO/TS 2009). After the contaminant colonies were counted, the inhibition ability of the media was identified with the help of specifying the range of contaminant burden. The ranges were divided into four groups: one total inhibition group with the identification of no contaminant colonies and three partial inhibition groups, including the ones with less than 10, the ones between 10 and 100, and the ones with more than 100 colonies (ISO/TS 2009, Jones et al. 1975, O'Grady et al. 2014). The growth of Brucella colonies was observed during incubation. The colony diameter of the observed colonies was measured. FM and F+ER were compared and contrasted based on the day of incubation on which the colony growth appeared for the first time.

RESULTS

Inoculation of 11 organ samples caused gross contamination on TSA. It was observed that contaminants were totally inhibited on FM and F+ER media for 11 samples. The inhibition abilities of FM and F+ER to suppress the contaminants and the contamination level on TSA are listed in Table 1.

As it is shown in Table 1, all of the inoculations on F+ER led to isolation. The isolates were then identified through biotyping procedure. The inoculation of sample 5 did not end up with a pure Brucella growth; therefore, the passage of this sample from FM was contaminated. The identification of sample 5 taken from FM as could not be carried out via biotyping because of this contamination.

The growth rate of Brucella colonies varied in FM and in F+ER. In Table 2, the data about the day of the incubation on which colonies were observed for the first time and colony growth and size were listed., it was possible to recognize and measure the colonies on F+ER for 8 samples but not on FM, on the same day of incubation. 5 samples were observed both on FM and F+ER on the same day of incubation. However, for 4 samples out of 5 the colonies were larger on F+ER than the ones on FM. There is only I sample (no: 8) on which larger Brucella colonies were observed on FM. Sample 9 was the only one which showed equal colony growth rate on both FM and F+ER.

The colony growth on the days following incubation was checked. It was apparent that colonies on F+ER became larger than the colonies on FM. In some samples (sample 4, 6, 8), the differences of colony size between FM and F+ER was observable only on the first days of incubation. In these samples, profusion of Brucella growth spread on the whole surface of media on the following days. It was the reason for not being able to measure a single colony and compare the sizes.

DISCUSSION

For 8 samples out of 14, F+ER enables us to passage the Brucella colonies one day earlier. It also provides an easy passaging for 4 samples as it gives the chance of gathering larger colonies with longer colony diameter. Brucella bacteria fastidious are microorganisms and require a longer incubation period than the contaminants growing fast in the samples (Alton et al. 1988, Marin et al. 1996, Stack et al. 2002). For growth on primary isolation, most strains need complex media which contain multiple amino acids, thiamin, biotin, nicotinamide and pantothenic acid (Corbel 2001). In addition, it was also stated that seeing colonies on selective media might take a few more days than the usual incubation period on the nonselective media (Alton et al. 1988). In a study by Hunter and Kearns (1977), in the medium they used, which included erythritol, the colonies were larger and noticeable after only 3-day incubation period. It can be stated that addition of erythritol to FM for isolation of B.melitensis strains contributes positively to the isolation success. Contrary to other studies (Her et al. 2010, Hunter and Kears 1977), the effect of erythritol addition on isolation and colony growth was elaborated by using B. melitensis strains in this study.

A possible energy source for many strains is erythritol. *B.melitensis* can oxidize amino acids but few carbohydrates other than D-glucose and erythritol (Corbel 2001). Growth analysis of *Brucella* spp. in media which contain erythritol revealed that Brucella spp. will use erythritol as a source of carbon

preferentially over the presence of glucose in the media (Anderson and Smith 1965, Petersen et al. 2013). Stack et al. (2002) pointed out that they could not identify the Brucella bacteria colonies in some of the milk samples which are artificially infected. They suggested that the reason was that contaminants in the milk samples hid the Brucella colonies. In this sense, the high burden of contaminants in the samples might lead to a decrease in the isolation performance. Therefore, enhancing the growth of Brucella colonies as, it is in this study, might overcome this difficulty. Karagul and Ikiz (2017) stated that although a medium in their study had the lowest inhibition ability; it did not have the lowest isolation rate. This fact might be interpreted as the positive effect of the erythritol component it included.

Erythritol is also mentioned as a sugar alcohol which has an impact on the tissue tropism of Brucella bacteria in ruminants (Garcia Lobo and Sangari Garcia 2005, Her et al. 2010, Keppie et al. 1965). It was explained that the presence of erythritol in the placentas of goats, cows, and pigs was the reason for the existence of Brucella in these sites and the following accumulation of big amounts of bacteria, which eventually leads to abortion (Keppie et al. 1965, Petersen et al. 2013). However, it is stated that erythritol does not stimulate (Poester et al. 2013, Seleem et al. 2008,) but hinders the growth of S19 strain (Garcia Lobo and Sangari Garcia 2005, Sperry and Robertson 1975). It was also shown that all of the erythritol degradation enzymes in S19 were identified, except for the D-erythrulose 1-phosphate dehydrogenase (Sperry and Robertson 1975).

At first, the ability of *B.abortus* to use erythritol was believed to be directly related to virulence, as shown by the fact that the *B.abortus* vaccine strain S19 was recognized as deficient in the use of erythritol (Petersen et al. 2013). The findings of the study by Karagul and Ikiz (2018) revealed that adding erythritol component to the selective media will contribute to the growth of *Brucella* strains except S19 and it might be suggested as a way of augmenting isolation sensitivity.

Alton et al. (1988) suggested passaging *Brucella* suspect colonies before the contaminants spread on the media's surface and they also recommended checking them three days after the incubation. However, contaminants might cover the surface of the medium completely earlier than the end of 3 days (Karagul and Ikiz 2017). In this study, it was possible to recognize the *Brucella* colonies on F+ER for some samples at the end of the second day of incubation. It is very helpful that may be used to support the Brucella isolation in the samples with high

contaminant burden. In another study, the colony growth rate of 14 reference strains out of 20 was observed to be fastest in Brucella medium including erythritol (Karagul 2016).

Contaminants were totally inhibited on both of the FM and F+ER for most of the samples. Therefore, the effect of adding erythritol has just offered a chance for earlier isolation. Total inhibition of contaminants on F+ER also confirms that erythritol addition does not have a negative influence on the inhibition ability of the medium. The most effective medium was FM while controlling unwanted organisms, especially when there is a high level of contamination (Hunter and Kearns Considering this, Karagul and Ikiz (2017, 2018) stated that the isolation rate can diminish in the samples in which contaminant organism burden rises up qualitatively and quantitatively while the number of the target bacteria lowers. While developing media, components such as erythritol which provokes growth and antimicrobial agents which provide inhibition can be added to the media (Karagul and Ikiz 2018). In this study, isolation could not be achieved by FM in the sample (no: 5), in which the contaminants were partially inhibited. Even if the F+ER had inhibited the contaminants partially too, the isolation was carried out. In this respect the isolation from this sample on F+ER might show the influence of erythritol on the isolation success. A study supporting this influence recommended using a combination of the contaminant inhibitors of FM in addition to the more enriched base of Barrow & Peel's Medium which includes erythritol for a better medium (Hunter and Kearns 1977).

The link between *Brucella* spp. and erythritol has been apparent recently. It was shown by some studies that there was a correlation between *B.abortus* strains's ability to utilize erythritol and the virulence of the strain with smaller utilization rates which lead to lower virulence growth (Petersen et al. 2013). Therefore, using a selective medium containing erythritol might provide some preliminary data in terms of the relationship between virulence and erythritol.

Consequently, the addition of erythritol to FM, a common selective medium for *Brucella* spp isolation, provides advantage for the isolation of *B.melitensis* strains. Conducting some follow-up studies consisting of *other Brucella* species-biovar and samples with gross contaminants burden is recommended. With the help of these studies, the advantages of erythritol addition can be displayed in comparison with other media.

Table 1: Identification and inhibition results of the selective media

Sa	A	Count of	Identification	Inhibition	Identification	Inhibition ability of
Sample	Animal	Contaminants	result for	ability of	result for	F+ER
le	al	on TSA	FM	FM	F+ER	
1	Sheep	<10cfu	B.melitensis bv3	TI	B.melitensis bv3	TI
2	Sheep	>100cfu	B.melitensis bv3	TI	B.melitensis bv3	TI
3	Sheep	>100cfu ^a	Rev1	TI	Rev1	TI
4	Goat	>100cfu ^a	B.melitensis bv1	PI>100CFUb	B.melitensis bv1	PI(10-100)°CFU
5	Goat	>100cfu ^a	None ^b	PI>100CFUb	B.melitensis bv1	PI(10-100)°CFU
6	Sheep	10-100cfu	B.melitensis bv1	TI	B.melitensis bv1	TI
7	Sheep	>100cfu ^a	B.melitensis bv3	TI	B.melitensis bv3	TI
8	Sheep	>100cfu ^a	B.melitensis bv3	TI	B.melitensis bv3	TI
9	Sheep	>100cfu ^a	B.melitensis bv3	PI(10-100)°CFU	B.melitensis bv3	PI(10-100)°CFU
10	Goat	<10cfu	B.melitensis bv1	TI	B.melitensis bv1	TI
11	Sheep	>100cfu ^a	B.melitensis bv3	TI	B.melitensis bv3	TI
12	Sheep	>100cfu ^a	B.melitensis bv1	TI	B.melitensis bv1	TI
13	Sheep	>100cfu ^a	B.melitensis bv3	TI	B.melitensis bv3	TI
14	Goat	>100cfu ^a	B.melitensis bv1	TI	B.melitensis bv1	TT

a. Diffuse growth of contaminants

Table 2: The growth of Brucella colonies on selective media regarding the day of incubation

လွှ	FARRR	REL MEDIUM	FARRELL MEDIUM	WITH ERYTHRITOL
Sample	Incubation Day: Colony size	Incubation Day: Colony size	Incubation Day: Colony size	Incubation Day: Colony size
1	3 days: None	7 days: >1mm	3 days:≥1mm	7 days: >2mm
2	5 days: ≤1mm	-	5 days: ≥1mm	-
3	5 days: ≤1mm	-	5 days: ≥1mm	-
4	2 days: None	3 days: Diffuse growth ^a	2 days: ≤0.5mm	3 days: Diffuse growth ^a
5	2 days: None	3 days: Contamination	2 days: ≤0.5mm	3 days: Diffuse growth ^a
6	2 days: None	3 days: Diffuse growth ^a	2 days: Diffuse growth ^a	3 days: Diffuse growth ^a
7	2 days: None	3 days: ≤1mm	2 days:≤1mm	3 days: ≥1mm
8	3 days: ≥1mm	6 days: Diffuse growth ^a	3 days: ≤1mm	6 days: Diffuse growth ^a
9	3 days: ≥1mm	-	3 days:≥1mm	-
10	3 days: ≥0,5mm	4 days: ≥1mm	3 days: ≥1mm	4 days: ≥2mm
11	2 days: None	3 days: ≥0,5mm	2 days: ≥0,5	3 days: ≥2mm
12	2 days: ≥0,5mm	5 days: ≤2mm	2 days: ≥1mm	5 days: ≥2mm
13	2 days: None	3 days:≤1mm	2 days: ≤0,5mm	3 days: ≥1mm
14	2 days: None	3 days:≥0,5mm	2 days: ≥0,5mm	3 days: ≥1mm

a: It was not possible to measure the colony size because of the diffuse growth on whole medium surface

b. More than 100 CFU contaminants were observed

c. Between 10 and 100 CFU contaminants

TI: Contaminant were totaly inhibited.

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RESEARCH ARTICLE

The Assessment of Laboratory Safety in Terms of Biosafety: An Application in Afyon Kocatepe University

İbrahim KILIÇ1*, İlkay DOĞAN2, Sinan SARAÇLI3

¹Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Biostatistics, Afyonkarahisar, Turkey
²Gaziantep University, Faculty of Medicine, Department of Biostatistics, Gaziantep, Turkey
³Afyon Kocatepe University, Faculty of Science And Literature, Department of Statistics, Afyonkarahisar, Turkey

ABSTRACT

In this descriptive research, it was aimed to determine of risk levels and assessment of laboratory safety in terms of biosafety according to the academicians in Afyon Kocatepe University. In addition, a comparison was made between the faculty of veterinary medicine and other faculties in terms of taking risks and precautions. The questionnaires used as data collection techniques were applied to 90 academicians working in Afyon Kocatepe University. As a result of the research, it was determined that the risk of zoonotic disease was the highest in the laboratories, the risk averages were moderate and the precaution was below the moderate level. On the other hand, the highest level of risk is ventilation problems, problems related to training needs and risks related to chemicals.

Keywords: Biosafety, Laboratory safety, Risk, Afyon Kocatepe University.

Laboratuvar Güvenliğinin Biyogüvenlik Açısından Değerlendirilmesi: Afyon Kocatepe Üniversitesi'nde Bir Uygulama

ÖZ

Betimsel bir nitelik taşıyan bu araştırmada, Afyon Kocatepe Üniversitesi'nde görev yapan akademisyenlerin çalıştıkları ve/veya sorumlu oldukları laboratuvarlardaki güvenlik durumunun biyogüvenlik açısından değerlendirilmesi ve risk düzeylerinin belirlenmesi amaçlanmıştır. Ayrıca, risk ve önlem alma bakımından veteriner fakültesi ve diğer fakülteler arasında bir karşılaştırma yapılmıştır. Veri toplama aracı olarak geliştirilen anket, Afyon Kocatepe Üniversitesi'nde görev yapan 90 akademisyene uygulanmıştır. Araştırma sonucunda, laboratuvarlarda en çok zoonoz hastalık riski olduğu, genel risk ortalamasının orta düzeyde, önlem alma durumunun ise orta düzeyin altında olduğu tespit edilmiştir. Diğer taraftan, risk düzeyinin en fazla olduğu hususlar; havalandırma sorunu, eğitim gereksinimine yönelik sorunlar, kimyasallara ilişkin riskler şeklinde sıralanmıştır.

Anahtar Kelimeler: Biyogüvenlik, Laboratuvar güvenliği, Risk, Afyon Kocatepe Üniversitesi

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ORCID ID; İK: 0000-0003-0595-8771, İD: 0000-0001-7552-6478, SS: 0000-0003-4662-8031

*Corresponding author e-mail: kilicbrahim@hotmail.com

GİRİŞ

Biyogüvenlik kavramı 2000'li yılların başından itibaren gittikçe önemi artan ve üzerinde bilimsel araştırmalar gerçekleştirilen konulardan biridir. Biyogüvenlik tüm ekosistemi ve bunun içerisinde yer alan insanın sağlığını korumayı hedefleyen bir konu olarak gelişmektedir ve farklı bilim alanı veya disiplinlerin gerektiren çalışma yapmasını bir konudur. Biyogüvenliğe yönelik bir sistemin gerçeklestirilmesi ve geliştirilmesi uzun vadeli, profesyonel ve planlıprogramlı yaklaşımlar gerektiren bir süreçtir. Bu sistemin en önemli aşaması, belirli bir sistematiği gerektiren takip ve değerlendirme sürecidir. Bu süreç sonucunda muhtemel tehlikelerin önceden tespit edilmesi ve zamanında harekete geçilebilmesi sağlanır (Demir 2009). Biyogüvenlik çok geniş bir kavram olup insan, hayvan, gıda, savunma, çevre, ekosistem, bilimsel çalışmalar ve daha birçok unsur ve konuyu içermektedir. Biyogüvenliğin insana doğrudan etkisinin yanı sıra, insan dışında sayılan diğer faktörlerin biyogüvenliğinin de doğrudan veya dolaylı olarak insan sağlığını tehdit etme riski bulunmaktadır. Bundan dolayı, biyogüvenliğin -ve dolayısı ile insan sağlığının- sağlanmasında yönetsel açıdan bazı yasal gereklilikler ile bazı kontrol, denetim, yaptırım ve zorunlulukların dikkate alınması gerekmektedir (Celen 2014).

İnsan sağlığı ve onun dengeli beslenmesinde en önemli bileşenlerden biri hayvansal gıdalardır ve bu gıdaların üretildiği kurum-kuruluşlarda, sağlıklı ve kaliteli ürün çıktısı alınması, hayvan refahı kapsamında vüksek refaha sahip, sağlıklı havvanlarla kısa vadede olmasa da uzun vadede karlı ve sürdürülebilir yapılması, tüketici algısına ilişkin vetiştiricilik memnuniyet sağlanması, gıda güvenliği ve gıda güvencesi biyogüvenlik ile doğrudan ilintili olan konular arasındadır. Hayvancılığın süreç içerisinde tüketici odaklı bir sektörel faaliyete dönüşmesi, ekosistemi dikkate alan ekolojik tarım ve tarımsal iyiuvgulamalar ile sürdürülebilir kaliteli politikalarının son zamanlarda değer kazanması biyogüvenlik konusunu ön plana getirmektedir. Bununla birlikte, yetiştiricilikte teknolojik olanaklar kullanarak hayvandan en yüksek derecede verim amaçlayan işletmelerin entansif almayı benimsemeleri ve yığınsal veya yoğun üretimin yaygınlasması da biyogüvenlik ve buna bağlı konuları daha da önemli hale getirmektedir (Köseman ve Şeker 2016).

Biyogüvenlik, modern biyoteknoloji uygulama teknikleri ile üretilen ürünlerin ekosistem kapsamında çevre, insan ve hayvan sağlığı üzerinde meydana getirebileceği negatif etkilerin saptanmasını, buna bağlı olarak risklerin belirlenmesini, belirlenen risklerin meydana gelme ihtimalinin düşürülmesini ve söz konusu risklerin olusması durumunda muhtemel

zararların kontrol edilmesi amacıyla alınabilecek önlemleri içermektedir. Biyogüvenlik unsurları ise risk tanımı, risk analizi, risk değerlendirme, risk yönetimi ve risk iletişimi olmak üzere beş önemli ana bileşenden oluşmaktadır. Risk değerlendirme ve yönetme süreci bunlar arasında en önemli konulardır (Başkaya ve ark. 2009).

Bu bilgiler doğrultusunda biyogüvenlik kavramının geniş bir kapsamı olduğu ve pek çok konuyla ilişkili olduğu söylenebilir. Bunlardan birisi de laboratuvar güvenliğidir ki biyogüvenlikle iç içe düşünülebilir. Son yıllarda özellikle tıp ve veteriner hekimliği başta olmak üzere ilgili tüm disiplinlerde risk taşıma potansiyeli olan madde ve materyalle çalışılan laboratuvarlarda görev yapan bireylerin ve çevrenin korunması için alınacak önlemlerin yeterli düzeyde standardize edilmesi önemli görülmektedir. Biyogüvenlik en önemli yönüyle laboratuvar personelini, yardımcı personeli ve çevreyi potansiyel tehlikede olan mikrobiyolojik ajanlardan koruma sürecini ifade eder. Korunma süreci, -kavramsal olarak ifade edilirse tecrit süreci- amaca uygun yerinde kullanımlarda temel olarak iki teknik savunma hattını içermektedir. İlk koruma, biyogüvenlik ve laboratuvar ekipmanlarının yerinde -olması gerektiği gibi- kullanımı ve risk altındaki personelin korunması, ikinci koruma ise laboratuvar dışında kalan diğer çevrenin korunması olarak ifade edilebilir (Ceyhan 2005). Burada çevreyi, personeli ve toplumu koruma düzeyinin artışına göre 1'den 4'e kadar olacak şekilde düzenlenen biyogüvenlik seviyeleri (BGS) tanımlanmalıdır. Bunlar (Yılmaz 2012):

- BGS-1: Bağışıklık sisteminde problem olmayan bireylerde hastalığa sebep olmazken, laboratuvar personeli için en düşük seviyelerde bulaşa sebep olan ajanlarla çalışılması için uygundur.
- BGS-2: Tüm çevre ve çalışanlar için ortalama bulaş riski taşıyan ajanlarla çalışmak üzere uygun olan biyogüvenlik seviyesidir.
- BGS-3: İnhalasyon yolu ile alındığında öldürücü enfeksiyonlara neden olabilen yerli veya egzotik etkenlerle çalışılan ve çeşitli klinik, tanı, eğitim, araştırma ya da üretim faaliyetinin yapıldığı laboratuvarlar içindir.
- BGS-4: Yüksek düzeyde risk içeren, tedavisi veya bağışıklanması olası olmayan, laboratuvar enfeksiyonları ve ölümcül hastalıklara sebep olabilen tehlikeli ve egzotik ajanları barındıran araştırmalar veya çalışmalar için uygun olan seviyedir.

Yukarıdaki bilgiler çerçevesinde bu araştırmada, Afyon Kocatepe Üniversitesi'nde görev yapan akademisyenlerin çalıştıkları ve/veya sorumlu oldukları laboratuvarlardaki güvenlik durumunun biyogüvenlik açısından değerlendirilmesi ve risk düzeylerinin belirlenmesi amaçlanmıştır.

MATERYAL ve **METOT**

Betimsel bir nitelik taşıyan bu araştırmada veri olarak anket tekniğinden toplama aracı yararlanılmıştır. Anket formunun oluşturulmasında, biyogüvenlik ve laboratuvar güvenliği ile ilgili literatürden ve Ergör ve ark. (2003) tarafından "Laboratuvarda Biyogüvenlik Calısmaları ve Dokuz Eylül Üniversitesi Deneyimi" isimli çalışmadan yararlanılmıştır. Diğer taraftan bu çalışma, Kılıç ve Doğan (2011) tarafından yapılan" Veteriner Fakültesi Biyogüvenlik Laboratuvarlarındaki Durumuna Yönelik Akademisyen Görüşleri: Afyon Kocatepe Üniversitesi'nde Bir Uygulama" başlıklı çalışmanın (AKÜ) laboratuvarları üniversite açısından genişletilmiş halini yansıtmaktadır. Çalışmada etik izin AKÜ 2018/66 nolu Etik Kurul Kararı ile alınmıştır. Bu doğrultuda çalışmada iki kısımdan oluşan anket uygulanmıştır. Birinci kısımda öğretim elemanlarının bazı bireysel özelliklerine yer verilirken, ikinci bölümde ise biyogüvenlik bakımından akademisyenlerin laboratuvar güvenliği ile ilgili bazı sorunları içeren faktörlere ilişkin genel görüşlerini betimlemek üzere Likert (1932)derecelendirmenin kullanıldığı (1="hiç risk yok", 2="az riskli", 3="orta düzeyde riskli", 4="çok riskli" ve 5="tamamen riskli") 16 maddeden oluşan ölçek ver almaktadır.

Çalışmanın evreni, Afyon Kocatepe Üniversitesi Veteriner Fakültesi, Tıp fakültesi, Sağlık Yüksekokulu (hemşirelik, fizik tedavi, beslenme), Fen Edebiyat Fakültesi fizik, kimya ve biyoloji bölümlerindeki laboratuvarlarda görev yapan akademisyenler ile sınırlandırılmıştır. Bu çerçevede, bu birimlerde görev yapan ve anket uygulamasını kabul eden 103 akademisyene anket uygulanmış ve eksik hatalı ve güvenirliği düşük anketler dikkate alınarak 90 anket formu değerlendirmeye alınmıştır.

Toplanan verilerin analizi için frekans, yüzde dağılımı, aritmetik ortalama ve standart sapma gibi betimsel istatistikler uygulanmıştır. Çalışmada, elde edilen verilerin analizinde SPSS 18.0 for Windows programı kullanılmıştır.

BULGULAR

Çalışmada, örneklemde yer alan katılımcıların bazı kişisel değişkenlerine ilişkin sıklık ve yüzde dağılımları Tablo 1'de sunulmuştur. Tablo 1'deki bulgulara göre, akademisyenlerin %33.3'ü kadın, %66.7'si erkek olup, %74.4'ü evli %25.6'sı bekârdır. Ankete katılan akademisyenlerin %3.3'ü "25 ve altı" yaş grubunda, %38.9'u "26-35" yaş grubu arasında, %47.8'i "36-45" yaş grubu arasında ve %10'u "46 ve üzeri" yaş grubunda yer almaktadır. Katılımcıların %11.1'inin

unvanı "Prof. Dr.", %28.9'unun "Doç. Dr.", %28.9'unun "Dr. Öğr. Üyesi", %8.9'unun "Arş. Gör. Dr.", %20'sinin "Arş. Gör." ve %2.2'sinin "Uzman" olduğu tespit edilmiştir. Akademisyenlerin %10'u "0-3 yıl" arasında, %26.7'si "4-7 yıl" arasında, %31.1'i "8-11 yıl" arasında ve %32.2'si "12 yıl ve üzeri"nde Afyon Kocatepe Üniversitesi'nde hizmet vermektedir. Akademisyenlerin laboratuvardaki çalışma sürelerine göre dağılımı %8.9'u "0-3 yıl", %25.6'sı "4-7 yıl", %30'u "8-11 yıl" ve %25.6'sı "12 yıl ve üzeri" şeklindedir.

Laboratuvarda günlük çalışma sürelerine göre dağılım incelendiğinde; %4.4'ünün "1 saat ve daha az"; %37.8'inin "2-3 saat"; %14.4'ünün "4-5 saat"; %16.7'sinin "6-7 saat" ve %26.7'sinin ise "8 saat ve üzeri" olduğu saptanmıştır. Katılımcıların %13.3'ü sigara kullandığını belirtirken %86.7'si de sigara kullanmadığını belirtmiş olup, %87.8'inin laboratuvar güvenliği ve/veya biyogüvenlik ile ilgili herhangi bir kurs, eğitim veya sertifika programına katılmadığı belirlenmiştir. Akademisyenlerin %41.1'i Veteriner Fakültesi, %27.8'i Tıp Fakültesi, %18.9'u Sağlık Yüksekokulu ve %12.2'si ise Fen Edebiyat Fakültesi'ndeki laboratuvarlarda görev yapmaktadırlar.

Örneklem grubunu oluşturan akademisyenlerin sahip oldukları sağlık sorunlarına ve laboratuvarların taşıdığı hastalık risklerine göre dağılım Tablo akademisyenlerin Buna sunulmuştur. göre, %18.9'unda alerjik hastalıklar, %7.8'inde astım hastalığı görülürken, %54.4'ü ise hiçbir sorununun olmadığını bildirmiştir. Diğer taraftan, akademisyenlerin %10'u ise laboratuvarların hiçbir hastalık riski taşımadığını belirtirken, %35.6'sı zoonoz hastalıklar, %13.3'ü kanser, %12.2'si alerjik hastalıklar, %10'u astım ve diğer solunum sistemleri hastalıkları, %11.1'i diğer bakteriyel hastalıklar ve %7.8'i de yanıklar, yaralanmalar, zehirlenmeler şeklinde riskler olduğunu belirtmistir.

Tablo 3'te araştırmaya katılan akademisyenlerin çalıştıkları laboratuvarların biyogüvenliğe ilişkin herhangi bir risk taşıma durumu ve bu riskler karşısında önlem alınma durumuna yönelik görüşleri verilmiştir. Bulgular incelendiğinde, akademisyenlerin %23.3'ü "tamamen riskli", %33.3'ü "çok riskli", %24.4'ü "orta düzeyde riskli", %18.9'u "az veya hiç riskli değil" şeklinde yanıt vermişlerdir. Genel risk ortalaması 5'li Likert derecelemesinde orta düzeyi gösteren 3'ün üzerinde 3.52±1,08 (X±SS) olarak hesaplanmıştır. Risk durumuna göre önlem alınma düzeyi bakımından ankete katılan akademisyenlerin %24.4'ü "hiç önlem alınmıyor", %34.4'ü "az önlem alınıyor", %32.2'si "orta düzeyde önlem alınıyor" ve %8.9'u ise "çok ve tamamen önlem alınıyor" şeklinde vermişlerdir. Bu bulgulara laboratuvarlardaki genel önlem alma durumu orta düzeyin altında kalmıştır ($\overline{X} \pm SS$; 2,29 \pm 0,98). Tablo 3'teki bulgulara göre, gerek risk düzeyi ve gerekse önlem alma düzeyi bakımından veteriner fakültesi ve diğer fakültelerde görev yapan akademisyenlerin görüşleri arasında anlamlı bir farklılık bulunamamıştır (p>0,05).

Biyogüvenlik veya laboratuvar güvenliğini ilgilendirebilecek sorunlara yönelik risk seviyelerine ait betimsel istatistikler Tablo 4'te verilmiştir.

Buna göre, aritmetik ortalama değerleri dikkate alındığında laboratuvar güvenliği ile ilgili konular en riskli olandan en risksiz olana doğru; havalandırma sorunu, eğitim gereksinimine yönelik sorunlar, kimyasallara ilişkin riskler, temizlik sorunu, yapısal sorunlar, elektrik, su, akıntı vb. riskler, ergonomik sorunlar, kişisel koruyucu sorunu, atık sorunu, tıbbi sorunlar, ortamdaki sıcaklık, nem vb. sorunlar, depolama sorunu, güvenlik sorunu, kesici, delici vb. aletle yaralanama sorunu, sinek, böcek vb. sorunlar ve yangın durumu şeklinde sıralanmıştır. Akademisyenlerin %46,6'sı havalandırma, %42,2'si eğitim gereksinimi ve %44,4'ü kimyasal sorununa yönelik riskin yüksek olduğunu (çok+tam seçenekleri ile) belirtmişlerdir.

Tablo 1. Katılımcıların bireysel özelliklerine göre dağılımı

Table 1. Distribution of participants according to their individual characteristics

Değişken	Grup	n	%	Değişken	Grup	n	0/0
Cinsiyet	Kadın	30	33.3	Laboratuvarda çalışma	0-3 yıl	8	8.9
	Erkek	60	66.7	süreleri	4-7 yıl	23	25.6
Yaş	25 ve altı	3	3.3	•	8-11 yıl	27	30.0
	26-35	35	38.9		12 yıl ve +	32	35.6
	36-45	43	47.8	Laboratuvarda günlük	1 saat ve -	4	4.4
	46 ve üzeri	9	10.0	ortalama çalışma süresi	2-3 saat	34	37.8
Medeni	Evli	67	74.4		4-5 saat	13	14.4
durum	Bekar	23	25.6		6-7 saat	15	16.7
Mesleki	Prof. Dr.	10	11.1	•	8 saat ve +	24	26.7
unvan	Doç. Dr.	26	28.9	Sigara içme durumu	İçiyor	12	13.3
	Dr. Öğr. Üy.	26	28.9		İçmiyor	78	86.7
	Arş. Gör. Dr.	8	8.9	Eğitimine katılma durumu	Katıldı	11	12.2
	Arş. Gör.	18	20.0	_	Katılmadı	79	87.8
	Uzman	2	2.2	Çalıştığı birim	Vet. Fak.	37	41.1
Fakültedeki	0-3 yıl	9	10.0		Tıp Fak.	25	27.8
çalışma süresi	4-7 yıl	24	26.7		SŶO	17	18.9
	8-11 yıl	28	31.1		FEF	11	12.2
	12 yıl ve +	29	32.2	Toplam		90	100,0

Tablo 2. Katılımcıların sahip olduğu sağlık sorunlarına ve laboratuvarların taşıdığı hastalık risklerine göre dağılım **Table 2.** Distribution according to the health problems of the participants and the disease risks of the laboratories

Sağlık sorunları	n	%	Hastalık riskleri	n	%
Hastalık yok	49	54.4	Hastalık riski yok	9	10.0
Alerjik hastalıklar	17	18.9	Zoonoz hastalıklar	32	35.6
Astım	7	7.8	Kanser	12	13.3
Kalp hastalıkları	5	5.6	Alerjik hastalıklar	11	12.2
Ortopedik hastalıklar	3	3.3	Astım veya diğer solunum sistemi hastalıkları	9	10.0
Görme - işitme kusuru	4	4.4	Diğer bakteriyel hastalıklar	10	11.1
Diğer (psikiyatrik, romatizmal,	5	5.6	Diğer (yanıklar, zehirlenmeler, yaralanma vb.)	7	7.8
metabolik vb. hastalıklar)					

Tablo 3. Laboratuvarlarda biyogüvenlik ile ilgili herhangi bir risk taşıma ve önlem alınma durumuna ilişkin katılımcı görüşleri

Table 3. Participants' opinions regarding the carrying out of any risk related to biosafety in laboratories

Daylatan		Ge	enel	Vet. Fak.	Diğ. Fak.
Değişken		n	%	x ±ss	$\overline{X} \pm SS$
Risk düzeyi	Hiç risk yok (1)	8	8.9	3.57±1.11	3.45±1.06
•	Az riskli (2)	9	10.0		
	Orta düzeyde riskli (3)	22	24.4	p:	>0.05 (ÖD)
	Çok riskli (4)	30	33.3	•	,
	Tamamen riskli (5)	21	23.3		
	$\overline{X} \pm SS$	3.5	52±1.08		
Önlem düzeyi	Hiç önlem yok (1)	22	24.4	2.21±0.95	2.32±0.98
·	Az önlem alınıyor (2)	31	34.4		
	Orta düzeyde önlem alınıyor (3)	29	32.2	p:	>0.05 (ÖD)
	Çok önlem alınıyor (4)	5	5.6	•	,
	Tamamen önlem alınıyor (5)	3	3.3		
	$\frac{1}{X} \pm SS$	2.2	29±0.98		

Tablo 4. Laboratuvar güvenliği ile ilgili sorunlara yönelik risk düzeylerine ilişkin betimsel istatistikler **Table 4.** Descriptive statistics on risk levels related to laboratory safety problems

	Taşıdığı risk düzeyi						
	1	2	3	4	5		
	Hiç	\mathbf{Az}	Orta	Çok	Tam	\overline{X} S	SS
%	3.3	21.1	36.7	17.8	21.1	3.32 1.	.04
$^{0}/_{0}$	5.6	20.0	34.4	26.7	13.3	3.22 1.	.09
%	7.8	17.8	38.9	18.9	16.7	3.19 1.	.11
%	4.4	13.3	35.6	13.3	33.3	3.58 1.	.12
%	4.4	12.2	41.1	17.8	24.4	3.46 1.	.01
%	5.6	22.2	27.8	14.4	30.0	3.41 1.	.11
%	4.4	23.3	40.0	17.8	14.4	3.14 1.	.12
$^{0}/_{0}$	7.8	25.6	35.6	16.7	14.4	3.05 1.	.14
%	5.6	23.3	41.1	21.1	8.9	3.04 1.	.01
%	10.0	18.9	46.7	13.3	11.1	2.97 1.	.02
%	14.4	25.6	37.8	12.2	10.0	2.78 1.	.20
%	12.2	38.9	21.1	15.6	12.2	2.77 1.	.24
%	17.8	34.4	28.9	10.0	8.9	2.58 1.	.31
%	18.9	38.9	23.3	12.2	6.7	2.49 1.	.14
%	17.8	34.4	27.8	13.3	6.7	2.57 1.	.16
%	15.6	36.7	32.2	10.0	5.6	2.53 1.	.01
	% % % % % % % % % % % % % % % % % % %	% 3.3 % 5.6 % 7.8 % 4.4 % 5.6 % 4.4 % 5.6 % 10.0 % 14.4 % 12.2 % 17.8 % 18.9 % 17.8	Hiç Az % 3.3 21.1 % 5.6 20.0 % 7.8 17.8 % 4.4 13.3 % 5.6 22.2 % 4.4 23.3 % 7.8 25.6 % 5.6 23.3 % 10.0 18.9 % 14.4 25.6 % 12.2 38.9 % 17.8 34.4 % 17.8 34.4 % 15.6 36.7	Hiç Az Orta % 3.3 21.1 36.7 % 5.6 20.0 34.4 % 7.8 17.8 38.9 % 4.4 13.3 35.6 % 4.4 12.2 41.1 % 5.6 22.2 27.8 % 4.4 23.3 40.0 % 7.8 25.6 35.6 % 5.6 23.3 41.1 % 10.0 18.9 46.7 % 14.4 25.6 37.8 % 12.2 38.9 21.1 % 17.8 34.4 28.9 % 17.8 34.4 27.8 % 17.8 34.4 27.8 % 15.6 36.7 32.2	Hiç Az Orta Çok % 3.3 21.1 36.7 17.8 % 5.6 20.0 34.4 26.7 % 7.8 17.8 38.9 18.9 % 4.4 13.3 35.6 13.3 % 4.4 12.2 41.1 17.8 % 5.6 22.2 27.8 14.4 % 4.4 23.3 40.0 17.8 % 5.6 23.3 41.1 21.1 % 10.0 18.9 46.7 13.3 % 14.4 25.6 37.8 12.2 % 12.2 38.9 21.1 15.6 % 17.8 34.4 28.9 10.0 % 18.9 38.9 23.3 12.2 % 17.8 34.4 27.8 13.3 % 17.8 34.4 27.8 13.3 % 15.6 <	Hiç Az Orta Çok Tam % 3.3 21.1 36.7 17.8 21.1 % 5.6 20.0 34.4 26.7 13.3 % 7.8 17.8 38.9 18.9 16.7 % 4.4 13.3 35.6 13.3 33.3 % 4.4 12.2 41.1 17.8 24.4 % 5.6 22.2 27.8 14.4 30.0 % 4.4 23.3 40.0 17.8 14.4 % 5.6 23.3 41.1 21.1 8.9 % 10.0 18.9 46.7 13.3 11.1 % 14.4 25.6 37.8 12.2 10.0 % 12.2 38.9 21.1 15.6 12.2 % 17.8 34.4 28.9 10.0 8.9 % 18.9 38.9 23.3 12.2 6.7 <tr< td=""><td>Hiç Az Orta Çok Tam X S % 3.3 21.1 36.7 17.8 21.1 3.32 1. % 5.6 20.0 34.4 26.7 13.3 3.22 1. % 7.8 17.8 38.9 18.9 16.7 3.19 1. % 4.4 13.3 35.6 13.3 33.3 3.58 1. % 4.4 12.2 41.1 17.8 24.4 3.46 1. % 5.6 22.2 27.8 14.4 30.0 3.41 1. % 4.4 23.3 40.0 17.8 14.4 3.05 1. % 5.6 23.3 41.1 21.1 8.9 3.04 1. % 10.0 18.9 46.7 13.3 11.1 2.97 1. % 14.4 25.6 37.8 12.2 10.0 2.78 1.</td></tr<>	Hiç Az Orta Çok Tam X S % 3.3 21.1 36.7 17.8 21.1 3.32 1. % 5.6 20.0 34.4 26.7 13.3 3.22 1. % 7.8 17.8 38.9 18.9 16.7 3.19 1. % 4.4 13.3 35.6 13.3 33.3 3.58 1. % 4.4 12.2 41.1 17.8 24.4 3.46 1. % 5.6 22.2 27.8 14.4 30.0 3.41 1. % 4.4 23.3 40.0 17.8 14.4 3.05 1. % 5.6 23.3 41.1 21.1 8.9 3.04 1. % 10.0 18.9 46.7 13.3 11.1 2.97 1. % 14.4 25.6 37.8 12.2 10.0 2.78 1.

öğretim elemanlarının görev yaptıkları laboratuvarlardaki biyogüvenliğe yönelik durumların

TARTIŞMA ve SONUÇ

Son yıllarda, tüm ekosistemde olduğu gibi "özellikle insanlar için potansiyel patojenik tehlike içeren materyal, infeksiyoz mikroorganizmalar veya onların genetik ya da toksik bileşenleri ile yapılan çalışmaların, insan ve çevre için güvenli şekilde yapılmasını sağlamak amacıyla laboratuvar alt yapı, tasarım, ekipman, teknik ve uygulamalarının en uygun kombinasyonu" olarak tanımlanabilen (Ceyhan 2005) biyogüvenlik, son zamanlarda üzerinde hassasiyetle durulan bir kavramdır. Afyon Kocatepe Üniversitesi

ve buna ilişkin risk düzeylerinin belirlenmesi amacıyla betimsel nitelikte yapılan bu çalışmada 90 akademisyenin görüşleri alınmıştır. Laboratuvar güvenliği ile ilgili ulusal ölçekte uygulamalı sınırlı sayıda çalışma (Ergör ve ark. 2003; Şeker ve Yardımcı 2003; Kılıç ve Doğan 2011; Gürler 2011) yapılmakla birlikte, farklı işletmelerde yapılan çalışmalar söz konusudur. Köseman ve Şeker (2016) tarafından yapılan "Malatya İlinde Sığırcılık İşletmelerinin Mevcut Durumu: II. Hayvan Sağlığı ve Ahır Hijyeni

Perspektifinde Biyogüvenlik Uygulamaları" adlı çalışmada, tesadüfi örnekleme yöntemiyle belirlenen 172 sığırcılık işletme sahibi ile anket yapılmıştır. Çalışmada; hayvan sağlığı ve işletmelerin hijyenine yönelik biyogüvenlik uygulamaları açısından birçok faktörün "kabul edilebilir" seviyede olduğu, ancak barınak koşulları bakımından önemli seviyede yetersizlikler olduğu bildirilmiştir.

Bu araştırmada genel olarak; akademisyenlerin çalıştıkları laboratuvarlarda diğer hastalık risklerine göre en yüksek zoonoz hastalık riski olduğu belirlenirken, bunu kanser riski, alerjik hastalıklar riski, astım ve diğer solunum sistemleri hastalıkları riski, diğer bakteriyel hastalık riski ve yanık, yaralanma, zehirlenme riski izlemistir. Ankete katılanların her on kişide biri de laboratuvarların hiçbir hastalık riski taşımadığını belirtmişlerdir. Kılıç ve Doğan (2011) tarafından yapılan çalışmada da benzer sonuçlar bulunmuş olup, laboratuvarların taşıdığı en önemli hastalık riskinin zoonoz hastalıklar belirtilmiştir. Ceyhan (2005), tüm kurum ve kuruluslarda olduğu gibi laboratuvarlarda da güncel biyogüvenlik kriterlerine uygun alt yapı, teknik, uygulama ve sistemlerin kurulması, personelin hastalanma risklerini önemli ölçüde azaltabileceğini bildirmiştir.

Bu araştırmaya göre, Afyon Kocatepe Üniversitesi akademisyenlerinin çalıştıkları laboratuvarlarda risk düzevinin en fazla olduğu konular; havalandırma sorunu, eğitim gereksinimine yönelik sorunlar ve kimyasallara iliskin riskler seklinde sıralanırken, risk düzeyi en az olanlar ise güvenlik sorunu, kesici, delici vb. aletle yaralanama sorunu, sinek, böcek vb. sorunlar şeklinde sıralanmıştır. Kılıç ve Doğan (2011) tarafından yapılan araştırmada da en yüksek risk düzeyine sahip problemlerin sırası ile "havalandırma "kimyasallara ilişkin riskler", "eğitim sorunu", gereksinimine yönelik sorunlar" ve "temizlik sorunu" olduğu bildirilirken, Ergör ve ark. (2003) benzer şekilde, DEÜ Araştırma ve Uygulama Hastanesi Merkez Laboratuvarının sorunlarını önem sırasına göre "havalandırma, güvenlik kabini vb.", "temizlik" ve "eğitim gereksinimi" seklinde sıralamıslardır. Günay ve ark. (2017)'na göre mikrobiyoloji laboratuvar çalışanları, işleri gereği uzun saatler çalışmakta ve ergonomik açıdan olumsuz birçok faktörle karşı karşıya kalabilmektedirler. Laboratuvar çalışanları arasında artan kas-iskelet sistemi problemleri laboratuvar ergonomisine yönelik bilgilendirici ve düzenleyici araştırmalar yapılmasına neden olmustur.

Araştırma sonuçlarına göre, akademisyen görüşlerinden hareketle, laboratuvarlardaki genel risk ortalamasının (düzeyinin) orta düzeyin üzerinde olduğu saptanırken, laboratuvar güvenliği ile ilgili önlem alma durumunun ise orta düzeyin altında (yetersiz) olduğu tespit edilmistir. Veteriner

fakültesindeki akademisyenler diğer fakültelere göre risk düzeyini daha yüksek, önlem alma düzeyini daha düşük bulsalar da, hem risk düzeyi ve hem de önlem alma düzeyi bakımından veteriner fakültesi ve diğer fakültelerde görev yapan akademisyenler arasında istatistiksel açıdan anlamlı bir farklılık tespit edilmemiştir.

Bu çalışmada elde edilen bulgu ve sonuçlar dikkate alındığında, özellikle sağlık bilimleri alanında faaliyet gösteren laboratuvarlarda biyogüvenliğe yönelik risk düzeylerinin azaltılmasına yönelik önlemler alınması gerektiği ortaya çıkmaktadır. Özellikle havalandırma sorunu, eğitim gereksinimine yönelik sorunlar, kimyasallara ilişkin riskler, temizlik sorunu ve yapısal laboratuvarlardaki sorunlar öncelikle çözülmelidir. Şeker ve Yardımcı (2003)'ya göre her laboratuvar için karşı karşıya kalınabilecek tehlikelerin ve risklerin ortaya konulduğu bir biyogüvenlik el kitabı geliştirmelidir. Ayrıca biyogüvenlik ile ilgili konularda eğitim almış uzman kişilerin belirli sorumluluklar alarak ilgili birimlerde çalıştırılmasının önemi vurgulanmıştır.

Biyogüvenlik uygulamalarının dikkate alınarak ilgili kişi, kurum, kuruluş, yönetici veya birimler tarafından gerekli önlemlerin alınması, pek çok açıdan hayati düzeyde önemli ve gerek bireysel gerekse kurumsal açıdan yararlı görülmektedir. Özellikle veteriner fakültesi laboratuvarlarında, hayvanlardan insanlara geçebilen zoonoz hastalık risklerinin (ve bulaşma riskinin) azaltılması elzem bir durum olarak ortaya çıkmıştır. Bununla birlikte tüm laboratuvarlarda; kanser, alerjik hastalıklar, astım ve diğer solunum sistemleri hastalıkları ve diğer bakteriyel hastalık risklerine karşı daha fazla önlem alınması gerektiği vurgulanmıştır.

Üniversitelerin bilimsel faaliyet üretme sürecinde en önemli veri toplama kaynağı olan laboratuvarların güvenliği; biyogüvenlik ile ilgili yapılan bilimsel çalışmalar, ilgili birimler tarafından yapılan kontrol ve denetlemeler ve Dünya Sağlık Örgütü ve Uluslararası Çalışma Örgütü gibi kurum ve kuruluşların çalışma havatında mesleki risklerden korunmak gelistirdiği-belirlediği standartlar dikkate alınarak değerlendirmeli ve gereği yapılmalıdır. Zira bu koşulların sağlanması, laboratuvarları kullanan bilim insanlarının uygulama ve bilimsel faaliyet üretme sürecinde sağlık risklerini azaltarak motivasyonlarını ve buna bağlı olarak verimlilik ve performanslarını artıracaktır. Bununla birlikte, farklı araştırma merkezi ve üniversitelerdeki örneklem gruplarıyla yapılacak çalışmalar ile ülkemizdeki biyogüvenlik kapsamında laboratuvar güvenliği ile ilgili mevcut durumun ortaya çıkarılması ve elde edilen sonuçlar doğrultusunda öneriler geliştirilmesinin, konuyla ilgili sınırlı sayıdaki literatüre katkısının yanı sıra ilgili kişi, kurum-kuruluş yöneticilere de veri kaynağı olacağı değerlendirilmektedir.

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RESEARCH ARTICLE

Pathologic Examination of the Protective Effect of Glycyrrhizin on Cyclophosphamide-induced Hemorrhagic Cystitis in Rats

Okan ERDOĞAN¹, Hikmet KELE޲*

¹Banaz Distinc Health Directorate, 64500, Usak, TURKEY ²Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, 03030, Afyonkarahisar, TURKEY

ABSTRACT

Cyclophosphamide (CP) is a widely used anti-neoplastic agent in humans and small animals and also has some adverse effects in them. The most common adverse effect of CP is hemorrhagic cystitis (HS). The use of Mesna may prevent HS, but is insufficient for some side effects. In this study, the effect of glycyrrhizin (GLY), which is one of the active substances of licorice root, was examined against CP-related side effects. The experiment was designed with 49 male Sprague Dawley rats. The Control group received only physiological saline and the CP group only CP. CP+Mesna group received CP and also three dose of Mesna. The animals in the CP+GLY100 and CP+GLY200 groups were received three doses of GLY, 100 mg/kg and 200 mg/kg dose, respectively. The previous protocol was applied to the CP+Mesna+GLY100 and CP+Mesna+GLY200 groups and only the first GLY application was replaced with Mesna. In this study, it was recorded that bladder macroscopy and histopathology were better preserved in GLY applicated groups compared to CP group. However, it was observed that this protection was slightly weaker than the Mesna and GLY-Mesna combinations. From this point of view, it was observed that GLY could be preventive in CP-related HS, in addition it could also support to Mesna applications.

Keywords: Cyclophosphamide, Glycyrrhizin, Hemorrhagic cystitis, Mesna, Pathology

Ratlarda Siklofosfamid ile İndüklenen Hemorajik Sistitisde Glycyrrhizinin Koruyucu Etkilerinin Patolojik Olarak İncelenmesi

ÖZ

Siklofosfamid (CP) yaygın olarak kullanılan bir anti-neoplastik ajandır ve bazı yan etkilere sahiptir. Siklofosfamidin en belirgin yan etkisi hemorajik sistitistir (HS). Mesna kullanımı HS'yi önleyebilmekte ancak diğer bazı yan etkiler için yetersiz kalmaktadır. Bu çalışmada, CP ilişkili HS'ye karşı, meyan kökünün aktif içeriklerinden olan glycyrrhizinin (GLY) etkisi incelenmiştir. Çalışma, 49 adet, erkek Sprague Dawley rat ile dizayn edilmiştir. Kontrol grubuna sadece fizyolojik tuzlu su, CP grubuna ise sadece CP verilmiştir. CP+Mesna grubuna CP ve ayrıca 3 doz Mesna uygulanmıştır. CP+GLY100 ve CP+GLY200 gruplarına; CP ve sırasıyla 100 mg/kg ile 200 mg/kg olmak üzere üç doz GLY uygulanmıştır. Bir önceki protokol CP+Mesna+GLY100 ve CP+Mesna+GLY200 gruplarınada uygulanmış olup sadece ilk GLY uygulaması Mesna ile değiştirilmiştir. Çalışmada GLY uygulanan gruplarda CP grubuna kıyasla mesane makroskobisi ve histopatolojisinin daha iyi bir şekilde korunmuş olduğu kaydedilmiştir. Ancak, bu korumanın Mesna ve GLY-Mesna kombinasyonlarına kıyasla biraz daha zayıf kaldığı izlenmiştir. Buradan yola çıkılarak GLY'in CP ilişkili HS'de önleyici olabileceği aynı zamanda Mesna uygulamalarına destek verebileceği kanaatine varılmıştır.

Anahtar Kelimeler: Glycyrrhizin, Hemorajik sistitis, Mesna, Patoloji, Siklofosfamid

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ORCID ID; OE: 0000-0002-8850-0573, HK: 0000-0002-2308-0811

*Corresponding author e-mail: hkeles@aku.edu.tr

INTRODUCTION

Hemorrhagic cystitis (HS) is a complication that is difficult to treat and occurs due to different etiologies (Del Pizzo et al. 1998, Manikandan et al. 2010). Considering the cases, it is observed that cyclophosphamide (CP) and ifosfamide, which are used for chemotherapeutic purpose, play a leading role in the cause of HS (Philips et al. 1961, deVries and Freiha 1990, West 1997). In the chronological process, various methods have been tried to prevent and/or cure CP-associated HS, but success has been limited and changed for each patient (Ballen et al. 1999). In clinical medicine, Mesna (2-mercaptoethan sodium sulfonate) is mostly preferred to prevent CPassociated HS (Soysal 2004). Mesna, which may be ineffective in relieving some clinical, macroscopic and microscopic symptoms (Gressier et al. 1995), also has side effects such as allergic reactions, dermatosis and hypersensitivity in the skin (Khaw et al. 2007, Dorris et al. 2011, Lin and Keefe 2012).

In recent studies, a tendency towards plant and extracts has been clearly observed in order to prevent damage caused by CP-induced HS. In these applications, the active ingredient can be used alone or in combination with Mesna. Ankaferd Blood Stopper® is a product consisting of Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and Urtica dioica. This product, which is also used for blocking the bleeding, in other parts of the body, is also used against HS (Goker et al. 2008). Against the CP-induced urotoxicity, morning glory (Ipomoea obscura) (Hamsa and Kuttan 2011),; ternatin (Vieira et al. 2004), a flavonoid extracted from chamomile (Egletes viscosa Less.);, curcumin, an extraction product of turmeric (Curcuma longa) (Arafa 2009),; berberine (Xu and Malavé 2001) obtained from Berberis spp.,; active ingredients of Mandevilla (Mandevilla velutina) (Santos et al. 2010),; Pyllanthus niruri plant (Boeira et al. 2011),; resveratrol (Keles et al. 2014) extracted from grape seed,; ellagic acid (Kankaya 2017) found abundantly in blackberry, pomegranate and rosehip species, were found to be efficient in preventing HS in experimental studies in mice and rats.

Glycyrrhiza glabra plant, also known as "Meyan" in our country, is consumed in different ways (Asımgil 1997, Baytop 1999a, 1999b). One of the active agents of this plant is glycyrrhizine (GLY). Glycyrrhizin has been shown to have a therapeutic effect in neoplasias with various etiologies (Ito et al. 1988, Lin et al. 1999, Shiota et al. 1999, Sohn et al. 2003, Ram et al. 2006, Asl and Hosseinzadeh 2008, Kim et al. 2012, Park et al. 2013). Anti-coagulative (Assafim et al. 2006), anti-inflammatory (Francischetti et al. 1997), anti-edematous (Ohnishi et al. 2011), and anti-hemorrhagic (Ieong et al. 2018) properties were reported in GLY applications. Glycyrrhizin

suppresses nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression (Wang et al. 2011, Uto et al. 2012). It has been suggested that NO is responsible for CP-related bladder damage (Oter et al. 2004) and NO increase was reported in non-CP cases, especially in the urothelial damage and hemorrhage occurred areas (Keles et al. 2018).

There was no experimental or field study in which GLY was used against CP-related HS in the literature review. In this study and context, the protective effects of GLY against controlled HS were investigated pathologically.

MATERIALS and METHODS

Cyclophosphamide (CP) (Endoxan®, 150 mg/kg Eczacibasi Istanbul, Turkey), Mesna (Uromitexan®, 400 mg, Eczacibaşi Baxter, Turkey), and Glycyrrhizin (GLY) (Sigma, Cas 53956-04-0) were purchased commercially. This study was carried out at Afyon Kocatepe University Experimental Animal Research and Application Center with permission of AKUHADYEK-477-15. The animals were given ad libitum standard rodent feed and tap water during the experiment. They were housed in polycarbonate cages, maintained on 12-hours light/12-hours dark cycle at room temperature (22±0.5°C) and in appropriate humid environment. The experiment was started at the end of the 7 day adjustment period.

In the study, 49 male Sprague Dawley rats (220-250 g) were divided into seven groups of seven rats each. Hemorrhagic cystitis model was created according to our previous study (Keles et al. 2014). The animals in the Control group were given only 2-ml saline and the animals in the CP group were given 2 ml of CP (150 mg/kg, i.p). CP+Mesna group was given CP (150 mg/kg, i.p.) and a total of 90 mg/kg Mesna was administered in three equal doses in 2-ml saline by i.p. The first injection was given 20 min before the CP injection, and the second and third injections were administered 4 and 8 h after CP injection, respectively. CP+GLY100 and CP+GLY200 groups were given CP (150 mg/kg, i.p.) and a total of 300 mg/kg and 600 mg/kg GLY were administered by gastric gavage (g.g.) in three equal doses in 2-ml saline, respectively. The first gavage was given 20 min before the CP injection, and the second and third gavages were administered 4 and 8 h after CP injection. Same applications were made CP+Mesna+GLY100 and CP+Mesna+GLY200 groups but only the first GLY application was replaced with Mesna (30 mg/kg i.p). After 48 h of HS induction, rats were killed by using injection of ketamine HCL (80 mg/kg, i.p.) and xylazine HCL (10 mg/kg, i.p.)

Following an abdominal incision, the bladders were removed, emptied and fixed in 10 % buffered formalin. Processed tissues were blocked with parafin, and then sectioned into 5-µm sections and stained with hematoxylin and eosin (HE). These slides were examined under a light microscope (Nikon Eclipse Ci attached Kameram® Digital Image Analyze System) and graded as mild (+), moderate and severe (+++)for hemorrhage, (++),desquamation, degeneration, inflammation, edema, and congestion.

Statistical analysis

At the end of the experiment, data with normal distribution was analyzed using one-way analysis of variance (ANOVA) and least significance difference (LSD) test were used to determine the statistical significance of the data. For statistical significance, p<0.05 was accepted.

RESULTS

Clinical and Macroscopical Results

After drug applications, the animals were taken to the corners of the cage for a period of time. Compared to the Control group, an increase of water consumption and a limited decrease of feed consumption were recorded in the experimental groups. Furthermore, intense hematuria with hand manipulations especially in animals of CP group was observed.

No macroscopic findings were observed in the bladders of the animals in the Control group (Figure 1A). The animal in the CP group had thickened wall of bladder, hemorrhages on both mucosa and serosa (Figure 2A) and often had a coagulated blood mass in the lumen. The bladders of the animals in the CP+Mesna group were macroscopically similar to those in the Control group (Figure 3A). The bladders of animals in the CP+GLY100 group had a serosal, mucosal and luminal hemorrhages on the thicker bladder wall compared to the Control group, but the lesions were milder than the CP group (Figure 4A). The bladder findings of the animals in the CP+GLY 200 group were similar to the bladder findings of the animals in the CP+GLY 100 group, but were less severe (Figure 5A). The bladder findings of CP+Mesna+GLY100 group were much lighter than group. Lesions were less severe CP+GLY100 and CP+GLY200 groups (Figure 6A). The bladder findings of the animals in the CP+Mesna+GLY200 Group were considerably mild compared to the CP group. The observed lesions were also lighter than the lesions detected in the CP+GLY200 CP+GLY100, CP+Mesna+GLY100 groups. The bladders in this group were similar to the Control group and the CP+Mesna group (Figure 7A).

Histopathological Results

Microscopical examinations revealed degenerative and desquamative findings, as well as acute inflammatory changes in the bladder tissues with varying intensity of congestion, edema and hemorrhages.

The levels of congestion in the CP, CP+GLY100 and CP+GLY200 groups were significantly higher than the Control group and other groups. In the CP+Mesna+GLY100 and CP+Mesna+GLY200 groups, the congestion was quite lesser compared to the CP+Mesna group and even at the Control group level.

There were no signs of edema in the Control group and CP+Mesna+GLY100 groups. Significantly lower edema in the CP+GLY200 group was similar to the CP+Mesna group. The most severe edema level was observed in the CP group. Compared to the CP group, the low edema in the CP+GLY200 group was relatively higher in the CP+GLY100 group.

No signs of hemorrhage were observed in the Control, CP+Mesna, CP+Mesna+GLY100 and CP+Mesna+GLY200 groups. The most severe hemorrhages occurred in the CP group. Mild hemorrhages were also observed in the CP+GLY100 and CP+GLY200 groups, but the difference between the groups was statistically insignificant.

While no signs of inflammation were observed in the Control, CP+Mesna+GLY100 and CP+Mesna+GLY200 groups, it was determined that the level of inflammation in CP+Mesna group was not statistically significant compared to the first three groups. However, it was noted that the level of inflammation in CP+GLY200, CP, and CP+GLY100 groups was statistically significant compared to other groups.

No degeneration signs were observed in the Control group and the most severe degenerative findings were observed in the CP group. It was observed that degeneration observed in CP+GLY100 and CP+GLY200 groups was statistically significant compared to CP+Mesna group. The lowest level of degeneration was observed in CP+Mesna+GLY200 group and the degeneration level in this group decreased significantly compared to CP+Mesna group.

There was no statistically significant change in the level of desquamation between Control, CP+Mesna+GLY100, and CP+Mesna+GLY200 groups, whereas the increase in desquamation in CP, CP+Mesna, CP+GLY100, and CP+GLY200 groups was statistically significant compared to these three groups (Table 1 and Figures 1B-7B).

Table 1. Histopathological parameters in study groups

Groups	Hemorrhage	Desquamation	Degeneration	Inflammation	Edema	Congestion
Control	0±0	0,28±0,487	0±0	0±0	0±0	1,0±1,0
CP	4,0±0,816	2,42±1,40	3,57±1,133	3,28±0,951	4,57±0,787	3,85±1,215
CP+M	0±0	1,71±0,951	1,71±1,253	0,14±0,377	0,71±1,496	2,14±0,899
CP+GLY100	<i>3,5</i> ±1,397	1,71±0,487	2,0±0	3,14±1,676	4,0±0,577	<i>3,71±0,487</i>
CP+GLY200	3,28±0,756	2,42±1,397	2,71±0,755	3,43±0,534	3,57±0,534	3,0±0,577
CP+M+GLY100	0 ± 0	0±0	1,0±0	0 ± 0	0 ± 0	1,28±0,487
CP+M+GLY200	0±0	0,14±1,299	0,43±0,534	0 ± 0	0,14±0,378	1,28±0,487

All values are given as mean \pm standard deviation. p<0.05

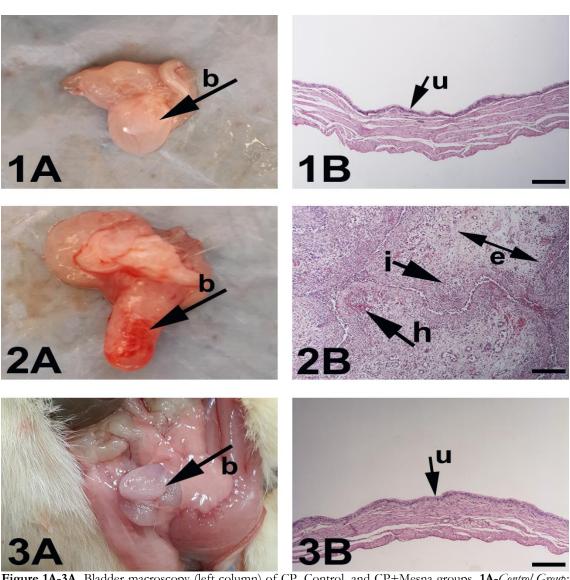


Figure 1A-3A. Bladder macroscopy (left column) of CP, Control, and CP+Mesna groups. 1A-Control Group: normal macroscopic appearance. 2A-CP Group: thickened wall and serosal hemorrhages. Coagulated blood and mucosal hemorrhages were observed in the lumen. 3A-CP+Mesna Group: any macroscopic lesion. Figure 1B-3B. Bladder histology (right column) of CP, Control, and CP+Mesna groups. Scale bar is 200 μm in the hematoxylin-eosin stained figures. Arrows pointing events; b:bladder, u:urothelium, i:inflammation, e:edema, h:hemorrhage 1B-Control Group: normal histological view of bladder. 2B-CP Group: severe congestion, hemorrhages, edema, inflammation, degeneration, and desquamation. 3B-CP+Mesna Group: histological view of normal bladder.

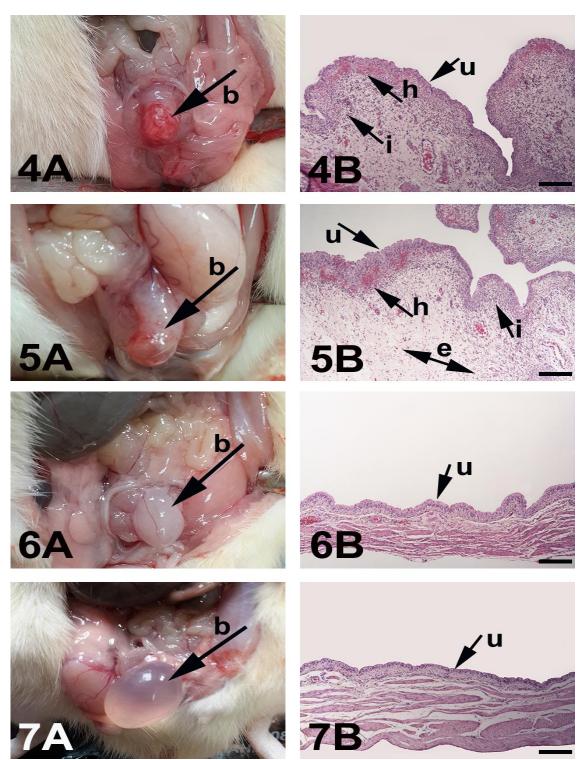


Figure 4A-7A. Bladder macroscopy (left column) of CP+GLY100, CP+GLY200, CP+Mesna+GLY100, and CP+Mesna+GLY200 groups. 4A-CP+GLY100 Group: thickened wall, serosal and mucosal hemorrhages. 5A-CP+GLY200 Group: lighter lesions, similar to CP+GLY100 group. 6A-CP+Mesna+GLY100 Group: significantly lighter hemorrhagic and edematous lesions. 7A-CP+Mesna+GLY200 Group: the best macroscopic appearance, similar to Control and CP+Mesna groups.

Figure 4B-7B. Bladder histology (right column) of CP+GLY100, CP+GLY200, CP+Mesna+GLY100 and CP+Mesna+GLY200 groups. Scale bar is 200 µm in the hematoxylin-eosin stained figures. Arrows pointing events; b:bladder, u:urothelium, i:inflammation, e:edema, h:hemorrhage 4B-CP+GLY100 Group: Congestion, hemorrhage, edema, inflammation, degeneration, and desquamation, which are less severe than CP group. 5B-CP+GLY200 Group: A slightly lighter lesion than the CP+GLY100 group. 6B-CP+Mesna+GLY100 Group: Very mild vascular findings, epithelium is preserved. 7B-CP+Mesna+GLY200 Group: any microscopical lesion.

DISCUSSION

The use of G. glabra root for medical purposes dates back to Christ (Akan and Balos 2008, Çınar 2012). This plant, which is widely used in our ancient medical tradition, is still used today (Uygun 2015). It has been reported that GLY, one of the active agents of this plant, inhibits coagulation and platelet aggregation in humans, and when combined with Bothrops jararaca snake venom in rats, it decreases the formation of thrombosis and also substantially eliminates venom-induced hemorrhages (Assafim et al. 2006). Glycyrrhizin which also has antiinflammatory properties (Francischetti et al. 1997), inhibits the migration of leukocytes to the site of inflammation via selectins (Rao et al. 1994). In experimental cerebral hemorrhage models of rats, which reduces inflammation following GLY hemorrhage (Ieong et al. 2018) and brain edema (Ohnishi et al. 2011), prevents possible brain injury.

In the light of the information given in the literatures; in this study, GLY, which is thought to reduce CP associated bladder lesions, was applied to animals at doses of 100 and 200 mg/kg. At the end of the study, serosal, mucosal, and luminal hemorrhagic surface along with the thickness of the bladder wall were decreased as compared to the CP group. In the microscopical examination, it was observed that the symptoms such as inflammation, congestion, edema, and hemorrhage, which were severely formed in the CP group, were alleviated in animals treated with 100 mg/kg GLY, and these lesions were very mild in 200 mg/kg GLY treated animals.

In some experimental studies (Vieira et al. 2004, Kankaya 2017), the active ingredients were administered alone and/or combined with a single Mesna application for protection from precursor acrolein damage and successful results were reported from these studies. In accordance with these studies, we also examined 100 and 200 mg/kg GLY alone and in combination with Mesna to observe the success of the active substance in four groups of animals. The success of the GLY-Mesna combinations was higher than the groups treated with GLY alone, and there was a positive correlation with the increase in the dose of GLY.

Kankaya (2017), who analyzed ellagic acid in CP-associated HS model in rats, reported that this active substance yielded successful results both macroscopically and microscopically, and correlated this success with active substance-related antioxidant capacity. Ankaferd and epinephrine applications against CP have been reported to prevent congestion and edema in both agents (Kilic et al. 2016). It has been reported that Berberin completely blocked the bladder edema and hemorrhages at increasing doses of the CP-associated HS model (Xu and Malavé

2001). Mandevilla velutina has been shown to inhibit CP-associated HS (Santos et al. 2010). It was reported that CP-associated edema, hemorrhage, and congestion decreased in the bladder in different doses of resveratrol (Keles et al. 2014). In this study, hemorrhage were found to be at the Control group levels in GLY-Mesna combined groups. The high level of edema in low-dose GLY alone was similar to Control group and GLY-Mesna combined groups. Similar to other vascular findings, such as hemorrhage and edema, GLY-Mesna combinations were also beneficial in preventing congestion.

A positive condition has been reported in CP-associated bladder inflammation due to the action of ellagic acid (Kankaya 2017) The content of *Mandevilla velutina* has been shown to inhibit the inflammation of the bladder in experimental HS cases (Santos et al. 2010). *Ipomoea obscura* has been shown to have an anti-inflammatory effect in this context (Hamsa and Kuttan 2011). Anti-inflammatory properties of *Phyllanthus niruri* plant were investigated by Boeira et al. (2011). In this study, it was found that anti-inflammatory effect was limited in both doses of GLY alone, but anti-inflammatory effect was significant in GLY-Mesna combinations.

The combination of ellagic acid with Mesna was found to be favorable against CP-associated epithelial damage and degeneration (Kankaya 2017). Researchers who found Ankaferd and epinephrine effectivity in preventing necrosis and ulceration referred to Ankaferd for epithelial regeneration (Kilic et al. 2016). Curcuma longa content was reported to has been urothelial preservative in CP-induced HS (Arafa 2009). In this study, when degenerative and desquamative changes are considered, it was seen that GLY had a successful or contingent effect and this effect was quite high in GLY-Mesna combined groups.

In conclusion, CP-associated macroscopic and microscopic lesions in the bladder were observed to be quite mild in the groups treated with GLY alone, and GLY-Mesna combinations were found to have been even more advanced and also a positive correlation was observed for the increased dose. Mesna is administered by i.p. route in clinical applications. As known, the i.p. route means the maximum absorption rate and maximum quantity for many drugs and active substances after the intravenous (i.v.) route. Unfortunately, GLY is a substance that is not suitable for i.p. or i.v. administration and is required to administrate by g.g. in our study. In g.g. applications, bioavailability is very low compared to the previous administration routes and this g.g. route may limit the success achieved as a result of the study. We believe that more promising results can be achieved with the development of i.p. or i.v. forms of GLY.

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RESEARCH ARTICLE

Effects of Bedding Change Frequency on Lipid Peroxidation, Antioxidant Status, and Histopathological Alterations in Rats

Damla ARSLAN-ACAROZ1*, Engin GOKSEL2, Hasan Hüseyin DEMIREL1, Sinan INCE3

¹Department of Laboratory and Veterinary Health, Bayat Vocational School, Afyon Kocatepe University, Afyonkarahisar, Turkey

²Experimental Animal Research and Application Center, Afyon Kocatepe University, Afyonkarahisar, Turkey

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

ABSTRACT

The aim of this research was to evaluate the effect of bedding change frequency on oxidative stress parameters of rats. In the experiment; animals were randomly and equally divided into 4 groups, each group consisted of 6 male rats. Group 1; bedding change every day, Group 2; bedding change once in 2 days, Group 3; bedding change once in 4 days, and Group 4; bedding change once in a week. Dust-free wood shavings were used as bedding material and the study lasted for 2 months. At the end of experimental period, MDA levels were found to be increased in blood, liver, kidney, heart, brain, and lung of groups with longer intervals of bedding change whereas GSH levels of these tissues were decreased. It was also found that SOD and CAT activities were higher in erythrocyte and lung tissues in Group 1 than the other groups. Moreover, notable histopathological alterations were observed in the tissues of longer intervals of bedding change (especially, group 3 and 4). As a result, it has been determined that long periods of bedding change in animals causes oxidative stress, tissues damages, and these alterations adversely affect the life quality of laboratory animals.

Keywords: bedding, rat, oxidative stress

Sıçanlarda Altlık Değişim Sıklığının Lipid Peroksidasyon, Antioksidan Durum ve Histopatolojik Değişiklikler Üzerine Etkileri

ÖZ

Bu araştırmanın amacı sıçanlarda altlık değişim sıklığının oksidatif stres parametreleri üzerine etkisi değerlendirmektir. Çalışmada hayvanlar her grupta 6 erkek sıçan olacak şekilde rastgele ve eşit olarak 4 gruba ayrıldı. Gruplar: Grup 1; her gün altlığı değişen, Grup 2; 2 günde bir altlığı değişen, Grup 3; 4 günde bir altlığı değişen ve Grup 4; haftada bir altlığı değişen şeklinde oluşturuldu. Çalışma 2 ay olacak şekilde planlandı ve altlık malzemesi olarak tozsuz ağaç talaşı kullanıldı. Çalışmanın sonunda, daha uzun aralıklarla altlık değişimi olan gruplarda kan, karaciğer, böbrek, kalp, beyin ve akciğer dokularında MDA düzeylerinin arttığı, bu dokulardaki GSH düzeylerinin ise azaldığı görülmüştür. Ayrıca, eritrosit ve akciğer dokularında SOD ve CAT aktivitelerinin Grup 1'de, diğer gruplardan daha yüksek olduğu belirlendi. Ayrıca, daha uzun aralıklarla altlıkları değişen grupların (özellikle grup 3 ve 4) beyin, akciğer, kalp, karaciğer ve böbrek dokularında histopatolojik değişiklikler gözlendi. Sonuç olarak, hayvanlarda uzun aralıklarla altlık değişiminin oksidatif strese, doku hasarlarına yol açarak laboratuvar hayvanlarının yaşam kalitesini olumsuz etkilediği belirlenmiştir.

Anahtar Kelimeler: altlık, sıçan, oksidatif stress

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ORCID ID; DAA: 0000-0001-9230-6725, EG: 0000-0002-2098-3978, HHD: 0000-0002-4795-2266,

SI: 0000-0002-1915-9797

*Corresponding author e-mail: darslan@aku.edu.tr

INTRODUCTION

One of the main considerations of animal welfare is microenvironment. Microenvironment comprises bedding change frequency, bedding material, and housing density (Domer et al. 2012). At the laboratory, rat cages are regularly cleaned between 2-3 times per week depending on animal facility conditions (Mason et al. 2006). In the cleaning process of animals, they are moved from one cage to another or their cages are transported with animals inside a laboratory or between laboratories which are routine animal facility procedures (Castelhano-Carlos and Baumans 2009). The cleaning process may affect acute behavioural and physiological responses of rats; they react to these procedures (Mason et al. 2006; Castelhano-Carlos and Baumans 2009). Bedding type, is a vital factor for laboratory animals, has an impact on the health and well-being of laboratory animals. Various bedding materials are preferred for laboratory animals such as wood shavings, paper, corn cobs, and chips (Potgieter and Wilke 1993; Yildirim et al. 2017). Bedding materials are closely related to aeration and moisture content which affects microbial quality (Potgieter and Wilke 1993). Wood shavings have recently been widely preferred as bedding material choice for laboratory animals. They are composed of fine particles of wood, also it is low-cost material. (Dean 1999; Yildirim et al. 2017).

The imbalance between the production of free radicals and antioxidants causes oxidative stress. This imbalance leads to biomolecular (lipids, proteins, and DNA) and cellular damage so it has an essential impact on the organism (Durackova 2010). The harmful effects of free radicals are balanced by the action of antioxidants (Valko 2006). One of the main biomarkers of oxidative stress parameter is malondialdehyde (MDA). Also, the measurements of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) take a pivotal place to determine oxidative statue (Sisein 2014).

In this study, we aimed to evaluate the effect of bedding change frequency on live weight gain, oxidative stress parameters, and histopathological changes of tissues of rats.

MATERIAL and METHODS

In the study, dust-free wood shavings were used as bedding material. All reagents and chemicals were obtained from commercial sources. Healthy male 60 days of age Wistar albino rats (250–300 g) were obtained from Animal Breeding Laboratories of Afyon Kocatepe University, Turkey.

The rats were housed under optimal conditions (25 °C, 50–55% relative humidity, 12 h/12h light/dark cycle). Also, standard rodent diet and clean water

were given *ad libitum* to animals. In addition, animals were acclimatised to the condition of the animal breeding laboratory for 7 days before the experiment started.

The rats were randomly divided into 4 equal groups; each group included 6 male rats. The experimental period was 60 days and the experimental groups were as follows:

Group 1; the bedding was changed every day. Group 2; the bedding was changed every 2 days. Group 3; the bedding was changed every 4 days. Group 4; the bedding was changed one a week. The protocols of experimental design were also approved by the Animal Care and Use Committee of Afyon Kocatepe University (Reference Number: 49533702-73) and were consonant with the principles of NIH.

Erythrocytes preparation was performed according to the method of Winterbourn et al. (1975) and homogenates were prepared according to Kucukkurt et al. (2008).

Kidney, liver, heart, brain, and lung tissues from each animal were collected from rats. Then, their tissues stained with haematoxylin-eosin (H&E) which were analyzed under Olympus light microscope (Bx51) equipped with a camera (Olympus DP20 Tokyo, Japan).

MDA (Draper and Hardley 1990; Ohkawa et al. 1979) and GSH (Beutler et al. 1993) levels of whole blood and tissue homogenates were determined based on spectrophotometric methods. Also, SOD (Sun et al. 1988) and CAT (Luck 1955; Aebi 1974) activities of erythrocyte lysate and tissue homogenate were measured by spectrophotometrically. The haemoglobin and protein contents of tissues were assayed according to methods of Drabkin and Austin (1935), and Lowry et al. (1951), respectively.

Statistical analyses

Obtained data were analysed using one-way ANOVA by using SPSS (20.0). Data were stated as mean and standard deviation (±SD). Also, differences between the groups were specified by Duncan post-hoc test. A difference was considered to be significant as p < 0.05.

RESULTS

Effect on body weight gain

The effect of bedding change frequency on body weight gain of animals was investigated from the beginning of the study. It was found statistically insignificant (p> 0.05) and shown in Figure 1.

Effect on MDA and GSH

MDA is a naturally occurring end product of lipid peroxidation. The effect on bedding change

frequency on MDA levels of blood, kidney, liver, heart, brain, and lung tissues was investigated.

MDA levels of blood, kidney, liver, heart, brain, and lung tissues were found to be high level in the long interval of bedding change groups compared to group 1 (p<0.05) and shown in Table 1. On the contrary, GSH levels of blood, kidney, liver, heart, brain, and lung tissues were found to be low in the long interval of bedding change groups compared to group 1 (p<0.05) and shown in Table 2.

Effects on SOD and CAT activities

SOD and CAT (as antioxidant enzymes) activities were determined in erythrocyte, kidney, liver, heart, brain, and lung tissues of rats and they are shown in Table 3 and Table 4, respectively. SOD and CAT activities were found to be low in erythrocyte and lung in the long interval of bedding change groups compared to group 1 (p<0.05). In addition, SOD and

CAT activities were found to be statistically insignificant in the other tissues.

Histopathological examination

Histopathological alterations in brain, lung, heart, liver, and kidney of experimental groups were shown in Figure 2. Neuronal degeneration and focal gliosis in brain (Figure 2A4), oedema and interalveolar septal thickening in lung (Figure 2B3 and B4), slight hyaline degeneration in heart (Figure 2C4), sinusoidal hyperemia (Figure 2D3), degeneration and necrosis in vena centralis of liver (Figure 2D4) have been observed in group 3 and group 4. Also, there was a focal mononuclear cell infiltration in kidney and necrotic changes in tubulus (Figure 2E4). In group 1 and group 2, no fundamental histopathological changes were observed in brain, lung, heart, liver, and kidney of rats (Figure 2A-E1, and 2A-E2, respectively).

Table 1. Effects of bedding change frequency on glutathione levels in blood, kidney, liver, heart, brain, and lung tissues of male rats.

	Blood	Kidney	Liver	Heart	Brain	Lung
	(nmol/ml)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)
Group 1	3.32 ± 0.52^{d}	4.52±0.59b	3.25±0.49b	3.49 ± 0.83^{b}	6.55±1.15 ^b	4.16±0.52 ^b
Group 2	4.58 ± 0.26^{c}	4.54 ± 0.43^{b}	4.24 ± 0.62^{a}	4.33 ± 0.76^{b}	6.86 ± 0.86 ^b	4.33 ± 0.76^{b}
Group 3	6.01 ± 0.91^{b}	4.53 ± 0.71^{b}	4.22 ± 0.48^{a}	9.08 ± 1.12^{a}	7.08 ± 0.55^{b}	9.21 ± 1.76^{a}
Group 4	11.15 ± 1.25^{a}	5.42 ± 0.57^{a}	4.54 ± 0.56^{a}	9.26 ± 0.91^{a}	8.74 ± 1.00^{a}	9.26 ± 0.91^a

Values are mean \pm Standard deviations; n = 6.

Table 2. Effects of bedding change frequency on malondialdehyde levels in blood, kidney, liver, heart, brain, and lung tissues of male rats.

	Blood	Kidney	Liver	Heart	Brain	Lung
	(nmol/ml)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)	(nmol/g tissue)
Group 1	47.41 ± 4.54^{a}	23.91 ± 4.07^{a}	21.61 ± 2.03^{a}	20.07 ± 1.72^{a}	19.54 ± 2.05^{a}	21.14±1.85a
Group 2	45.09 ± 5.02^{a}	22.77 ± 5.09 ab	19.24±1.37b	20.00 ± 1.13^a	17.43±1.45b	$19.19 \pm 1.45^{\mathrm{ab}}$
Group 3	34.62 ± 4.55 b	18.65 ± 2.35 bc	18.87 ± 1.33 b	19.79 ± 1.59^a	17.19 ± 0.80 b	18.96 ± 3.61 ab
Group 4	23.11±3.67°	18.06 ± 1.18^{c}	16.63±1.43°	17.27 ± 1.15 b	15.83±1.19b	16.85 ± 1.42^{b}

Values are mean \pm Standard deviations; n = 6.

Table 3. Effects of bedding change frequency on superoxide dismutase activities in erythrocyte, kidney, liver, heart, brain, and lung tissues of male rats.

	Erythrocyte	Kidney	Liver	Heart	Brain	Lung
	(U/gHb)	(U/µg protein)	(U/μg protein)	(U/μg protein)	(U/μg protein)	(U/µg protein)
Group 1	29.18 ± 1.10^{a}	1.97±0.61	2.25 ± 0.60	3.70 ± 0.87	5.20 ± 1.49	6.06 ± 0.92^{a}
Group 2	$18.94 \pm 2.65^{\mathrm{b}}$	1.75 ± 0.59	2.08 ± 0.80	4.01 ± 1.20	4.44±1.53	3.73 ± 0.96^{b}
Group 3	17.49 ± 1.18^{b}	2.07 ± 0.78	1.78 ± 0.30	4.24 ± 1.16	3.57 ± 0.83	3.66 ± 1.08 ^b
Group 4	12.41±1.61°	1.84 ± 0.56	2.39 ± 0.50	2.97 ± 0.90	3.45 ± 0.67	3.44 ± 0.88^{b}

Values are mean \pm Standard deviations; n = 6.

a,b,c,d In the same column values with different letters show statistically significant differences (p < 0.05).

 $^{^{}a,b,c}$ In the same column values with different letters show statistically significant differences (p < 0.05).

 $^{^{}a,b,c}$ In the same column values with different letters show statistically significant differences (p < 0.05).

Table 4. Effects of bedding change frequency on catalase activities in erythrocyte, kidney, liver, heart, brain, and

lung tissues of male rats.

	Erythrocyte	Kidney	Liver	Heart	Brain	Lung
	(k/gHb)	(k/µg protein)	(k/µg protein)	(k/µg protein)	(k/μg protein)	(k/µg protein)
Group 1	15.43±2.26 ^a	1.04 ± 0.27	0.81 ± 0.13	0.55 ± 0.07	0.35 ± 0.10	2.47 ± 0.79^{a}
Group 2	14.46 ± 3.86^{a}	1.26 ± 0.36	0.69 ± 0.15	0.56 ± 0.07	0.26 ± 0.07	1.89 ± 0.17^{b}
Group 3	8.82 ± 1.89^{b}	1.31 ± 0.24	0.61 ± 0.07	0.55 ± 0.09	0.29 ± 0.09	1.77 ± 0.34^{b}
Group 4	4.12±1.16°	1.34 ± 0.39	0.74 ± 0.12	0.49 ± 0.07	0.23 ± 0.05	1.67 ± 0.20^{b}

Values are mean \pm Standard deviations; n = 6.

 a_p, b_p In the same column values with different letters show statistically significant differences (p < 0.05). k; nmol/min

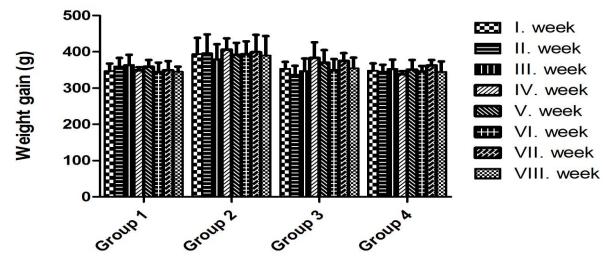


Figure 1. Weight gain of male rats during the experimental period (Mean \pm Standard deviations; n = 6).

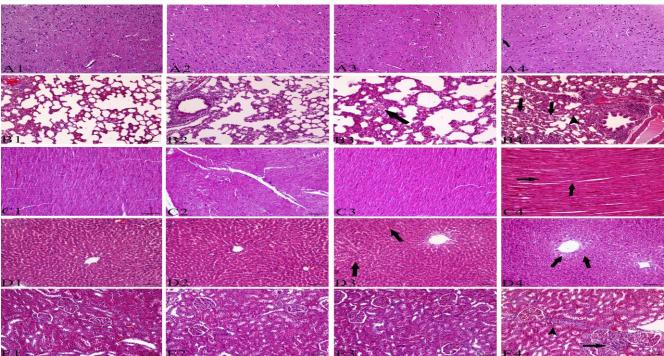


Figure 2. Effect of bedding change frequency in the brain (A), lung (B), heart (C), liver (D), and kidney tissues of male rats. Representative figures were stained with H&E. The original magnification was 20x and the scale bars represent 100 μm. Arrows and arrowheads indicate degeneration and focal gliosis in neurons of brain (Figure 2A4), oedema in lung (arrow) and thickness in interalveolar septal tissue (arrowheads) (Figure 2B3 and B4), mild hyaline degeneration in heart (Figure 2C4), sinusoidal hyperemia in liver (Figure 2D3), necrosis and degeneration in vena centralis (Figure 2D4), focal mononuclear cell infiltration (arrow) and necrotic alterations in tubules of kidney (arrowhead) (Figure 2E4). (1) Group 1; bedding change every day, (2) Group 2; bedding change once in 2 days, (3) Group 3; bedding change once in 4 days and (4) Group 4; bedding change once in a week.

DISCUSSION

Cage cleaning is important in terms of hygiene. Also, long term of cage cleaning and/or bedding change causes illness in animals. Burn et al. (2006) reported that totally 320 male Sprague Dawley and Wistar rats were kept in cages containing aspen wood or absorbent paper for 5 months and these cages were cleaned twice a week, once a week and once every two weeks. In the study, non-aggressive behaviours were determined at the highest level in once a week cage cleaned group. The incidence of sneezing and the pathological alterations of the lungs in aspen bedding animals have been reported to be higher than those used in paper bedding. At the end of the study, it has been stated that the frequency of cage cleaning affects the rats in terms of social housing but the types of bedding have a significant effect on rat health. On the other hand, Yildirim et al. (2017) performed a study with 48 male and female Sprague-Dawley rats and evaluated the effect bedding types (wood shavings, perlite, and corn cobs) on body weight. They observed that bedding material had no significant effect on body weight among the groups. In accordance with our study, it was observed that weight gain in rats was not affected by bedding change frequency. This may be due to the type of bedding or the duration of the study.

Totally, 48 female Sprague Dawley rats were divided into 4 equal groups and animals were kept in pine chips, eucalyptus pulp, vermiculite, and wire meshed bedding for 14 days. After this period, GSH levels were determined in heart, lung, and liver tissue of rats. Liver and lung GSH levels were observed at the highest level in the pine chips group and the level of heart GSH was the highest in the vermiculite group. Moreover, 48 male and 48 female Sprague-Dawley rats were kept with different types of beddings such as wood shavings, perlite, and corn cob for 30 days. At the end of the study CAT, SOD, GSH, GPx, and MDA parameters of blood samples were evaluated. It was reported that CAT, GPx, SOD, and GSH levels decreased significantly in the perlite groups, whereas MDA levels increased in the perlite groups (Yildirim et al. 2017). In the present study, it was determined that oxidative stress parameters increased in the long interval of bedding change groups. Also, it was observed that intracellular antioxidant enzyme levels in lung and blood tissues were depleted with long interval of bedding change. This situation shows that oxidation is the result of long interval of bedding change and it has a negative effect on the living conditions of animals.

Horn et al. (2012) performed a study which evaluated the effect of bedding type (grinded with poplar, cellulose and 50:50 mixture for 60 days) on lung histopathology of rats (192 male and 192 female). Peribronchial lymphoid hyperplasia was observed in

all groups. The perivascular eosinophilic filtration and metaplasia in the cellulose group, multifocal foreign body bronchopneumonia were observed in aspen and cellulose groups. Focal interstitial pneumonia was also observed in the cellulose group. Effects of sanitation frequency, cage density, and bedding type have effects on animal wellbeing and health of animals. Ferrecchia et al. (2014) evaluated four different bedding materials (1/4 of irradiated corn cob, aspen wood chips, reclaimed wood pulp, and recycled newspaper) in 100 mice. No lesions were detected in a corn cob, paper chip, or aspen chip beddings. However, mice housed on reclaimed wood pulp demonstrated significant nasal pathology which included epithelial multifocal submucosal oedema. necrosis, inflammatory cell infiltrates, haemorrhage, congestion. In the present study, it was observed that of bedding interval change caused histopathological changes in the brain, lung, heart, liver, and kidney tissues of the rats.

In conclusion, it was determined that long interval of bedding change in animals triggered oxidative stress, caused cellular damage in tissues and adversely affected the quality of life of the animals.

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RESEARCH ARTICLE

The Detection of SSRs From The Terrier Dog Normal and Tumoral Mammary Gland Tissue EST Libraries

Zehra OMEROGLU ULU¹, Merve CELİK², Salih ULU¹, Nehir OZDEMIR OZGENTURK^{1*}

¹Faculty of Art and Science, Molecular Biology and Genetics, Yildiz Technical University, Istanbul, Turkey
²Medical Biotechnology Ph.D. Program, Institute of Health Sciences, Actbadem Mehmet Ali Aydınlar University, 34752 İstanbul,
Turkey.

ABSTRACT

Dogs share a common environment with humans and knowledge of the specific dog breed diseases is very useful in developing a model for human cancer studies. ESTs represent part of the transcribed genome of an organism and are an important resource for identifying microsatellites. Simple Sequence Repeats (SSRs), or microsatellites, which contain repetitive DNA sequences, are among the most powerful genetic markers known. The development of EST-SSRs has become a fast, efficient, and low-cost option for genomic studies. In this study, to determine SSRs from EST libraryof mammary gland tissue of the Terrier dog that has 2304 ESTs; SSRIT and IMEx software, which have web-based versions and are easily accessible, were used. SSRIT finds motifs from 2 to 10 base lengths and adjusts the minimum number of repeats by eliminating single nucleotide motifs. IMEx finds perfect and imperfect microsatellites separately. It can find motifs of different lengths from 1 to 6 and the minimum number of repeats can be set. In addition, the appropriate primer for the desired SSR region can be designed. The 2, 3, 4, 5 and 6 nucleotide motifs were found for normal tissue ESTs whereas 5 nucleotide motifs were not found for tumoral tissue ESTs.

Keywords: SSR, EST-SSR, Terrier, Cancer, Dog

Terrier Köpek Normal ve Tümörlü Meme Dokusu EST Kütüphanelerinden SSR'ların Belirlenmesi

ÖZ

Köpekler, insanlarla ortak bir çevreyi paylaşır ve belirli köpek ırklarının hastalıkları hakkında bilgi, insan kanser çalışmaları için bir model geliştirmede çok yararlıdır. EST'ler, bir organizmanın transkribe edilen genomunun bir parçasını temsil eder ve mikrosatellitleri tanımlamak için önemli bir kaynaktır. Tekrarlayan DNA dizilerini içeren Basit Dizi Tekrarları (SSR'ler) veya mikrosatellitler, bilinen en güçlü genetik belirteçler arasındadır. EST-SSR'lerin gelişimi, genomik çalışmalar için hızlı, verimli ve düşük maliyetli bir seçenek haline gelmiştir. Bu çalışmada, 2304 EST içeren Terrier köpeğin meme bezi dokusunun EST koleksiyonundan SSR'lerin belirlenmesi için; Web tabanlı sürümleri olan ve kolayca erişilebilen SSRIT ve IMEx yazılımları kullanılmıştır. SSRIT, 2 ila 10 baz uzunluğundaki motifleri bulur ve tekli nükleotid motiflerini ortadan kaldırarak minimum tekrar sayısın ayarlar. IMEx mükemmel ve kusurlu mikrosatellitleri ayrı ayrı bulur. Farklı uzunluklarda 1 ile 6 arasındaki motifleri bulabilir ve minimum tekrar sayısı ayarlanabilir. Ek olarak, istenen SSR bölgesi için uygun primer tasarlanabilir. Normal doku EST'leri için 2, 3, 4, 5 ve 6 nükleotidli motifler bulunurken, tümörlü doku EST'leri için 5 nükleotidli motif bulunamamıştır. **Anahtar Kelimeler:** SSR, EST-SSR, Terrier, Kanser, Köpek

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ORCID ID; ZOU: 0000-0002-8884-4683, MC: 0000-0002-7801-6067, SU: 0000-0002-4505-0197,

NOO: 0000-0001-7093-7403

*Corresponding author e-mail: nehirozdemir@yahoo.com

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INTRODUCTION

It has been shown that the development of experimental technologies, as well as the development of information technologies, have been accompanied by the accumulation of different biological data (Bishop et al. 2015). The accumulation of the data in the biology has increased the need for software to automate the operations that are impossible to do Developments in the manually. bioinformatics have made it much easier to form large sets of biological data (Feagan et al. 2007, Collins et al. 2003). Recently, genome studies have been progressing rapidly. These genomic studies are aimed to determine when and how genes are active. Because, if the working mechanisms of the genes are understood, how these genes react to the disease can also be found (Kanehisa et al. 2003).

Dogs are affected by a large number of diseases and different dog breeds are affected by different diseases. Many dog species are particularly susceptible to inherited diseases such as cancer, heart disease, rheumatoid arthritis, autoimmune disorders, deafness and blindness (Helmen et al. 1993, Perez-Alenza et al. 2000). Dogs share a common environment with humans and they are exposed to the same carcinogens, therefore, dogs may serve as models of human diseases (Pinho et al. 2012, Antuofemo et al. 2007). Knowledge of the specific dog breed diseases is very useful in developing a model for human cancer studies. Dog is a useful model organism for human breast cancer studies due to its similarity to the human genome, genetic and morphological structure (Ozdemir Ozgenturk et al. 2018).

Researchers began collecting different types of data such as the expressed sequence-tagged sites or ESTs that would influence the nature of the sequence databases (Nagaraj et al. 2007). An EST is a short subsequence of a cDNA obtained as a result of sequence analysis. It is usually 300-500 base pairs in length and is not functional in the coding of proteins (Adams et al. 1991, Liu et al. 2013). ESTs provide a fast and inexpensive way to obtain data on the discovery of new genes, genome mapping, identification of coding regions, gene expression and regulation (Ozdemir Ozgenturk et al. 2018). ESTs represent part of the transcribed genome of an organism and are the important resource for identifying microsatellites (Li et al. 2014).

Simple Sequence Repeats (SSRs), or microsatellites, which contain repetitive DNA sequences, are among the most powerful genetic markers known. SSRs are 2-6 bp in length, short tandem-repeat sequences that are present highly in eukaryotic genomes. The repeating sequence is usually simple, consisting of two, three or four nucleotides, which can be repeated 10 to 100 times (Akagi et al. 1996, George et al. 2015,

Seyedabadi et al. 2017, Zhang et al. 2014). They have been used to study the gene mapping, genetic diversity assessing and population structure of many species because of their abundance and hypervariability throughout the genome. These markers generally show high degree polymorphism between different individuals in a species as well as between different species (Zalapa et al. 2012, Varshney et al. 2005).

SSRs could be developed from ESTs, which are especially associated with functional genes. With the high-throughput sequencing technology, the development of EST-SSRs has become a fast, efficient, and low-cost option for genomic studies (Li et al. 2014).

There are several software tools to determine SSRs in EST libraries. Many desktop and web-based tools were developed such as TRF (Tandem Repeat Finder), **SSRIT** (Simple Sequence Repeat Identification Tool), TROLL (Tandem Repeat Occurrence Locator), WebSat (A Web Software for Marker MicroSatellite Development), (Imperfect Microsatellite Extractor) (Benson et al. 1999, Temnykh et al. 2001, Castelo et al. 2002, Martins et al. 2009, Mudunuri et al. 2007).

SSRIT (Simple Sequence Repeat Identification Tool) was developed in 2001 by S. Temnykh, G. DeClerk, A. Lukashova and colleagues to detect SSRs in a set of 57.8 Mb size rice. SSRIT accepts the data in FASTA format. By eliminating single nucleotide motifs, it can find motifs from 2 to 10 base lengths and adjust the minimum number of repeats. It gives SSR file in table format as output. This table can give information about the motif, length, beginning and ending base number of the SSR and the length of the loaded sequence for analysis (Temnykh et al. 2001).

IMEx (Imperfect Microsatellite Extractor) was developed by SB Munduri in 2007. This program, which includes desktop and web-based versions, finds perfect and imperfect microsatellites separately. It can find motifs of different lengths from 1 to 6 and the minimum number of repeats can be set. The imperfection percentage to be detected can be adjusted. The program can analyze the data in a file, but the data to be uploaded must be in FASTA or Plain format. The output is in a table format and shows the motif, motif length, beginning and ending sequence. In addition, the appropriate primer for the desired SSR region can be designed (Martins et al. 2009).

In the present study, we aimed to gain the gene expression profile of mammary gland tissue based on the total 2304 ESTs as well as results of a comparison of normal and tumoral stages. These results facilitate the use of functional genomics approaches aiming at

creating gene expression patterns of tumoral and normal mammary gland. In this study, identification of SSRs from Terrier dog EST library was performed by bioinformatics analysis.

MATERIALS and METHODS

Expressed Sequence Tags: 2304 EST, sequenced from normal and tumoral mammary gland tissue cDNA libraries prepared from Terrier dogs by Nehir Ozdemir Ozgenturk et al. were used.

SSRs Detection: It was aimed to determine the correct SSRs by comparing the two common tools to avoid mistakes caused by software weaknesses. In this study, SSRIT was used to determine SSRs in Terrier EST library that has 2304 EST. IMEx was used to control the found SSRs.

For each software, the 2304 EST sequences were divided into ten parts to search. Each piece was pasted to the search window individually.

In SSRIT software, parameters from the main page were set to maximum 6 nucleotides motifs-length and minimum 5 repeats. Output was generated and the results were tabulated.

In IMEx software, basic search mode has been selected from the main page. Parameters from the main page were set to "perfect" as the repeat type, 2-6 nucleotides as motifs-length and 5 as minimum repeat number. Output was generated the results were tabulated.

Statistical Analysis: Data were analyzed using paired t-test. A difference was regarded as significant if p<0.05.

RESULTS

SSR motifs of the EST library have been determined by SSRIT software and the found SSRs have been controlled by IMEx software. Table 1 to 4 show the SSR results of each software separately in tabular form. Normal mammary gland tissue and tumoral mammary gland tissue SSR results differ from each other. The 2, 3, 4, 5 and 6 nucleotide motifs were found for normal tissue ESTs whereas 5 nucleotide motifs were not found for tumoral tissue ESTs. CT is the most common motif in 2 nucleotides motifs for both normal and tumoral EST library. There are some common results with the two software. As the number of nucleotides in the motif increased, the repeat number and the total number of the motifs found in the EST library decreased. Based on the statistical analysis of normal and tumoral SSRs, there is no statistically significant difference between samples. Tables 5 to 8 are given as supplemental tables.

Normal EST SRRs

2, 3, 4 and 6 nucleotide (nt) long motifs were counted by all two software and total number of motifs and most common motifs were reported. As shown in Table 1 and 2, CT is the most common motif in 2 nucleotides motifs for normal EST library. The most common motifs in 3 nucleotides motifs are GGA and CTG. 4 and 5 nucleotides motifs are not common for two software. TCGGGG is the most common motif in 6 nucleotides motifs for normal EST library.

Tumoral EST SSRs

2, 3, 4 and 6 nucleotides long motifs were counted by all two software and the total number of motifs and most common motifs were reported. As shown in Table 3 and 4, CT is the most common motif in 2 nucleotides motifs for tumoral EST library. The most common motif in 3 nucleotides motifs is CAG with 7 repeats number. In motifs with 4 nucleotides, the most abundant and the motifs with the longest repeats are the same and are 5 repeats of AATG, AATA, and GATG. 5 nucleotides motifs were not found for tumoral ESTs. CCGGCT is the most common motif in 6 nucleotides motifs for tumoral EST library.

Table 1. SSRIT Results for Normal ESTs (Normal EST'ler için SSRIT Sonuçları)

Motif Length	Amount of Motif Types	Total Amount of Motifs	The Most Common Motifs	Amount of The Most Common Motifs
2 nucleotides	12	102	$(CT)_{14}$	22
3 nucleotides	16	25	(GGA) ₇ (CTG) ₉	3
4 nucleotides	9	11	(ATAA) ₅ (TTTC) ₁₈	2
5 nucleotides	1	1	(CCCCG) ₆	1
6 nucleotides	1	1	(TCGGGG) ₅	1

^{*(}Motif)_{repeat number}

Table 2. IMEx Results for Normal ESTs (Normal EST'ler için IMEx Sonuçları)

Motif Length	Amount of Motif Types	Total Amount of Motifs	The Most Common Motifs	Amount of The Most Common Motifs
2 nucleotides	12	102	(CT) ₁₄	22
3 nucleotides	16	25	(GGA) ₇ (CTG) ₉	3
4 nucleotides	7	10	(TCTT) ₁₇	3
5 nucleotides	2	2	(CGCCC) ₆ (AACCC) ₅	1
6 nucleotides	2	2	(TCGGGG) ₅ (TCCTTC) ₅	1

^{*(}Motif)_{repeat number}

Table 3. SSRIT Results for Tumoral ESTs (Tümör EST'leri için SSRIT Sonuçları)

Motif Length	Amount of Motif Types	Total Amount of Motifs	The Most Common Motifs	Amount of The Most Common Motifs
2 nucleotides	12	96	(CT) ₁₅	19
3 nucleotides	9	17	$(CAG)_7$	4
4 nucleotides	3	3	(AATG) ₅ (AATA) ₅ (GATG) ₅	1
6 nucleotides	1	2	(CCGGCT) ₅	2

^{*(}Motif)_{repeat number}

Table 4. IMEx Results for Tumoral ESTs (Tümör EST'leri için IMEx Sonuçları)

Motif Length	Amount of Motif Types	Total Amount of Motifs	The Most Common Motifs	Amount of The Most Common Motifs
2 nucleotides	12	96	$(CT)_{15}$	19
3 nucleotides	9	17	(CAG) ₇	4
4 nucleotides	3	3	(AATG) ₅ (AATA) ₅ (GATG) ₅	1
6 nucleotides	1	2	(CCGGCT) ₅	2

^{*(}Motif)repeat number

Table 5. Statistical data of 2 nucleotides lenght SSRs (2 nükleotid uzunluklu SSR'ların istatiksel verileri)

2 marala adi dan lamada	(SSRIT	results)	(IMEX results)	
2 nucleotides length	Normal	Tumor	Normal	Tumor
Mean	8,5	8	8,5	8
Variance	37,545	32,545	37,364	21,636
Observations	12	12	12	12
Pearson Correlation	0,624		0,435	
Hypothesized Mean Difference	0		0	
df	11		11	
t Stat	0,337		0,296	
$P(T \le t)$ one-tail	0,371		0,386	
t Critical one-tail	1,796		1,796	
$P(T \le t)$ two-tail	0,743		0,773	
t Critical two-tail	2,2	201	2,2	201

Table 6. Statistical data of 3 nucleotides lenght SSRs (3 nükleotid uzunluklu SSR'ların istatiksel verileri)

2 my alootidaa lamath	(SSRIT	results)	(IMEX results)	
3 nucleotides length	Normal	Tumor	Normal	Tumor
Mean	1,19	0,81	1,19	0,81
Variance	0,862	1,362	0,862	1,362
Observations	21	21	21	21
Pearson Correlation	-0,565		-0,565	
Hypothesized Mean Difference	0		0	
df	2	0	2	0
t Stat	0,94		0,94	
$P(T \le t)$ one-tail	0,179		0,179	
t Critical one-tail	1,725		1,725	
$P(T \le t)$ two-tail	0,358		0,358	
t Critical two-tail	2,086		2,086	

Table 7. Statistical data of 4 nucleotides lenght SSRs (4 nükleotid uzunluklu SSR'ların istatiksel verileri)

A mural cost dans lamasth	(SSRIT	results)	(IMEX results)	
4 nucleotides length	Normal	Tumor	Normal	Tumor
Mean	1	0,273	1,111	0,333
Variance	0,4	0,218	0,861	0,25
Observations	11	11	9	9
Pearson Correlation	-0,677		-0,629	
Hypothesized Mean Difference	0		0	
df	10		8	
t Stat	2,39		1,793	
$P(T \le t)$ one-tail	0,0	19	0,055	
t Critical one-tail	1,8	1,812 1,86		86
$P(T \le t)$ two-tail	0,0	38	0,111	
t Critical two-tail	2,2	28	2,3	06

Table 8. Statistical data of 6 nucleotides lenght SSRs (6 nükleotid uzunluklu SSR'ların istatiksel verileri)

6 marglootidaa lomath	(SSRIT results)		(IMEX results)	
6 nucleotides length	Normal	Tumor	Normal	Tumor
Mean	0,5	1	0,667	0,667
Variance	0,5	2	0,333	1,333
Observations	2	2	3	3
Pearson Correlation	-1		-1	
Hypothesized Mean Difference	0		0	
df	1		2	
t Stat	-0,333		()
$P(T \le t)$ one-tail	0,398		0,5	
t Critical one-tail	6,314		2,92	
P(T<=t) two-tail	0,795		1	
t Critical two-tail	12,7	706	4,3	803

DISCUSSION

In recent years, some studies have been published according to different dog breeds, but the information is largely dispersed and lacks functional interpretation. The aim of this paper is to present such a resource which allows the functional interpretation of the SSR data. This study and the associated database enables the integrated interpretation of SSR data from normal and tumoral mammary gland tissue of Terrier dog.

In the previous study (Ozdemir Ozgenturk et al. 2018), we have generated 2304 ESTs from a normal mammary tissue of healthy Terrier dog and a tumoral mammary tissue of another sick Terrier dog with breast cancer. To further understand the molecular mechanism and differences between tumoral and normal mammary gland tissue (Alcıgır et al. 2018), gene expression profiles were compared. This study will help to identify the genes in the mammary tissue of Terrier dog, which is used as a model organism in cancer research (Pinho et al. 2012), will be an important source for future studies and will help to understand the molecular biology of cancer.

The lack of sufficient simple sequence repeat (SSR) markers has hindered the progress of genetic selection breeding and molecular research for Terrier dog. To this end, we aimed to develop and characterize expressed sequence tag (EST)–SSRs from the mammary gland tissue of Terrier dog. Totally, potential EST-SSR markers were identified and distributed in the coding and non-coding regions. These EST-SSR markers produced in this study will greatly facilitate the Terrier dog genetic diversity and molecular breeding studies (Kaur et al. 2015).

SSRs of normal and tumoral mammary gland tissues were found with SSRIT software and controlled with IMEx software. There are differences between the data of two mammary gland tissues.

In this study, a total of 140 EST-SSRs were determined in SSRIT software for normal tissue and no significant difference was observed between them when controlled by IMEx software. A total of 118 EST-SSRs for tumoral tissue in the SSRIT software were detected and the results were observed exactly the same when controlled by the IMEx software. Results of the SSRs mined from EST libraries using SSRIT and IMEx showed dinucleotide repeats as the most abundant SSR motif type and CT is the most frequent repeats for both normal and tumoral EST libraries. Among these markers, di- and tri- repeat types were the abundant types.

Although there were some differences between the two software's work, the absence of any major differences between the results of these two programs proved the validity of SSRs and supported each other's results.

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