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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Relationship of Barn and Animal Hygiene with Milk Yield, Milk Composition and Mastitis in Holstein and Simmental Cows [*]

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Abstract: This study was conducted to determine the effect of barn hygiene on animal hygiene in Holstein and Simmental cows and the relationship between animal hygiene and milk yield, milk composition and somatic cell count (SCC). In the study, some hygiene traits of four different barns with 40 heads of Holstein and Simmental cows in the early and middle lactation period, the degree of cleanliness of the upper rear leg, lower rear leg, abdomen, udder and tail head of cows, the milk yield and composition of animals, and the SCC were determined. In the study, it was determined that bedding was more dirtiness in Holstein barns than Simmental barns. In terms of body hygiene, breed and breed X lactation period interaction were determined to be effective (P<0.05) on the scores of cleanliness of the lower rear leg, the upper rear leg and the abdomen. It was determined that there was a high correlation between the cleanliness scores of the lower and upper rear legs and the abdominal region of the body and the daily milk yield; between the cleanliness scores of the lower legs and tail head and the milk lactose ratio (P<0.05). In this study, it was concluded that the accumulation of manure on the passageway in free-stall barns causes the bedding material to become dirty and slippery, and the dirtiness of the bedding causes dirty of the upper leg (side) and abdominal region of the animals' bodies. In addition, it has been concluded that daily milk yield can be effective in dirtiness in cows' tails and that high tail dirtiness can cause a decrease in milk lactose ratio.

Keywords: Barn hygiene, cleanliness score, daily milk yield, dairy cow, lactose.

Holştayn ve Simmental İneklerinde Barınak ve Hayvan Hijyeninin Süt Verimi, Süt Bileşimi ve Mastitis ile İlişkisi

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Öz: Bu araştırma, Holştayn ve Simmental ineklerinde barınak hijyeninin hayvan hijyeni üzerine etkisi ve hayvan hijyeni ile süt verimi, süt bileşimi ve somatik hücre sayısı arasındaki ilişkiyi belirleyebilmek amacıyla yapılmıştır. Araştırmada, laktasyonun erken ve orta döneminde olan 40'ar baş Holştayn ve Simmental ineklerin bulunduğu dört farklı ahırın bazı hijyen özellikleri, ineklerin arka bacak alt ve üst kısım, karın, meme ve kuyruk bölgesi temizlik skorları ile hayvanların süt verimi, süt bileşimi ve somatik hücre sayısı (SHS) belirlenmiştir. Çalışmada, Holştayn ahırlarındaki yataklık materyallerinin Simmental ahırlarından daha kirli olduğu tespit edilmiştir. Vücut hijyeni açısından arka bacak alt kısım, arka bacak üst kısım ve karın bölgesi temizlik skoru üzerinde ırk ve ırk X laktasyon dönemi interekasyonun etkili (P<0.05) olduğu belirlenmiştir. Ayrıca, vücudun arka bacak alt ve üst kısım ile karın bölgesinin temizlik skoru ile günlük süt verimi arasında; kuyruk ve arka bacak alt kısım temizlik skoru ile süt laktoz oranı arasında yüksek korrelasyon olduğu belirlenmiştir (P<0.05). Bu araştırmada, serbest gezinmeli ahırlarda servis yolundaki gübre birikiminin, yataklıkların kirlenmesine ve kayganlaşmasına sebep olduğu, yataklıkların kirliliğinin ise hayvanların vücutlarının arka bacak üst kısmı (yan) ve karın bölgesinde kirlenmeye sebep olduğu sonucuna varılmıştır. Ayrıca, günlük süt veriminin ineklerin kuyruklarındaki kirlilikte etkili olabileceği ve kuyruk kirliliğinin süt laktoz oranında düşüşe sebep olabileceği sonucuna varılmıştır.

Anahtar kelimeler: Barınak hijyeni, günlük süt verimi, laktoz, sütçü sığır, temizlik skoru.

[*] *This research article was summarized from the first author's master's thesis.*

INTRODUCTION

Under the criteria for good housing in the welfare of dairy cattle evaluation of whether or not it's connected to animals, ease of movement, access to the walking range or pasture, animals hitting during sleep to the equipment of barn, the cleanliness of recreation and walking ranges, the resting period and the cleanliness of different parts of the animal body, so many traits such as cleaning focuses on animal hygiene (Anonymous 2009; Asan & Metin, 2016). Bartussek et al., (2000) emphasized the importance of the floors that the animal is in constant contact with, in the index he developed as "Animal Needs Index" and stated that many features such as softness, cleanliness, slipperiness, dryness of the lying area, ground condition of the movement and exercise areas and cleanliness of the barn should be evaluated in terms of animal welfare. Structural traits of the floors of the places where the animals are indirect in contact, such as the stall area, passageway, activty areas and feeding area in free-stall housing; the wetness and dirtiness in these places are caused by the presence of manure and urine wastes on the floor, adversely affect the health of the legs and udders, impairing the welfare conditions of the animals (McDaniel & Wilk, 1991). DeVries et al., (2012) reported that the areas in front of the floors and managers of the areas where animals walk, stand and lie in dairy cattle barns are the most manure-intensive areas and that the risk of mastitis can be reduced by improving barn hygiene and therefore the hygiene of animals by cleaning the manure in these areas. Depending on the inadequacy of cleaning processes, barn structure and structural problems in dairy cattle create faeces and urine wastes accumulated in the barn, as well as mud found in areas outside the barn, which are transmitted to the animal's body and form dirt. Dirtness occurring in different parts of the cows' bodies is one of the most important welfare indicators affecting the quality of life and productivity of dairy cattle raised in free-stall barn (Sant'anna & Paranhos da Costa, 2011). It has been reported in some previous studies that cows have poor body hygiene, that is, if the body is dirty, foot diseases occur, milk yield decreases, the SCC in the milk and subclinical mastitis cases increase (DeVries et al., 2012; Uzal, 2008; Şahanoğlu, 2014).

This study was conducted to evaluate the characteristics and animal hygiene in stables where Holstein and Simmental cows were breeding in early and middle lactation period and to determine the effect of barn hygiene on animal hygiene and the relationship between animal hygiene and milk yield, milk composition and SCC.

MATERIAL AND METHOD

This study was carried out at a private dairy cattle enterprise in Amasya, Turkey. The living material of the study consisted of a total of 80 cows of Holstein and Simmental breeds. For the research, 40 Holstein (average age of 29 months) and 40 Simmental (average age of 32 months) cows of similar ages, in the first lactation and the early (first 100 days of lactation) and middle (between 100-200 days of lactation) period of lactation, were selected by examining the records of the farm. The selected cows were divided into four groups according to the factors of breed and lactation period with an equal number of animals in each group. In the classification into groups, Holstein cows that are in the early period of lactation are defined as group 1, Holstein cows that are in the middle period of lactation are defined as group 2, Simmental cows that are in the early period of lactation are defined as group 3 and Simmental cows that are in the middle period of lactation are defined as group 4. During the study, no changes were made in the conditions of care and nutrition of the cows, and the farm conditions maintained.

In the study, animal hygiene was evaluated on the same day as the barn hygiene of each group for weekly periods for a month, and then milk samples were taken from each cow in all groups and analyzed for milk composition and SCC.

Barn hygiene scoring: The research was carried out in a free-stall dairy cattle farm. The stalls were designed opposite each other, with the cows facing each other, and rubber pad material was used on the floor. The mean each stall dimensions were 124.5 X 244 cm. In the study, the stall in the barns where each group is located was examined one by one, and the softness, cleanliness and slipperiness of the stall floor and the slippery level of the activity areas of the animals (passageways) were determined by Bartussek et al., (2000) was scored according to the method reported (Table 1). The scoring of barn hygiene was done by the same searcher in weekly periods for one month. Wellington were worn on the feet to assess the accumulation of manure on the passageway in the barn, and they were marked with a pencil measuring 2 cm from the front end of the wellington and 4 cm from the heel. The level of manure in the wellington was measured by walking from one end to the other end of the manure road for 20 minutes before the passageway was empty and the manure road was cleaned with a scraper. The same process was repeated 20 minutes after manure stripping. Before and after the stripping of the manure, the difference between the manure dirtiness levels determined on the wellington was taken and the scoring was made. According to this; 0 points if dirty at a level of 0.5 cm of wellington or clean (clean), 1 point if dirty at a level of 0.5-1 cm between dirty (less), 2 points if dirty at a level of between 1-3 cm of wellingtons is dirty (dirty), 3 points if contaminated at a level of 3 cm or more (very dirty) are given (Aydın, 2017).

Animal hygiene scoring: Body hygiene assessment of cows in each group was performed before milking in the evening on the day of taking milk samples in this study. While the cows were in the barn without being cleaned before milking, the hind leg of each animal was evaluated by the same researcher with scores between 1 to 5 according to the level of cleanliness of the lower rear leg (hind leg), upper rear leg (side), tail area (tail head), udder and abdominal area Reneau et al., (2005) used the method, and 1 point was given if the area examined was very clean, and 5 points were given if it was very dirty.

Table 1. Scoring of the stall floor and passageway of the barn.

Score	Stall floor			Passageway
	Softness	Cleanliness	Slipperiness	Slipperiness
2.5	> 60 mm straw			
2.0	30-60 straw			
	> 6 mm sand o.s.			
1.5	soft rubber			
	< 30 mm straw			
	< 60 mm sand			
1.0	wood, hard rubber or plastic matting, asphalt	clean	good grip	good grip
0.5	concrete, metal or plastic grids	medium	medium	medium
0	concrete slats	dirty	slippery	slippery
-0.5	concrete slats worse than above	very dirty	very slippery	very slippery

Milk analysis and SCC: In the study, milk samples were taken from each cow in all groups in the evening milking when the barn and animal hygiene scores were made. To determine the milk yield and composition and the SCC, a total of 40 ml of milk samples were taken from the four udder lobes of each animal in all groups at the end of the evening milking and placed in milk sample containers. Chemical tablets (Microtabs II), that stall microbial proliferation without influencing milk components and SCC, were added in milk samples and milk samples were transported to laboratory under cold chain (+4^{0}C) conditions and they were analyzed. To determine the ratio of fat, protein, lactose and dry matter in milk and the SCC; milk samples were heated at 40°C in a water bath before analysis. Milk composition and SCC were determined using the Combi 150 (Bentley) analyzer, which was created by integrating the somatic cell counting device (Somacount 150) and the milk component measuring device (Bentley 150) and works with the flow cytometry analysis method. In addition, from the records of the milking system of the enterprise, the daily milk yield, number of milked days and lactation milk yield records of each cow in all groups were obtained (Akdağ et al., 2017).

Statistical analysis: The conformity of the data obtained in this study to the normal distribution was determined by the Kolmogorov-Smirnov test, and the analysis was performed after applying the logarithmic transformation (log_{10}SCC) SCC did not show a normal distribution. The mod score was used for the hygiene characteristics of the barn where the research groups were located. Analysis of variance was used to compare the animal body hygiene scores, milk yield, milk composition and the SCC belonging to each group. The Duncan test was performed to evaluate differences among the groups. A correlation test was applied to determine the relationships between milk yield, milk composition and SCC and animal hygiene scores, and pearson correlation was used to determine the phenotypic correlation coefficients. In statistical calculations, the GLM (General Linear Model) procedure was used from the SPSS program package (SPSS, v21). Probability values less than 0.05 were taken to be significant (P<0.05).

RESULTS AND DISCUSSION

In this study, barn hygiene scores related to the softness, slipperiness and cleanliness of the stall floor in the barns of all groups, the level of passageway slipperiness and the accumulation of manure on the passageways are given in Table 2. The softness and slipperiness degrees of the beds received the same score in all four barns, but the Simmental barns were determined to be cleaner in terms of cleanliness of the beds than the Holstein barns. The level of the slipperiness of the passageways, which is the activity area of animals, was evaluated as moderately clinging for Simmental barns and slippery for Holstein stables. In addition, it was found that the accumulation of manure on the passageways is greater in Holstein barns than in Simmental barns. The evaluation of the bearings as slippery in all barns is because the soft rubber pads used in the stall turn into a deformably slippery structure in this study. It is considered that manure accumulation on the passageways is a factor in evaluating the bedding in the Holstein barns as dirty and the passageway as slippery. Graves et al., (2010), reported that the regular cleaning of the passageway in cattle barns affects the cleanliness of the stall by reducing the amount of manure that the animals will carry to the stall with their feet. Aydın, (2017) reported that the regular operation of the scrapers determined the cleanliness of the beds and the promenade in his research on the free stall dairy cattle. DeVries et al., (2012) reported that the areas in front of the floors and feeders in the barn where animals walk, stand and lie are the most fertilizer-intensive areas and that cleaning the manure in the environment improves barn and animal hygiene. Body hygiene in dairy cattle is based on the evaluation of manure-derived dirt (hygiene score) on different parts of the body.

Table 2. Some hygiene scores belong to the barns of Holstein and Simmental cows of different lactation periods.

Traits	Scores			
	Group 1	Group 2	Group 3	Group 4
Stall floor				
Softness	1.5	1.5	1.5	1.5
Cleanliness	0.5	0.5	1	1
Slipperiness	0	0	0	0
Passageway				
Slipperiness	0	0	0.5	0.5
Manure accumulation	2	1	0	1

Group 1: barn of early lactation Holstein cows; Group 2: barn of middle lactation Holstein cows; Group 3: barn of early lactation Simmental cows; Group 4: barn of middle lactation Simmental cows

In this study, the least-squares averages and standard errors of the body hygiene scores of Holstein and Simmental cows are given in Table 3.

Table 3. Comparison of the body hygiene scores of Holstein and Simmental cows in different lactation periods.

Traits	Tail head	Upper rear leg	Abdomen	Udder	Lower rear leg
	Means±SE	Means±SE	Means±SE	Means±SE	Means±SE
Breed					
Holstein	2.65±0.12	3.25±0.19^{a}	3.32±0.15^{a}	2.85±0.13	3.80±0.13^{a}
Simmental	2.52±0.13	2.62±0.13^{b}	2.87±0.10^{b}	2.65±0.13	3.12±0.10^{b}
Lactation period					
Early	2.62±0.13	3.07±0.16	3.20±0.15	2.67±0.13	3.45±0.11
Middle	2.55±0.12	2.80±0.17	3.00±0.12	2.82±0.13	3.47±0.13
Breed X Lactation period					
Group 1	2.75±0.18	3.60±0.23^{a}	3.55±0.19^{a}	2.85±0.19	3.85±0.16^{a}
Group 2	2.55±0.18	2.90±0.23^{b}	3.10±0.19^{ab}	2.85±0.19	3.75±0.16^{a}
Group 3	2.50±0.18	2.55±0.23^{b}	2.85±0.19^{b}	2.50±0.19	3.10±0.16^{b}
Group 4	2.55±0.18	2.70±0.23^{b}	2.90±0.19^{b}	2.80±0.19	3.15±0.16^{b}
Total	2.58±0.09	2.93±0.11	3.10±0.95	2.75±0.09	3.46±0.08

a,b: Different letters in the same column and for the same trait indicate a statistically significant difference (P<0.05); Group 1: Holstein cows in early lactation;
Group 2: Holstein cows in middle lactation; Group 3: Simental cows in early lactation; Group 4: Simmental cows in middle lactation

It was determined that the dirtiness degree of the upper part of the hind leg, the lower part of the hind leg and the ventral part of the abdomen of the Holstein cows was higher than that of the Simmental cows in this study (P<0.05). In addition, similar to the result of the breed factor, the effect of breed X lactation period interaction on the level of dirtiness in the same areas of the body was found to be significant (P<0.05), while the effect of the lactation period alone was found to be insignificant (P>0.05). It is believed that the excess manure accumulation on the passageway of Holstein barns is effective in determining the higher level of dirtness of the lower part of the hind leg of the body in Holstein cows. Cook, (2002) reported that the lower parts of the legs of dairy cattle were contaminated with manure while they were walking on the passageway and in exercise areas, and the manure on the ground and bedding during their rest caused the upper part and sides of the hind legs to become dirty. Bergsten and Pettersson, (1992) and Hughes, (2001) reported that the lower part of the leg was contaminated due to problems in walking range and the accumulation of manure, while the dirtness of the side of the leg (upper part of the hind leg) was a factor in stall hygiene and dirtness of the underlayment material. Erdem and Okuyucu, (2019) determined udder and leg hygiene scores to be higher in Holstein cows compared to Simmental and Holstein X Simmental crossbreeds (more dirty) and linked body dirtness to in-barn dirtness depending on climate. Aytekin et al., (2021) have reported that many factors such as barn type, stall structure, bedding material, mechanization structures, season and animal behaviour are effective in the body cleaning of animals in dairy cattle enterprises. In the same study, it was found that the ventrals of the udder and abdomen of cows are cleaner than the Jul and upper parts of the hind legs.

The least-squares means and standard errors of Holstein and Simmental cows regarding milk yield, milk composition and SCC in the early and middle periods of lactation are given in Table 4. It was determined that the difference between daily and lactation milk yields and milk fat, protein and dry matter ratios of the two breeds was significant (P<0.05). It has been determined that the lactation period affects only the milk yield lactation and SCC. The effect of breed X lactation period interaction on all characteristics except lactose ratio and SCC was significantly determined (P<0.05). Gürses and Bayraktar, (2012) determined the 100-day milk yield of 2719 kg and the 200-day milk yield of 5246 kg in Holstein cows raised in different regions in Turkey. Şekerden, (2002) reported the fat ratio as 3.3%, the protein ratio as 3.5% and the total dry matter ratio as 11.1% in Holstein cows. In the studies where the milk yield characteristics of the Simmental breed were examined, the milk yield of 305 days was determined as Koç, (2016) reported as 4227 kg, Sönmez et al., (2007) reported it as 4562 kg. Götz et al., (2015) reported that Simmental cattle have a fat content of more than 3.9% in milk and protein content of more than 3.5%, Chessa et al., (2015) reported the ratio of fat and protein as 3.86% and 3.44%, respectively. The fact that the results obtained in this study regarding the milk yield and composition of both breeds differ from the previous research results is related to many environmental factors such as lactation number, nutrition, season and environmental temperature (Alpan & Aksoy 2015). In this study, the mean SCC was determined as 17000 c/ml in the Holstein breed and 15677.5 c/ml in the Simmental breed before the logarithmic transformation was applied. It was determined that only the lactation period affected the SCC, and the SCC in the middle period of lactation was higher than in the early period (P<0.05). Similar to the results of this research, Koç, (2011) reported that the lactation period affects the SCC.

The phenotypic correlation coefficients body hygiene scores and milk yield, milk composition and SCC are given in Table 5. In the study, it was determined that the cleanliness scores of the lower rear leg, upper leg and abdominal region of the animals had a high positive correlation between daily milk yield (P<0.05). However, a negative correlation was found between the cleanliness score of the lower leg and the daily milk yield (P<0.05). In addition, it was determined that the milk lactose ratio was negative (P<0.05) with the score of cleanliness in the tail region and positive correlation with the lower part of the hind leg (P<0.05). These results show that as the daily milk yield increases, there will also be an increase in

dirtness in the upper back leg (side) and abdominal area. In addition, it shows that an increase in dirtness in the tail region may cause a decrease in the lactose ratio of milk. The decrease in milk lactose ratio suggests the risk of subclinical mastitis. Thus in some previous studies, a low milk lactose ratio was considered an indicator of subclinical mastitis in determined (Akdağ et al., 2017; Antanaitis et al., 2021; Riggio, 2012). Aytekin et al., (2021) in their study on Holstein and Swiss Brown cows, reported that daily milk yield was effective on the cleanliness scores of the tail, leg, foot, udder and abdomen of the cows.

Table 4. Comparison of the milk composition and SCC of Holstein and Simmental Cows in different lactation periods.

Traits	Number of milked days	Lactation milk yield(kg)	Daily milk yield (kg)	Fat (%)	Protein (%)	Lactose (%)	Total dry matter (%)	SCC
	Means±SE	Means±SE	Means±SE	Means±SE	Means±SE	Means±SE	Means±SE	Means±SE
Breed								
Holstein	129.2±6.96	3466.6±216.58^{a}	26.7±0.55^{a}	10.31±0.42^{a}	2.76±0.06^{b}	4.70±0.03	18.62±0.39^{a}	3.71±0.10
Simmental	109.7±7.94	2156.8±199.54^{b}	16.9±0.88^{b}	8.43±0.51^{b}	3.02±0.05^{a}	4.78±0.03	17.08±0.48^{b}	3.68±0.11
Lactation period								
Early	76.5±3.21^{b}	1695.27±131.86^{b}	20.49±1.22	9.61±0.50	2.91±0.65	4.73±0.02	18.02±0.46	3.52±0.10^{b}
Middle	162.5±3.36^{a}	3928.25±165.56^{a}	23.26±0.85	9.15±0.48	2.86±0.58	4.75±0.03	17.68±0.45	3.88±0.10^{a}
Breed X Lactation period								
Group 1	89.00±4.11^{c}	2277.25±152.06^{c}	26.88±0.94^{a}	10.14±0.64^{a}	2.85±0.08^{ab}	4.70±0.04	18.26±0.60^{a}	3.58±0.15
Group 2	169.45±4.11^{a}	4656.10±152.06^{a}	26.69±0.94^{a}	10.49±0.68^{a}	2.67±0.08^{b}	4.71±0.04	19.01±0.64^{a}	3.85±0.15
Group 3	64.00±4.11^{d}	1113.30±152.06^{d}	14.10±0.94^{c}	9.01±0.68^{ab}	2.98±0.08^{a}	4.77±0.04	17.76±0.64^{ab}	3.47±0.15
Group 4	155.55±4.11^{b}	3200.40±152.06^{b}	19.82±0.94^{b}	7.89±0.66^{b}	3.06±0.08^{a}	4.79±0.04	16.48±0.60^{b}	3.90±0.15
Total	119.50±2.05	2811.76±76.03	21.87±0.47	9.38±0.33	2.89±0.04	4.74±0.22	17.88±0.31	3.70±0.07

^{a,b,c,d}: Different letters in the same column and for the same trait indicate a statistically significant difference (P<0.05);SCC=$\log_{10}$SCC; Group 1: Holstein cows in early lactation; Group 2: Holstein cows in middle lactation; Group 3: Simental cows in early lactation; Group 4: Simmental cows in middle lactation.

Hughes, (2001) noted that high-yielding cows consume more feed and drink water and accordingly their faeces are more liquid. In the same study, it was reported that the tail contaminated with faeces soiled the sides and udder of the animal's body. Erdem and Okuyucu (2019) in their study on Holstein and Simmental cows, found that cleanliness in different parts of the body was effective in milk total dry matter, protein and lactose ratio. They reported that there was a negative correlation between the lower and upper rear legs and udder hygiene scores and the milk lactose and protein ratio, and a positive correlation with SCC. Sant'anna and Paranhos da Costa, (2011) reported that cleanliness in the udder, lower leg and side areas of the body is associated with the SCC, and cleanliness increases the risk of mastitis in udder health. Schreiner and Ruegg, (2003) reported that the dirtness formed in the udder and hind limb area of animals is directly related to the SCC found in milk, but the dirness formed in the udder area affects the SCC more than the dirtness in the hind limb area.

Table 5. Phenotypic correlation coefficients between animal hygiene scores of milk yield. milk composition and SCC.

Traits	Tail head	Upper rear leg	Abdomen	Udder	Lower rear leg
Daily milk yield	0.113	0.309**	0.234*	0.162	-0.244*
Lacttation milk yield	0.052	0.059	0.107	0.185	-0.169
Fat	0.190	0.141	0.183	0.019	-0.054
Protein	0.162	0.011	-0.033	-0.126	-0.021
Lactose	-0.256*	-0.091	0.056	-0.020	0.238*
Dry matter	0.163	0.122	0.106	0.075	-0.044
SCC	0.095	0.050	0.016	-0.059	-0.007

SCC=$\log_{10}$SCC; *: P<0.05; **: P<0.01

CONCLUSION

When all the results of this research are evaluated, it is understood that the accumulation of manure on the barns causes the passageway, where the animals carry out most of their movements, to remain wet and therefore slippery. Wet and dirtyned passageways cause animals to carry manure to the stalls with their feet and cause the beds to become dirty and slippery. If the bedding at the stalls is dirty, it can cause contamination of the upper part of the hind leg and abdominal region of the animals' bodies due to the lying-resting positions of the cows. In addition, it can be said that there is more dirty in the tail in cows that give high milk compared to cows with a low daily milk yield, this dirty will cause a decrease in the milk lactose ratio.

CONFLİCT OF İNTEREST

The authors declare that there is no actual, potential or perceived conflict of interest for this article.

ETHİCS COMMİTTEE INFORMATİON

This study is not subject to the permission of HADYEK in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of the Animal Experiments Ethics Committees". In addition, the authors have declared that Research and Publication Ethics are observed.

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