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Research Article

The Effects of Transport Stress on Certain Welfare Parameters and Behaviours in Red Karaman, Imroz, Sakız and Karakul Rams

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ABSTRACT

A total of thirty two rams from Red Karaman, Imroz, Sakız and Karakul breeds were used to investigate the influence of transport stress on certain hematological and biochemical stress indicators, and on individual, feeding, eliminative and abnormal behaviours during the 2 h resting period. Three blood samples were taken from each animal at home pen, at immediately after transport and after the two hours resting period. Breed had no significant influence on PCV and N:L ratio. However, Imroz rams had higher WBC count than Sakız and Karakul rams, and Red Karaman rams had higher Hb level than Karakul rams. Transportation stress usually caused an increase in hematological parameters. Plasma cortisol concentration increased after transport stress in rams from all breeds. While plasma cortisol concentration returned to initial level after the 2 h resting period in Imroz and Karakul rams, it did not decrease to its initial level in Red Karaman and Sakız rams. The effect of breed, as a main effect, on plasma CK, LDH and glucose levels was not significant. However, Imroz rams had higher plasma total protein concentration than that of Sakız rams. Breed × sampling time had significant influence on CK level. While the effect of sampling time on CK was significant for Red Karaman and Sakız rams, sampling time had no influence on CK for Imroz and Karakul breeds. Significant increase in CK level was observed immediately after transport in Sakız rams, and after the 2 h lairage period in Red Karaman rams. Percentages of lying, standing, idling and investigation behaviours were affected by breed of ram. While Imroz rams exhibited the highest lying behaviour, Red Karaman rams had highest mean for percentage of investigation behaviour. Red Karaman rams also exhibited highest frequencies of vocalisation and 'butting other animals' behaviour. In conclusion, from animal welfare point of view, breed differences in stress responses should be considered in the planning of transportation and lairage procedures.

Key Words: Breed effect, transport stress, welfare, behaviour

ÖZET

MORKARAMAN, İMROZ, SAKIZ VE KARAGÜL KOÇLARDA TRANSPORT STRESİNİN BAZI REFAH PARAMETRELERİ VE DAVRANIŞLAR ÜZERİNE ETKİSİ

Transport stresinin hematolojik ve biyokimyasal stres göstergeleri ile taşıma sonrası iki saatlik dinlenme süresince bireysel, beslenme, boşaltım ve anormal davranışlar üzerine etkisinin incelendiği çalışmada, Morkaraman, İmroz, Sakız ve Karagül ırklarından toplam otuz iki koç kullanılmıştır. Her hayvandan bulunduğu bölmede, transporttan hemen sonra

ve iki saatlik dinlenme periyodu sonrası olmak üzere toplam üç kez kan alınmıştır. PVC ve N:L oranı üzerine ırkın etkisi önemsiz bulunmuştur. Bununla beraber, WBC sayısı bakımından İmroz koçların, Sakız ve Karagül koçlardan daha yüksek değerlere sahip olduğu; Morkaraman koçların ise Hb düzeyinin Karagül koçlardan daha yüksek olduğu gözlenmiştir. Transport stresi genellikle hematolojik parametrelerin artmasına neden olmuştur. Çalışmada, transport sonrası tüm koçlarda plazma kortizol konsantrasyonunda artma olmuştur. İki saatlik dinlenme periyodunun ardından plazma kortizol seviyesi İmroz ve Karagül koçlarda transport öncesi düzeyine geri dönerken, Morkaraman ve Sakız koçların plazma kortizol düzeylerinde düşme gerçekleşmemiştir. Plazma CK, LDH ve glikoz seviyesi üzerine ırkın etkisi önemsiz bulunmuştur. Bununla beraber İmroz koçların plazma total protein konsantrasyonunun Sakız koçlardan daha yüksek olduğu gözlenmiştir. Irk × örnek alma zamanının CK seviyesi üzerine etkisi önemli bulunmuştur. Örnek alma zamanının CK seviyesi üzerine etkisi Morkaraman ve Sakız koçlarda önemli, İmroz ve Karagül koçlarda ise önemsiz bulunmuştur. Sakız koçlarda transporttan hemen sonra, Morkaraman koçlarda ise iki saatlik dinlenme periyodunun ardından CK seviyesinde önemli bir artış olduğu gözlenmiştir. Yatma, ayakta durma ve araştırma davranışları oranları üzerine ırkın etkisi önemsiz bulunmuştur. Yatma davranışını en sık İmroz koçlar sergilerken, araştırma davranışını en sık Morkaraman koçlar sergilemişlerdir. Ayrıca Morkaraman koçlarda vokalizasyon ve diğer hayvanlara toslama davranışının daha fazla olduğu gözlenmiştir. Sonuç olarak, hayvan refahı bakış açısıyla, transport ve kesim öncesi dinlendirme prosedürlerinin planlanmasında farklı ırkların stres yanıtlarının da farklı olduğu göz önünde bulundurulmalıdır.

Anahtar Kelimeler: Irk etkisi, transport stresi, refah, davranış

Introduction

In the last semi-centennial, parallel to the improvements in socioeconomic status, great increases in the animal products consumption in western countries have been observed. So as to meet the rising animal products need, animal production has turned into a big industry and the numbers of intensive farms have drastically increased. The animals' being exploitatively forced for higher production in too much crowded production facilities has aroused an important debate in the society. The consumers have begun to put more emphasis on issues like how the animals were reared in the farms, how they were transported and slaughtered and so the importance of the concept "animal welfare" has increased (Amon et al., 2001). In developed countries consumers are willing to pay more money to buy products from animals which were reared, transported and slaughtered in animal friendly conditions. In farm animals, the last few days before slaughter forms the most stressful period of their lives. Because of the management applications during transport and before slaughter, animals may display strong stress responses like behaviour abnormalities and aggression.

In sheep, the level of stress during transport is influenced by many factors like transport duration (De la Fuente et al., 2010; Hall et al., 1998b), transport intensity (Cockram et al., 1996) and genotype (Hall et al., 1998a). Among animals there are individual differences in terms of responses to stress. This variation may result from effects creating differences in genetic structure like the breed of the animal or selection for different production characteristics (Broom, 2005). In their studies investigating the response to transport stress of eight different sheep breeds, Hall et al. (1998a) reported that the plasma cortisol levels after transport of breeds reared in highlands were higher than those of breeds reared in lowlands. Kadim et al. (2006) found that plasma cortisol, adrenalin, dopamine and noradrenalin levels were significantly higher in Batina goat breed than Jabal Akdhar goat breed. Broom (2005) stated that the breed of the animal should also be taken into consideration when a transport is being planned.

Turkey is formed of various regions having different geographical and climatic conditions. Therefore in every region, sheep breeds which have adapted to the conditions of that region are being bred. Although there are lots of studies investigating comparatively the different production characteristics of sheep breeds in Turkey, the studies investigating the physiological and behavioural responses of these breeds to various stressors in terms of

animal welfare are limited. Moreover, there is not a study investigating comparatively the responses of indigenous breeds in Turkey to transport stress. This might be due to the very new improvement of animal welfare science in Turkey. In this study it was aimed to investigate comparatively the effects of transport stress on certain hematological and biochemical stress parameters, and on individual, feeding, eliminative and abnormal behaviours during the 2 h resting period following transport in indigenous Red Karaman, İmroz, Sakız and Karakul breed rams originating from different regions of Turkey.

Materials and methods

The research protocol of the current study was approved by the Ethic Committee of Istanbul University (Approval number: 193, Approval date: 30.12.2010).

Animals, handling and transport procedures

The animals used in the study were a total of 32 rams, which were eliminated from breeding at the age of 5-6 years, from Red Karaman, Imroz, Sakız and Karakul breeds. Prior to the study eight rams from each breed were placed in separate boxes in the Istanbul University Veterinary Faculty. The rams were fed *ad libitum* with concentrate having 89% dry matter, 17% crude protein, 12 MJ/kg DM energy and good quality alfalfa hay.

Red Karaman, Imroz, Sakız and Karakul rams were transported for 75 minutes and their physiological and behavioural responses to transport stress were determined. The dimensions of sheltered lorry used for transportation of animals were 2 m × 3.75 m. Different breed of rams were transported separately. In the transportation of different breeds, the same lorry, driver and route were used. Transport conditions and handling were the same for all groups. Transport time was 75 min, and stocking density during transport was about 0.94 m^2/ram. After transportation the rams were placed in a 13 m^2 box in the Istanbul University Veterinary Faculty.

Behavioural observations

In order to determine the individual, feeding and abnormal behaviours of rams during the resting period following transport, behaviours of the rams were observed for two hours. The observations were made by the same experienced observer sitting 1 m away from the box. In terms of individual and feeding behaviours, time-sampling observation method (Bogner, 1984) was used and at the end of each 5 minutes, firstly the posture (lying, standing) and then the behavioural activity (walking, idling, investigation, rumination, drinking) displayed by each animal were recorded on charts. Abnormal behaviours (licking or gnawing walls, feeder etc.; butting walls, feeder etc.; butting other animals), eliminative behaviours (defecation and urination), vocalisation, selfgrooming and allogrooming behaviours were recorded at the time they were observed as these behaviours are displayed by animals more seldom. The descriptions of behavioural traits investigated in the study were presented in Table 1 according to the reports of Dwyer et al. (2004), Ergul Ekiz and Özcan (2006) and Healy et al. (2002).

Blood sampling and analyses

So as to make blood sampling from the rams easier, the necks of the rams were shaved two days before the transportation date. Three blood samples from each ram were collected from the jugular vein by the same trained person throughout the study. The first blood sample was taken in the home pen when the rams were in resting condition. The second blood sample was collected when the rams were unloaded into resting pen. The third blood sample was taken at the end of 2 h resting period. Blood sampling process was completed approximately within 1 min for each collection in order to avoid excessive stress.

Two blood samples (EDTA samples for hematological analysis; heparinised samples for plasma cortisol and biochemical analysis) were collected at each sampling. Samples taken into heparinised tubes were centrifuged at 3500 rpm for 15 min within 1 h of collection, and plasma was stored at -85°C for further analysis.

Table 1. Description of behavioural traits investigated in the study.
Tablo 1. Araştırmada incelenen davranış özelliklerinin tanımları.

Behaviour	Description
A. Individual Behaviours	
Lying	Lying without showing any other behaviour
Standing	Standing without showing any other behaviour
Walking	Moving at a walk
Idling	Inactive state without showing any other behaviour
Investigation	Object licking or smelling
B. Feeding Behaviours	
Rumination	Chewing the rumen content, which comes to the mouth
Drinking	The ram meets its water need
C. Eliminative Behaviours	
Defecation	Voiding faeces
Urination	Voiding urine
D. Abnormal Behaviours	
Licking or gnawing walls, feeder etc.	The ram licks or gnaws the walls, feeder etc.
Butting walls, feeder etc.	The ram lowers his head and butts the walls, feeder etc.
Butting other animals	The ram lowers his head and butts another ram
E. Other Behavioural Activities	
Selfgrooming	The ram grooms or licks itself
Allogrooming	The ram grooms or licks another ram
Vocalisation	A low pitched 'rumble' or 'mmm' bleat made with the mouth closed or a louder 'baa' vocalisation made with the mouth open

Hematological parameters were determined using classical methods: The blood packed cell volume (PCV) was determined using the standard capillary microhematocrit method and was reported as percentage values. The hemoglobin concentration (Hb) was determined by the oxy-hemoglobin method (Richterich, 1969). The total number of white blood cells (WBC) was counted in a 1:10 dilution of blood sample in Turk's solution, by using the Thoma-Zeiss hemocytometer. Each sample was counted twice and the mean was calculated. Blood smears were prepared for the determination of N:L ratio. After drying, the smears were stained with May-Grünwald Giemsa stain, and one hundred cells (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) were counted on each slide using a light microscope at x100 magnification. N:L ratios were determined by dividing the number of neutrophils by the number of lymphocytes.

Cortisol concentrations in plasma were measured by using a diagnostic ELISA direct immunoenzymatic kit (DiaMetra, Foligno, Italy; Ref: DK0001; Lot No: 2186) and values were expressed as ng/ml. The sensitivity of the assay was 5 ng/ml. The intra- and inter-assay variations were 7 and 9.32% respectively. Plasma concentrations of total protein (TP; Ref: 1001291; Lot No: D195), glucose (GLU; Ref: 1001192; Lot No: 172), creatine kinase (CK; Ref: 1001050; Lot No: 2188:T) and lactate dehydrogenase (LDH; Ref: 1001260; Lot No: 2216T) were determined by a computer process controlled multiparametric autoanalyser (TMS 1024, Tokyo-Boeki Medical System, Tokyo, Japan) using their accompanying commercial kits (Spinreact, Girona, Spain).

Data editing and statistical analyses

Repeated measurement of ANOVA in SPSS 10.0 statistical package (SPSS, 1999) was used to analyse data for hematological and biochemical parameters. The model included sampling time as a within-subject effect and breed as a between-subject effect, and also sampling time × breed interaction. Significance

control was assessed by using the least significant difference procedure. In order to determine the effect of breed on hematological and biochemical parameters in the specific sampling time, one-way ANOVA and Duncan's multiple range tests were also performed.

The individual and feeding behaviour data, which were recorded by time-sampling method, were edited to percentages giving the rate of these behaviours within all observations. One-way ANOVA and Duncan's multiple range tests were used for the statistical analyses of individual and feeding behaviours. The abnormal behaviours, eliminative behaviours and other behavioural activities, which were recorded at the time they were observed, were presented as means of frequency in the study. As these characteristics did not fit in normal distribution, a logarithmic transformation was applied before statistical analyses. In the analyses of logarithmic data of these characteristics one-way ANOVA and Duncan's multiple range tests methods were used.

Results

The effects of breed, sampling time and their interaction on certain hematological parameters are presented in Table 2. Breed, as a main effect, did not influence the PCV and N:L ratio according to repeated measurements ANOVA statistics. However, differences among breeds in terms of Hb level and WBC count were significant. Imroz rams had higher WBC count at each sampling time than those values obtained from Sakız and Karakul rams. Karakul rams had also lower mean Hb level than Red Karaman rams. Sampling time, as a main effect, had a significant effect on PCV, Hb level, WBC count and N:L ratio ($P<0.001$). In general, not in all cases, there was a tendency with increases in hematological parameters after transport. Breed × sampling time interaction was significant only for PCV. This result indicated that while sampling time had significant effect on PCV for Imroz and Karakul rams, it was not significant for Red Karaman and Sakız rams.

The effect of breed, as a main effect, on plasma cortisol, creatine kinase (CK), lactate dehydrogenase (LDH) and glucose levels were not significant ($P>0.05$). However, Imroz rams had higher plasma total protein concentration than that of Sakız rams (Table 3). As a main effect, sampling time significantly influenced plasma cortisol, CK, glucose and total protein levels, but not LDH concentration. Transportation and related handling procedures caused an increase in plasma concentration of cortisol in all breeds investigated in the current study. However, there were differences among breeds in terms of trend of cortisol decline during the resting period. In Imroz and Karakul rams, after 2 h resting, plasma cortisol levels decreased to the level that was measured at home pen. However, plasma cortisol levels after lairage in Red Karaman and Sakız rams were higher than those of their initial levels, although there were significant decreases in cortisol concentration during resting. Plasma glucose concentration was higher at blood samples taken immediately after transport than that of initial sampling in all breeds investigated in the study. While breed × sampling time interaction was significant in terms of CK, it was not significant for LDH, glucose and total protein concentrations. In the Imroz and Karakul breeds, CK level did not change significantly due to sampling time, but significant differences were found in Red Karaman and Sakız breeds.

The effects of breed on percentages of individual and feeding behaviours observed during 2 h lairage following transportation are presented in Table 4. The percentage of time spent for lying was significantly higher in Imroz rams compared with that of other breeds ($P<0.001$). Imroz rams also exhibited higher idling behaviour than that of Red Karaman rams. However, percentage of investigation behaviour was lower in Imroz rams compared with Red Karaman rams. Investigation behaviour was also exhibited lower in Karakul rams than Red Karaman rams. The differences among breeds in terms of percentages of walking, drinking and rumination behaviours were not significant ($P>0.05$).

Table 2. Means for certain hematological stress parameters in rams from different indigenous breeds.
Tablo 2. Çeşitli yerli ırk koçlarda bazı hematolojik stres parametrelerine ait ortalama değerler.

Characteristics	Sampling time (ST)	Breed (B)				SEM	ANOVA Significance[e]	Significance of main effects[f]		
		Red Karaman	Imroz	Sakız	Karakul			B	ST	B x ST
PCV, %	At home pen	28.13	25.38 ^{y}	28.63	24.38 ^{y}	0.74	NS			
	After transport	28.63	28.50 ^{z}	28.63	25.38 ^{z}	0.70	NS	NS	***	*
	After lairage	26.75	25.63 ^{y}	28.13	23.88 ^{y}	0.69	NS			
	Significance [d]	NS	***	NS	*					
Hb, g/dl	At home pen	10.91 ^{a}	10.03 $^{ab, y}$	9.77 ^{ab}	8.64 $^{b, y}$	0.29	*			
	After transport	11.68 ^{a}	11.19 $^{a, z}$	9.96 ^{ab}	8.99 $^{b, z}$	0.36	*	*	***	NS
	After lairage	10.68 ^{a}	9.59 $^{ab, y}$	9.59 ^{ab}	8.65 $^{b, y}$	0.26	*			
	Significance [d]	NS	*	NS	*					
WBC, x10^3/µl	At home pen	10.03 $^{a, y}$	10.59 $^{a, y}$	7.73 $^{b, y}$	7.52 $^{b, y}$	0.39	**			
	After transport	10.57 $^{ab, yz}$	12.54 $^{a, z}$	8.99 $^{b, z}$	9.65 $^{b, z}$	0.46	*	*	***	NS
	After lairage	11.53 $^{ab, z}$	12.99 $^{a, z}$	10.01 $^{b, z}$	9.39 $^{b, z}$	0.49	*			
	Significance [d]	*	**	***	**					
N:L	At home pen	0.92 ^{y}	0.71 ^{y}	0.85 ^{y}	0.69 ^{y}	0.07	NS			
	After transport	1.92 ^{z}	1.11 ^{z}	1.77 ^{z}	1.34 ^{z}	0.14	NS	NS	***	NS
	After lairage	1.41 ^{z}	1.34 ^{z}	1.88 ^{z}	1.44 ^{z}	0.12	NS			
	Significance [d]	**	**	*	**					

$^{y, z}$ Differences between the means of sampling times carrying various letters in the same column are significant.
$^{a, b}$ Differences between the means of breeds carrying various letters in the same line are significant.
^{NS} Not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001.
^{d}: Significance level of differences between sampling times for the same breed according to repeated measurements of ANOVA statistics.
^{e}: Significance level of differences between breeds for the same sampling time according to one-way ANOVA statistics
^{f}: Significance of main effects according to repeated measurements ANOVA statistics

Table 3. Means for plasma cortisol, CK, LDH, glucose and total protein concentrations in rams from different indigenous breeds.
Tablo 3. Çeşitli yerli ırk koçlarda plazma kortizol, CK, LDH, glukoz ve total protein konsantrasyonlarına ait ortalama değerler.

Characteristics	Sampling time (ST)	Breed (B)				SEM	ANOVA Significance[e]	Significance of main effects[f]		
		Red Karaman	Imroz	Sakız	Karakul			B	ST	B x ST
Cortisol, ng/ml	At home pen	90.70 ^{x}	46.95 ^{y}	106.31 ^{x}	92.94 ^{y}	13.83	NS	NS	***	NS
	After transport	234.03 ^{z}	99.91 ^{z}	299.54 ^{z}	240.39 ^{z}	30.58	NS			
	After lairage	113.00 ^{y}	58.67 ^{y}	172.90 ^{y}	119.03 ^{y}	17.57	NS			
	Significance [d]	***	*	***	*					
CK, U/l	At home pen	123.43 ^{y}	185.75	115.17 ^{y}	158.88	12.52	NS	NS	***	*
	After transport	150.14 ^{y}	196.75	265.33 ^{z}	173.50	18.55	NS			
	After lairage	191.29 ^{z}	182.63	267.00 ^{z}	209.75	25.17	NS			
	Significance [d]	**	NS	*	NS					
LDH, U/l	At home pen	1253.63	1149.50 ^{y}	1096.00	977.63	38.95	NS	NS	NS	NS
	After transport	1280.75 ^{a}	1249.50 $^{a, z}$	1111.50 ^{ab}	974.25 ^{b}	40.80	*			
	After lairage	1243.63	1155.00 ^{y}	1113.50	1044.63	35.29	NS			
	Significance [d]	NS	**	NS	NS					
Glucose, mg/dl	At home pen	65.50 $^{ab, x}$	58.75 $^{b, y}$	71.14 $^{a, y}$	61.63 $^{b, y}$	1.36	**	NS	***	NS
	After transport	113.75 ^{z}	123.75 ^{z}	98.57 ^{z}	113.13 ^{z}	7.19	NS			
	After lairage	100.25 ^{y}	113.63 ^{z}	69.71 ^{y}	98.38 ^{yz}	6.49	NS			
	Significance [d]	**	***	***	**					
Total protein mg/l	At home pen	8.13 ^{a}	8.18 $^{a, y}$	7.24 ^{b}	7.49 ^{ab}	0.14	*	*	**	NS
	After transport	8.01 ^{ab}	8.64 $^{a, z}$	7.19 ^{b}	7.66 ^{b}	0.17	*			
	After lairage	7.76 ^{ab}	8.19 $^{a, y}$	7.06 ^{b}	7.44 ^{ab}	0.14	*			
	Significance [d]	NS	**	NS	NS					

$^{x, y, z}$ Differences between the means of sampling times carrying various letters in the same column are significant.
$^{a, b}$ Differences between the means of breeds carrying various letters in the same line are significant.
^{NS} Not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001.
^{d}: Significance level of differences between sampling times for the same breed according to repeated measurements of ANOVA statistics.
^{e}: Significance level of differences between breeds for the same sampling time according to one-way ANOVA statistics
^{f}: Significance of main effects according to repeated measurements ANOVA statistics

Table 4. Means for percentages of individual and feeding behaviours during the 2 h lairage period following transportation in rams from different indigenous breeds.
Tablo 4. Çeşitli yerli ırk koçlarda transport sonrası 2 saat dinlenme periyodunda bireysel ve beslenme davranışlarının oranlarına ait ortalamalar.

Behaviours	Breed				SEM	Sig[e]
	Red Karaman	Imroz	Sakız	Karakul		
Lying, %	0.00 [b]	25.50 [a]	0.00 [b]	0.00 [b]	2.82	***
Standing, %	100.00 [a]	74.50 [b]	100.00 [a]	100.00 [a]	2.82	***
Walking, %	12.50	2.50	8.50	12.00	1.49	NS
Idling, %	54.50 [b]	85.00 [a]	69.50 [ab]	79.50 [a]	3.54	**
Investigation, %	17.00 [a]	0.50 [b]	10.50 [ab]	1.00 [b]	2.47	*
Drinking, %	1.50	0.50	0.50	3.00	0.43	NS
Rumination, %	14.50	11.50	11.00	4.50	1.99	NS

[a, b] Differences between the means of breeds carrying various letters in the same line are significant.
[NS] Not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001.
[e]: Significance level of differences between breeds according to one-way ANOVA statistics.

The mean frequencies of eliminative, abnormal and other behavioural activities during the 2 h lairage period following transportation in Red Karaman, Imroz, Sakız and Karakul rams are given in Table 5. Red Karaman rams exhibited higher vocalisation behaviour than those of other breeds (P<0.01). The frequency of 'butting other animals' behaviour was also highest in Red Karaman rams. On the other hand, 'licking or gnawing walls, feeder etc.' behaviour was significantly higher in Sakız rams than those of Red Karaman and Imroz rams. However, breed influence was not significant in terms of mean frequencies of selfgrooming, allogrooming, butting walls, feeder etc. and eliminative behaviours.

Table 5. Means for frequency of eliminative, abnormal and other behavioural activities during the 2 h lairage period following transportation in rams from different indigenous breeds.
Tablo 5. Çeşitli yerli ırk koçlarda transport sonrası 2 saat dinlenme periyodunda boşaltım, anormal ve diğer davranış aktivitelerinin frekanslarına ait ortalamalar.

Behaviours	Breed				SEM	Sig[e]
	Red Karaman	Imroz	Sakız	Karakul		
Defecation	0.13	0.25	0.25	0.25	0.09	NS
Urination	0.50	0.13	1.00	0.50	0.14	NS
Licking or gnawing walls, feeder etc.	0.25 [b]	0.00 [b]	3.38 [a]	1.63 [ab]	0.47	*
Butting walls, feeder etc.	2.38	0.00	0.25	0.00	0.45	NS
Butting other animals	6.25 [a]	1.13 [b]	0.25 [b]	1.13 [b]	0.80	**
Selfgrooming	0.00	2.25	1.00	0.25	0.34	NS
Allogrooming	0.00	0.25	0.00	0.00	0.06	NS
Vocalisation	8.25 [a]	0.13 [b]	1.50 [b]	0.13 [b]	1.08	**

[a, b] Differences between the means of breeds carrying various letters in the same line are significant.
[NS] Not significant (P>0.05); * P<0.05; ** P<0.01.
[e]: Significance level of differences between breeds according to one-way ANOVA statistics.

Discussion

Transport and slaughter of farm animals are the final stages of an animal production system aimed to produce meat (Grigor et al., 2004). However, transportation of farm animals includes different procedures (such as collection of animals, driving of the flock to lorry and associated handling on the farm, loading,

journey, unloading, fatigue, physical discomfort, noise, vibration and social disruption), which may cause physical and emotional stress (De la Fuente et al., 2010; Terlouw et al., 2008). These stressors may lead some changes in blood constituents such as PCV, N:L ratio, plasma concentration of CK, LDH, glucose and cortisol. Therefore, these parameters were used to asses stress regarding to transportation in farm animals by numerous authors (Bórnez et al., 2009; Tadich et al., 2009).

In the current study, sampling time, as a main effect, had significant influence on PCV, Hb level, WBC count and N:L ratio (Table 2). However, there were differences among breeds in terms of effect of sampling time on PCV and Hb level. While mean values of PCV and Hb level increased in Imroz and Karakul rams after transportation, such an increase was not observed in Red Karaman and Sakız rams. On the other hand, elevated PCV and Hb level due to transportation in Imroz and Karakul rams had returned to initial level after 2 h resting. However, increased level of WBC and N:L ratio due to transportation in rams from all breeds investigated in the current study did not decrease to the level measured at home pen after the 2 h resting period. Kent (1997) noted that changes in PCV might be due to responses to dehydration, blood loss and mobilisation of red blood cells from the spleen in response to catecholamines. Hall and Bradshaw (1998) also noted that PCV rises as an initial response to stress, most probably as a consequence of spleen contractions, which forces erythrocytes into the circulation. Supporting the current result, Kannan et al. (2000) found an increase in N:L ratio due to transportation in goats. On the other hand, Tadich et al. (2009) did not find any change in PCV after transport and lairage. Contrary to the current result, Bórnez et al. (2009) observed significant increases in hemoglobin and PCV levels during lairage period in light lambs, however lairage time was 15 h in that study. Liotta et al. (2007) also reported higher PCV value after lairage compared with those of unloading level in 31 h and 58 h lairaged.

Karakul rams had lower Hb level than that of Red Karaman rams at three sampling times. On the other hand, mean WBC count at home pen, after transport and after resting were higher in Imroz rams than those of Sakız and Karakul rams. Although there were significant differences among breeds in terms of Hb level and WBC count at blood sampling taken immediately after transportation, significant breed effect for these parameters could not be attributed to differences between breeds in terms of response to transportation stress, since such differences were already observed at blood samples taken at home pen.

There was no significant influence of breed and breed × sampling time interaction, as a main effect, on plasma concentration of cortisol ($P>0.05$), however sampling time significantly ($P<0.001$) affected cortisol level (Table 3). Plasma cortisol level is a reliable indicator of stress experienced by an animal (De la Fuente et al., 2010). Plasma cortisol concentrations after transportation in rams from all breeds were higher than those values measured at home pen. These results indicate that transportation and related handling procedures caused an increase in plasma concentration of cortisol in all breeds investigated in the current study. Supporting the results of the current study, numerous authors (Ali et al., 2006; Hall et al., 1999; Kadim et al., 2009; Tadich et al., 2009) also reported significant increase in plasma cortisol level due to transport stress for various sheep breeds. Broom et al. (1996) found that loading, penning and start of transport caused an increase in plasma cortisol concentration. They also observed a greater plasma cortisol level during the first 180 min of journey in transported sheep than that of non-transported counterparts. Ali et al. (2006) explained the elevated cortisol level due to transportation stress by the stimulation of the hypothalamo-pituatry-adrenal axis.

A significant decrease in plasma cortisol concentration after the resting period of 2 h was observed in rams from all investigated breeds in the current study. However, there were slight differences among breeds in terms of trend of cortisol decline during the resting period. While

plasma cortisol concentration returned to its initial level after 2 h resting period in Imroz and Karakul rams, it did not decrease to its initial level in Red Karaman and Sakız rams. These results indicate inadequacy of 2 h lairage time for recovery of Red Karaman and Sakız rams from transportation stress. Kannan et al. (2000) found that cortisol values in goats decreased to the baseline level after 1 h lairage. Grigor et al. (2004) also observed that the plasma cortisol concentration had returned to normal level in veal calves after 1.5 h of lairage. On the other hand, Ekiz et al. (2011) reported that 30 min lairage time was not adequate in order to supply recovery from transportation stress in Kıvırcık lambs. In contrast to the current results, Hall et al. (1998a) found significant genotype influence on plasma cortisol concentration after transport in sheep and they concluded that sheep originated from highlands had higher cortisol responses to transportation stress. Kadim et al. (2006) also observed lower cortisol level in Jabal Akdhar goats than Dhofari and Batina goats.

Creatine kinase (CK) is released into the blood as a response to muscle damage, change in cell permeability resulting from injury or when there is a vigorous exercise. Lactate dehydrogenase (LDH) also increases in the blood after muscle and tissue damage (Bórnez et al., 2009; Kannan et al., 2003; Kent, 1997). Hence, CK and LDH have been used as indicators of trauma, high levels of physical activity or other damages occurred during handling and transport in farm animals (Bórnez et al., 2009; De la Fuente et al., 2010; Tadich et al., 2009). As a main factor, breed had no influence on CK and LDH in the current study (Table 3). However, there was a significant breed × sampling time interaction in terms of CK. While CK level did not change due to sampling time in Imroz and Karakul breeds, significant changes was observed in Red Karaman and Sakız breeds. CK concentration increased after 75 min transport in Sakız rams. This result might be due to possible trauma during loading, journey or unloading as reported by Ekiz et al. (2011) for Kıvırcık lambs and by Grigor et al. (2004) for veal calves. On the other hand, transportation and related handling procedures did not cause an increase in CK activity in Red Karaman rams, while plasma activity of CK in this breed increased significantly during lairage. This result might be due to injuries and bruises resulted from higher behavioural interaction between Red Karaman rams, especially butting each other, during lairage as shown in Table 5. On the other hand, no significant changes in CK and LDH during the transport and lairage in Imroz and Karakul rams indicate that these rams did not experience trauma or vigorous exercise causing muscular-tissue damage during transportation and lairage. Supporting results observed in Imroz and Karakul rams, Bórnez et al. (2009) and De la Fuente et al. (2010) did not find significant increase in CK and LDH activities due to transportation. Hall et al. (1999) also found no consistent CK changes during transport in sheep.

Plasma glucose concentration increased after transport compared with initial values at home pen in all breeds investigated in the study (Table 3). Bórnez et al. (2009) also reported an increase in plasma glucose level due to transportation in light and suckling lambs. They noted that an elevated plasma glucose level after transportation could be useful as an indicator of the intensity of stress experienced during loading, journey and unloading. Similar to the current result, Ali et al. (2006) found elevated plasma glucose concentration after transport in sheep and explained this result by the secondary effect of hypercortisolaemia and increased glucose production from the liver, reflecting increased sympatho-adrenal activity due to stress. An increase in plasma glucose level as a response to transportation stress was also reported for lambs (Ekiz et al., 2011) and goats (Kannan et al., 2000 and 2003).

Sakız rams had lower means for total protein level at all sampling times than those of Imroz rams (Table 3). Significant breed influence on total protein level was also reported by Kadim et al. (2006). There was a significant increase in total protein after transport in Imroz rams, while such an increase was not observed in other breeds. Bórnez et al. (2009) found non-significant differences in terms of total protein

level among blood samples taken on farm, after transport and after lairage in light and suckling lambs. Ekiz et al. (2011) also found similar total protein level among blood samples taken at home pen, immediately after transport and after lairage in Kıvırcık lambs.

Behaviours including postural changes have been used to asses an animal's response to stress by numerous authors (Cockram et al, 2000; Rutherford, 2002). The posture (lying or standing) of ram during the 2 h lairage period were significantly different ($P<0.001$) depending on the breed of ram (Table 4). Lying posture was not observed in Red Karaman, Sakız and Karakul rams during the 2 h lairage following transportation, while the percentage of time spent for lying was 25% in Imroz rams. Therefore, the percentage of time spent standing was significantly lower in Imroz rams compared with those of other breeds ($P<0.001$). An elevated lying behaviour might indicate either the adaptation of animal to the environmental conditions (rest) or a decrease in the ability of animal to stand (exhaustion) (Cockram, 2007). In the current study, higher lying behaviour observed in Imroz rams could not be due to exhaustion, since their CK and LDH levels were not higher than those of other breeds (Table 3). Therefore, a higher lying behaviour in Imroz rams might be due to adaption of Imroz rams to novel environments of lairage more easily than rams from other breeds. The percentage of time spent for lying during resting found for Turkish sheep breeds in the current study were lower than those reported by Cockram et al. (1997 and 2000). The difference between current result and above reports might be explained by the differences in journey times and stocking densities during transport between studies. Journey time in the current study was shorter than other studies, and therefore physical resting requirements of rams in the current study were lower. Furthermore, Cockram et al. (1997) noted that if the sheep were allowed sufficient space to lie down on the vehicle, the requirement of physical rest becomes less important. In the current study, stocking density during transport was about 0.94 m^2/ram, which was considerably higher than the legal requirements of European Union (EEC, 1995). On the other hand, report of Jarvis and Cockram (1995) for the time spent for lying during 4 h lairage after transport was similar with that of the result found for Imroz rams in the current study. Among the behavioural traits investigated in the current study, time spent for standing had the highest percentage for Red Karaman, Sakız and Karakul rams. Supporting the current result Cockram et al. (1997) found that the most observed behaviour in the first 2 h of lairage was standing in 16-week-old Suffolk × Greyface lambs.

The percentage of time spent for idling was significantly lower in Red Karaman rams compared with those of Imroz and Karakul rams ($P<0.01$). This result might be attributed to the difference between breeds in terms of investigation behaviour which was higher in Red Karaman rams than those of Imroz and Karakul rams ($P<0.05$). The percentages of time spent for walking, drinking and rumination were similar among rams of Turkish sheep breeds investigated in the current study. Several stressors (such as handling, transport, novel environment etc.) and some events, which give rise to pain, hunger, anxiety or illness, might cause a decrease or cessation of rumination (Fraser and Broom, 1990). Hence, an increase in the time spent for rumination is an indicator of good welfare (Arney, 2009). The percentages of time spent for rumination found in the current study ranged between 4.5 - 14.5 %, and these levels were similar with previous reports for non transported Kıvırcık lambs (Karaağaç et al., 2005) and Awassi lambs (Keskin et al., 2010), but lower than the reports for Kıvırcık ewes by Ergul Ekiz and Ozcan (2006) and Ergul Ekiz et al. (2009).

No significant differences among breeds in terms of eliminative behaviours, butting walls, feeder etc., self- and allo-grooming behaviours were observed in the current study. However, Red Karaman rams exhibited a higher frequency of "butting other animals" and "vocalisation" behaviours than rams of other breeds. A greater frequency of "butting other animals" behaviour in Red Karaman rams may be due to the effort for establishment of social

rank in this breed (Houpt, 2005). Tölü and Savaş (2007) noted that the frequency of butting behaviour increases with an increase in dominance rank. On the other hand, frequency of "butting other animals" behaviour in Red Karaman rams observed in the current study was lower than the reports of previous studies for crossbred lambs (Karaağaç et al., 2005). An increase in vocalisation behaviour is a sign of distress and arousal in adult animals (Sevi et al., 2001) and frequency of vocalisation behaviour has often been used in the evaluation of distress or arousal (Dwyer et al., 2004; Sevi et al., 2001). Hence, higher frequency of vocalisation observed in Red Karaman rams may indicate that rams from this breed were in more distress or arousal than those of other breeds. Boissy et al. (2005) also found significant genotype effect on high- and low- pitched bleats in lambs. They observed higher bleats in Berrichon-du-Cher crossbreds than purebred Romanov, Lacauna and F_1 lambs. They noted that Romanov lambs showed the most passive responses (such as weak level of exploration), which were indicators of behavioural inhibition resulted from discomfort due to novelty environment. Hall et al. (1998a) also noted differences between breeds suited to extensive husbandry systems and breeds that evolved in more intensive conditions in terms of responses to welfare challenges.

Conclusion

Results for hematological and biochemical parameters clearly indicated that 75 min transportation caused stress in rams from Turkish breeds investigated in the current study. After the 2 h resting period, plasma cortisol concentrations in Imroz and Karakul rams decreased to initial level. But, plasma cortisol concentration after 2 h resting in Red Karaman and Sakız rams did not return to the level measured at home pen, which indicates that 2 h lairage time was not adequate for recovery from transportation stress in Red Karaman and Sakız rams. Vocalisation behaviour and behavioural interactions, particularly butting each other were also higher in Red Karaman rams than other breeds. A greater behavioural interaction during resting period in Red Karaman rams also caused an increase in CK level after lairage.

As a consequence, the significant breed differences in terms of some physiological, biochemical and behavioural responses to stressors, which may be due to transportation and related handling procedures, and novelty environment of lairage unit, were observed in the current study. Hence, when an animal welfare approach is taken into consideration, breed differences in stress responses should be considered in the planning of transportation and lairage procedures.

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