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Research Article

Effect of Ram-Ewe Mixed Transportation on Certain Welfare Parameters in Red Karaman and Imroz Sheep

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ABSTRACT

The present study was conducted to investigate certain stress parameters of Ram-Ewe mixed transport group compared with single sex groups in indigenous Red Karaman and Imroz sheep. Sheep from each breed were divided into three transport groups: a. Ram group (8 rams from each breed), b. Ewe group (8 ewes from each breed) and c. Ram-Ewe mixed group (4 rams and 4 ewes from each breed). Sheep from six different sub-groups were transported for 75 min and then lairaged for 2-h, separately. The influence of transport group on concentrations of plasma cortisol, CK, LDH, glucose, and neutrophile: lymphocyte ratio were not significant ($P>0.05$). The differences among Ram, Ewe and Ram-Ewe mixed groups for percentages of "standing", "lying" and "walking" behaviours, and frequencies of "butting other animals" and "urination" during 2-h lairage period were significant. Ram group exhibited higher "lying" behaviour and lower "standing" behaviour than those of Ewe group and Ram-Ewe mixed group ($P<0.01$). "Time spent for walking" percentage was lower in Ram group than that of Ram-Ewe mixed group ($P<0.05$). Moreover, "butting other animals" was observed more frequently in Ram group than Ewe group ($P<0.01$). Sheep from Red Karaman breed had higher plasma cortisol level measured after transport ($P<0.001$) and after lairage ($P<0.01$) compared with those of Imroz breed. The percentage of "standing" behaviour ($P<0.01$) and the frequency of "vocalisation" behaviour ($P<0.001$) observed in Red Karaman sheep were higher than those of Imroz sheep. As a consequence, transportation resulted with stress responses in Red Karaman and Imroz sheep, but Ram-Ewe mixed transportation did not cause additional stress responses compared with single sex groups. Therefore, it was concluded that the mixed transportation of rams and ewes during the non-breeding season did not cause any negative result in terms of animal welfare.

Key Words: Sheep, male-female mixed transportation, transport management, stress

ÖZET

MORKARAMAN VE İMROZ KOYUN IRKLARINDA KOÇ-KOYUN KARIŞIK TRANSPORTUN BAZI REFAH PARAMETRELERİ ÜZERİNE ETKİLERİ

Araştırma, yerli Morkaraman ve İmroz koyun ırklarında Koç-Koyun karışık transportun bazı stres parametreleri üzerine etkilerini tek cinsiyet grupları ile karşılaştırmah olarak incelemek amacı ile yürütülmüştür. Her bir ırktan koyunlar üç transport grubuna ayrılmıştır: a. Koç grubu (her bir ırktan 8 koç), b. Koyun grubu (her bir ırktan 8 koyun) ve c. Koç-Koyun karma grup (her bir ırktan 4 koç ve 4 koyun). Altı farklı alt grupta yer alan koyunlar ayrı ayrı olmak üzere 75 dakika süre ile transport edilmiş ve sonrasında 2 saat süre ile dinlendirilmiştir. Transport grubunun plazma kortizol, CK, LDH ve glikoz konsantrasyonları ile nötrofil:lenfosit oranı üzerine etkisi önemsiz bulunmuştur (P>0,05). Transport sonrası 2 saatlik dinlenme sürecinde "yatma", "ayakta durma" ve "yürüme" davranışlarının oranları ile "ürinasyon" ve "diğer hayvanlara toslama" davranışlarmn frekansları bakımmdan transport grupları arasmda önemli farklılıklar olduğu belirlenmiştir. Koç grubu, Koyun grubu ve Koç-Koyun karma grup ile kıyaslandığında daha fazla yatma davranışı ve daha az ayakta durma davranışı sergilemiştir (P<0,01). Koç grubunda "yürüme" davranışı ile geçirilen zaman oranı Koç-Koyun karma gruba kıyasla daha düşük bulunmuştur (P<0,05). Ayrıca, Koç grubunda "diğer hayvanlara toslama" davranışı Koyun grubuna göre daha sık gözlenmiştir (P<0,01). Plazma kortizol düzeyi Morkaraman ırkmda transport sonrası (P<0,001) ve dinlenme sonrası (P<0,01) yapılan ölçümlerde İmroz ırkma kıyasla daha yüksek bulunmuştur. Morkaraman ırkmda "ayakta durma" davranışı oranı (P<0,01) ve "vokalizasyon" davranışı frekansı (P<0,001) İmroz ırkına göre daha yüksek gözlenmiştir. Sonuç olarak, Morkaraman ve İmroz koyunlarda transport işleminin stres yanıtı oluşumu ile neticelendiği belirlenmiştir. Ancak, koç ve koyunların karma olarak transport edilmesinin tek cinsiyet grupları ile karşılaştırıldığmda ilave bir stres yanıtı oluşumuna sebep olmadığı görülmüştür. Dolayısı ile koç ve koyunların üreme mevsimi dışında bir arada transport edilmesinin hayvan refahı açısmdan herhangi bir olumsuzluğa sebep olmadığı sonucuna ulaşılmıştır.

Anahtar Kelimeler: Koyun, erkek-dişi karışık transport, transport yönetimi, stres

Introduction

Farm animals can be exposed to numerous stressors in their lifetime. Transportation is probably one of the most stressful stages of life in livestock. Almost all of the farmed animals are transported at least once in their life-time for several reasons (Broom, 2005). Slaughtering is the final stage of life for animals fattened to produce meat or for animals culled from breeding herd for different reasons, such as poor performance, health problems, senility etc. These animals should be transported to abattoir for slaughter (Grigor et al., 2004).

Transportation and related handling procedures involve various stressors, such as gathering, novel and unfamiliar environment, loading and unloading, noise and vibration, journey, changes in temperature and humidity, mixing with unfamiliar animals (Fazio and Ferlazzo, 2003; Hall and Bradshaw, 1998; Knowles, 1998). According to Fisher et al. (2009) the effect of transportation on animal welfare is dependent on how it is managed. The authors noted that while efficiently planned and managed transportation have no influence on welfare of animals, it can affect animal welfare quite adversely, if something wrong happens (Fisher et al., 2009).

In commercial slaughterhouses, mixing of unfamiliar animals is a common practice because of insufficient lairage pen size and ignorance of its influence on animal welfare. Studies conducted with pigs (Bradshaw et al., 1996; de Jong et al., 2000) and cattle (Kenny and Tarrant, 1987) showed that mixing of unfamiliar animals can have an additional effect to transportation stress. Hall and Bradshaw, (1998) and Fauciatano, (1998) noted that mixing of unfamiliar animals may cause fight, and results in increased levels of plasma creatine kinase (CK), and salivary and plasma cortisol. Mixing of unfamiliar sheep may also change the structure of social group, and increased frequency of aggressions may be observed during the establishment of the new social rank (Miranda-de la Lama et al., 2011).

The effects of mixing with unfamiliar animals on welfare have been investigated extensively in pigs and cattle. However, limited work regarding mixing has been done in sheep. To have knowledge about risk factors regarding animal welfare during transport and lairage, such as mixing of male and female animals, may provide the opportunity to plan and manage the transportation in order to improve animal welfare. The present study was conducted to determine the levels of certain stress parameters at home pen, following transportation and after two hours of resting period in Red Karaman and Imroz sheep transported as all-Ram, all-Ewe and Ram-Ewe mixed groups. Furthermore, feeding, individual, abnormal and eliminative behaviours of these animals were investigated during two hours of resting period after transport.

Materials and Methods

Animal material, handling and transportation

The current study was carried out on indigenous breeds with 24 sheep from Red Karaman breed (12 ewes and 12 rams) and 24 sheep from Imroz breed (12 ewes and 12 rams). The ewes used in the present study were not in oestrus during the experimental period. Animals from each breed-gender subgroups had been finished at adjacent pens in farm animal care unit of the Istanbul University Veterinary Faculty. Animals from each subgroup had ad libitum access to the high quality alfalfa hay and concentrate feed (17% crude protein, 12 MJ/kg DM energy, 89% dry matter).

In the study, sheep from each breed were transported in three groups: a. Ram group (8 rams from each breed), b. Ewe group (8 ewes from each breed) and c. Ram-Ewe mixed group (4 rams and 4 ewes from each breed). Sheep from six different sub-groups were transported separately. Animals were transported at February 17-18, 2011.

Each sub-group was transported with the same lorry by the same driver in the same route. For each subgroup, transportation duration and stocking density were 75 minutes and 0.94 m^2/sheep, respectively. Immediately after transportation, animals from each sub-group were rested for two hours in a lairage unit of 13 m^2.

Blood collection and analyses

Three blood samples from V. Jugularis were collected from each animal by the same trained person in order to determine the stress responses of animals to transportation. For an easy blood sampling, necks of the animals were sheared 2 days before the transport. Blood sampling in each collection was completed within one minute in order to prohibit increased stress due to sampling process. Sampling times were:

1. In resting condition at the finishing pen.
2. Immediately after unloading of animals into the lairage pen.
3. Immediately after two hours of resting in the lairage pen.

At each sampling time, two blood samples from each sheep were collected: Heparinised samples were used for measurement of concentration of plasma cortisol, CK, lactate dehydrogenase (LDH), glucose and total protein, while EDTA samples were used for determination of neutrophile:lymphocyte (N:L) ratio. Plasma was extracted from the heparinised blood samples after centrifugation (3500 rpm, 15 min) performed within one hour of blood sampling, and then kept at -85°C until analysed.

N:L ratio was determined in May-Grünwald Giemsa stained blood smears by counting one hundred leukocytes (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) using a light microscope at ×100 magnification. The N:L ratio was calculated by formulae given below:

N:L ratio = No. of neutrophils / No. of lymphocytes

Commercial diagnostic ELISA kit (DiaMetra, Foligno, Italy) was used for determination of plasma cortisol concentration. Assay sensitivity, and intra- and inter-assay variations were 5 ng/ml, and 7 and 9.32% respectively. A multiparametric autoanalyser (TMS 1024, Tokyo, Japan) and commercial kits

(Spinreact, Girona, Spain) were used for determination of plasma glucose, total protein, CK and LDH concentrations.

Behavioural observations

Behavioural activities investigated in the present study were determined by methods previously reported by Dwyer et al. (2004), Ekiz et al. (2012a), Ergul Ekiz and Ozcan (2006) and Healy et al. (2002). The descriptions of individual, feeding, eliminative, abnormal behaviours and other behavioural activities investigated in the study had been presented in reports of Ekiz et al. (2012a) and also were given in Table 1.

In the study, behavioural observations were performed during the two hours of resting at lairage unit. The same experienced researcher observed the behavioural activities from 1 m away the lairage unit. Feeding and individual behaviours of animals were observed by time-sampling (Bogner, 1984) method. In this method, the posture and the behavioural activity of each sheep were marked on sheets at the beginning of every five minutes.

As eliminative and abnormal behaviours, vocalisation, allogrooming and selfgrooming were exhibited by animals more seldom; these behavioural activities were marked on sheets at the time when they were displayed during the two hours of observation period.

Table 1. Description of behavioural activities observed in the present study (Ekiz et al., 2012a).

Tablo 1. Araştırma kapsamında gözlemlenen davranış aktivitelerinin tanımları (Ekiz ve ark., 2012a).

Behaviour	Description
I. Individual Behaviours	
Lying	Lying without showing any other behavioural activity
Standing	Standing without showing any other behavioural activity
Walking	Moving at a walk
Idling	Inactive state without showing any behavioural activity
Investigation	Object smelling or licking
II. Feeding Behaviours	
Rumination	Chewing the rumen content, which comes to the mouth
Drinking	Sheep provides its water need
III. Eliminative Behaviours	
Defecation	Voiding faeces
Urination	Voiding urine
IV. Abnormal Behaviours	
Licking or gnawing walls, feeder etc.	Sheep licks or gnaws the walls, feeder etc.
Butting walls, feeder etc.	Sheep lowers its head and butts the walls, feeder etc.
Butting other animals	Sheep lowers its head and butts another animal
V. Other Behavioural Activities	
Selfgrooming	Sheep grooms or licks itself
Allogrooming	Sheep grooms or licks another animal
Vocalisation	Sheep bleats with the mouth closed or open

Data editing procedures and statistical analyses

Before the statistical evaluation, data recorded by time-sampling method for feeding and individual behavioural activities were arranged to percentage values, which give the proportion of each behavioural activity within total frequency of feeding and individual behavioural activities.

The eliminative behaviours, abnormal behaviours, and other behavioural activities were edited as frequencies of these behaviours during whole observation period. Moreover, normality of data for these traits were tested by

preliminary analyses. As results of preliminary analyses did not indicate normal distribution for the data of these characteristics, frequency data were normalised by logarithmic transformation.

GLM procedures at SPSS 10.0 programme (SPSS, 1999) was used in order to determine the effects of breed (Red Karaman and Imroz), transport group (Ram, Ewe and Ram-Ewe mixed) and transport group × breed interaction on behavioural characteristics, plasma CK, LDH, cortisol, total protein, glucose concentrations and N:L ratio. LSD method was used for significance control.

Repeated measurement of ANOVA was also used for data of CK, LDH, cortisol, total protein, glucose, and N:L ratio for each sub-factors (ram, ewe, mixed, Red Karaman and Imroz). The model of repeated ANOVA statistics included sampling time (at home pen, after transport, and after two hours of resting) as a within-subject effect. LSD method was used in order to assess significance control.

Results and Discussion

The influence of transportation group and breed on concentrations of plasma CK, LDH, cortisol, total protein, glucose, and on N:L ratio were given in Tables 2 and 3. The differences between Ram, Ewe and Ram-Ewe mixed transport groups for biochemical parameters, except total protein, were not significant ($P>0.05$). On the other hand, Ram group had higher total protein concentration measured at home pen ($P<0.01$), after transport ($P<0.05$) and after resting period ($P<0.05$) than Ewe group and Ram-Ewe mixed group. Therefore, a higher total protein level after transport and after lairage in Ram group than in the other groups could not be attributed to the transportation, because rams of this group also had higher plasma total protein concentration measured at home pen. Supporting the current results for cortisol concentration, Okeudo and Moss (2005) found similar cortisol values for ewes and rams. In the study of Sevi et al. (2001), who investigated influence of regrouping on the cortisol responses of ewes, plasma cortisol levels of ewes from regrouping, regrouping and relocation, and control treatments were found to be similar. Broom (2003) noted that if unfamiliar male cattle are kept together during transport or in lairage, stress responses in animals might be observed. Numerous authors (Bradshaw et al., 1996; Warriss and Brown, 1985) also reported elevated stress responses with increasing concentration of blood cortisol, glucose and lactate in pigs due to mixing of unacquainted animals during transport or in lairage. In contrast to studies in pigs and cattle stated above, a lack of negative influence of Ram-Ewe mixed transportation on biochemical stress parameters in the current study could be attributed to the differences between species with respect to stress responses. Supporting this fact, Sañudo et al. (1998) reported that sheep are less sensitive against to the stressors compared with pigs or cattle.

Plasma CK, LDH, total protein and glucose concentrations in Red Karaman and Imroz sheep were similar at each sampling times ($P>0.05$). Red Karaman and Imroz sheep also had similar plasma cortisol levels measured at home pen. On the other hand, concentration of plasma cortisol immediately after transport ($P<0.001$) and after lairage ($P<0.01$) in Red Karaman sheep were higher than those of Imroz sheep. These results indicate that cortisol responses of Red Karaman sheep to the transportation stress were higher than those of Imroz sheep. Hall et al. (1998) observed significant effect of sheep genotype on plasma cortisol levels after transport, and the authors noted that genotypes from highlands had greater cortisol responses to the stressors related with transportation. Kadim et al. (2006) reported lower plasma cortisol concentration in Jabal Akdhar goats compared with Batina and Dhofari breeds. Contrary, Ekiz et al. (2012a) found no significant differences in cortisol concentration among rams from Sakız, Imroz, Red Karaman and Karakul breeds at home pen, after transport and after lairage. On the other hand, reports of Ekiz et al. (2012a) in terms of CK, LDH and glucose, in which the differences between Red Karaman and Imroz rams for these parameters were not significant, supported the results of the current study.

Table 2. Effects of transport group and breed on plasma cortisol, CK and LDH concentrations[A].

Tablo 2. Transport grubu ve ırkın plazma kortizol, CK ve LDH konsantrasyonları üzerine etkisi.

Traits	Sampling time	Transport Group (TG)			Breed (B)		SEM	Significance of fixed effects		
		Ram	Ewe	Mixed	Red Karaman	Imroz		TG	B	TG × B
Cortisol, ng/ml	At home pen	68.83^{z}	38.56^{z}	50.68^{z}	66.421^{z}	38.954^{y}	7.790	NS	NS	NS
	After transport	166.97^{x}	165.20^{x}	207.99^{x}	255.23^{x}	104.87^{x}	17.601	NS	***	NS
	After lairage	83.22^{y}	57.68^{y}	80.22^{y}	103.79^{y}	43.62^{y}	10.327	NS	**	NS
	Significance [d]	***	***	***	***	***				
CK, U/l	At home pen	144.71	163.88^{y}	156.01^{y}	137.60^{y}	172.13^{y}	8.880	NS	NS	NS
	After transport	166.29	240.56^{x}	208.65^{x}	178.09^{x}	232.24^{x}	14.177	NS	NS	NS
	After lairage	181.50	244.25^{x}	217.98^{x}	189.89^{x}	239.27^{x}	15.224	NS	NS	NS
	Significance [d]	NS	***	*	**	***				
LDH, U/l	At home pen	1197.86^{y}	1132.25^{y}	1068.73^{xy}	1129.55^{y}	1136.35^{y}	32.631	NS	NS	NS
	After transport	1242.57^{x}	1182.69^{x}	1104.10^{x}	1159.66^{x}	1193.24^{x}	32.281	NS	NS	NS
	After lairage	1187.07^{y}	1174.69^{xy}	1029.84^{y}	1125.86^{xy}	1135.20^{y}	30.174	NS	NS	NS
	Significance [d]	**	*	*	*	**				

[A] : Data are least squares means.

[x, y, z] : Differences between the mean values carrying different letters in the same column are significant.

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

[d] : Significance level of differences between blood sampling times for the same transport group or breed.

Table 3. Effects of transport group and breed on plasma glucose, total protein concentrations and N:L ratio [A].

Tablo 3. Transport grubu ve ırkın plazma glukoz ve total protein konsantrasyonları ile N:L oranı üzerine etkisi.

Traits	Sampling time	Transport Group (TG)			Breed (B)		SEM	Significance of fixed effects		
		Ram	Ewe	Mixed	Red Karaman	Imroz		TG	B	TG × B
Glucose, mg/dl	At home pen	62.57 [y]	61.88 [y]	62.06 [y]	62.85 [y]	61.49 [y]	0.959	NS	NS	NS
	After transport	108.50 [x]	85.38 [x]	99.19 [x]	99.05 [x]	96.32 [x]	3.844	NS	NS	NS
	After lairage	101.71 [x]	87.25 [x]	99.19 [x]	100.42 [x]	91.68 [x]	3.764	NS	NS	NS
	Significance [d]	***	***	***	***	***				
Total protein mg/l	At home pen	8.11 [a, xy]	7.24 [b]	7.28 [b]	7.63 [x]	7.45 [y]	0.119	**	NS	NS
	After transport	8.25 [a, x]	7.23 [b]	7.49 [b]	7.70 [x]	7.61 [x]	0.143	*	NS	NS
	After lairage	7.94 [a, y]	7.18 [b]	7.28 [b]	7.48 [y]	7.46 [y]	0.122	*	NS	NS
	Significance [d]	*	NS	NS	*	*				
N:L	At home pen	0.82 [y]	0.97 [y]	0.99 [y]	1.05 [y]	0.81 [z]	0.068	NS	NS	NS
	After transport	1.56 [x]	1.79 [x]	1.45 [x]	1.94 [x]	1.26 [y]	0.101	NS	**	NS
	After lairage	1.37 [x]	1.73 [x]	1.58 [x]	1.62 [x]	1.49 [x]	0.112	NS	NS	NS
	Significance [d]	***	***	***	***	***				

[A] : Data are least squares means.

[x, y, z] : Differences between the mean values carrying different letters in the same column are significant.

[a, b] : Differences between the mean values of transport groups carrying different 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 blood sampling times for the same transport group or breed.

Red Karaman and Imroz sheep had similar N:L ratio measured at home pen and after lairage. But, N:L ratio measured after transport in Red Karaman sheep was higher than Imroz sheep ($P<0.01$). This result indicates that an increase in N:L ratio resulted from transport stress was higher in Red Karaman sheep, but elevated N:L ratio in Red Karaman sheep declined to the level observed for Imroz sheep after 2-h lairage period. Ekiz et al. (2012a) also found no significant difference between Sakız, Imroz, Red Karaman and Karakul rams in N:L ratio measured at home pen and after lairage; but contrary to the current results, the authors reported similar N:L ratio measured after transport for these breeds.

Sampling time had significant influence ($P<0.001$) on plasma cortisol concentration for all transport groups (Ram, Ewe and Ram-Ewe mixed) and breeds (Red Karaman and Imroz). Plasma cortisol concentrations after transportation were greater than those values obtained at home pen in animals from all sub-groups. An elevated plasma cortisol concentration related with transport stress was also reported in previous studies for various sheep breeds (Ali et al., 2006; Hall et al., 1999; Tadich et al., 2009) and for rams from Turkish sheep breeds including Red Karaman and Imroz sheep (Ekiz et al., 2012a). An increased plasma cortisol concentration caused from transportation stress might be due to the stimulation of the hypothalamo-pituatry-adrenal axis, as reported by Ali et al. (2006). After the lairage of 2-h, plasma cortisol level decreased significantly in all sub-groups. However, the trend of cortisol decline during lairage was different between Red Karaman and Imroz sheep. Plasma cortisol level measured for Imroz sheep returned to its pre-transport level after two hours of lairage, while it did not recover to the level obtained at home pen in Red Karaman sheep. The same trend was also observed in the study by Ekiz et al. (2012a). The authors reported that level of plasma cortisol after lairage returned to the level measured at home pen in Karakul and Imroz rams, but concentrations did not return to the pre-transport levels in Sakız and Red Karaman rams. With another point of view, two hours of resting duration was adequate for recovery from transport stress in Imroz sheep, while that resting period did not supply a recovery in Red Karaman sheep. In the literature, different durations of lairage have been reported for sheep to recover from transportation stress. Ekiz et al. (2012b) found that 30 min resting in Kıvırcık lambs was not adequate to obtain recovery from stress related with 75 min transport.

The influence of sampling time on CK and LDH concentrations were significant for all sub groups, except CK level of Ram sub-group (Table 2). CK and LDH might be the indicators of trauma, vigorous exercise or tissue damage resulted from pre-slaughter management and transport, since these enzymes are released into the blood after tissue damage, injury and/or vigorous exercise (Bórnez et al., 2009; Kannan et al., 2003; Kent, 1997). In the present study, elevated CK and LDH concentrations after transport indicate possible trauma due to loading, journey or unloading. An elevated CK concentration due to transportation was also found by Ekiz et al. (2012a) for Sakız rams and by Ekiz et al. (2012b) for Kıvırcık lambs.

Parallel to the trend in cortisol concentration, a significant increase in plasma glucose level and N:L ratio caused from transportation were observed in all sub-groups. These results suggest that transportation was stressful for all sub-groups. Similar trends of plasma cortisol and glucose levels during the transportation period was previously reported by Ekiz et al. (2012a; b) for sheep and by Kannan et al. (2003) for goat. Ali et al. (2006) explained the increased glucose concentration occurred during physical and psychological stress by the secondary impact of hypercortisolaemia and by an increase in glucose production from liver, reflecting the elevated sympatho-adrenal activity as a response to the stressor. Moreover, Bórnez et al. (2009) reported that increased plasma glucose concentration due to transport could indicate the amount of stress experienced during pre-slaughter period. Supporting the current results, an elevated glucose level (Ali et al., 2006; Bórnez et al., 2009; Ekiz et al., 2012b) and N:L

ratio (Ekiz et al., 2012b; Kannan et al., 2000) as a response to transportation stress were also reported in previous studies. The effect of transport group × breed interaction on plasma concentrations of biochemical stress parameters and N:L ratio was not significant. These results indicate that the influence of transport group on these parameters were similar in Imroz and Red Karaman sheep.

The influence of transportation group and sheep breed on individual and feeding behaviours are presented in Table 4. The posture of animals during the two hours of lairage period was significantly influenced by transportation group ($P<0.01$). Ram group exhibited higher lying behaviour and lower standing behaviour than the Ewe group and Ram-Ewe mixed group. Moreover, the percentage of time spent for walking was lower in Ram group than Ram-Ewe mixed group ($P<0.05$). On the other hand, differences among transportation groups in terms of the percentages of time spent for idling, investigation, rumination and drinking were not significant ($P>0.05$). Sevi et al. (2001) found increased locomotor activity in sheep subjected to group and pen exchange compared with that of sheep never regrouped (control group). The authors also noted that regrouped sheep spent less time for lying than the control group. Moreover, supporting the current result, the authors observed that rumination behaviour was not influenced by mixing treatment.

The effect of transport group × breed interaction on lying and standing behaviours were significant ($P<0.01$). Red Karaman rams and ewes did not exhibit lying behaviour during the 2-h lairage period; therefore, they stood during the whole resting period. Therefore, differences between transport groups in terms of standing and lying behaviours were not significant in Red Karaman sheep. However, Ram group in Imroz sheep exhibited significantly higher lying behaviour than those of Ewe and Mixed groups in this breed. According to the results of statistical analysis of overall dataset, breed had significant effect on standing and lying behaviours ($P<0.01$) and the percentage of time spent for standing was 89.21% in Imroz sheep. Cockram (2007) noted that an increase in lying behaviour in a novel environment might be due to the adaptation of animal to the new environment or due to the decreased standing ability resulting from exhaustion. In the present study, the difference between Imroz and Red Karaman breeds in terms of LDH and CK levels were not significant. This indicates that exhaustion level was similar in Imroz and Red Karaman sheep. Hence, a higher percentage of lying posture in Imroz breed might be explained by the adaptation of Imroz sheep to lairage unit earlier than Red Karaman sheep. Supporting the present result, Ekiz et al. (2012a) reported higher lying behaviour in Imroz rams than Sakız, Red Karaman and Karakul rams, and they explained this result with the better adaption performance of Imroz rams to the novel environments of lairage. The percentages of investigation, walking, rumination and drinking behaviours in Red Karaman and Imroz breeds were similar, while time spent for idling in Imroz sheep was higher than that of Red Karaman breed. Supporting the present result, Ekiz et al. (2012a) reported no significant influence of breed of ram on percentages of rumination, drinking and walking behaviours during the 2-h lairage. However, Ekiz et al. (2012a) observed a higher percentage for investigation behaviours in Red Karaman rams than Karakul and Imroz rams.

Least squares means and significance controls for frequency of eliminative, abnormal and other behavioural activities in transport groups and breeds are presented in Table 5. The influence of transport group on these behaveours were not significant ($P>0.05$), except for urination and butting other animals behaviours ($P<0.01$). Urination behaviour was lower in Ram group compared with that of Ram-Ewe mixed group. Moreover, Ram group exhibited higher "butting other animals" behaviour than Ewe group. Das et al. (2001) observed an increase in urination behaviour due to frightening in goats. Villalba et al. (2009) noted that animals showing a higher frequency of urination might be considered as more fearful.

Table 4. Effects of transport group and breed on percentages of individual and feeding behaviours during the two-hour lairage[A].

Tablo 4. Transport grubu ve ırkın iki saatlik dinlenme sürecinde bireysel ve beslenme davranışlarının oranları üzerine etkisi.

Behaviours	Transport Group (TG)			Breed (B)		SEM	Significance of fixed effects		
	Ram	Ewe	Mixed	Red Karaman	Imroz		TG	B	TG × B
Lying, %	13.43[a]	0.00[b]	2.75[b]	0.00	10.79	1.616	**	**	**
Standing, %	86.57[b]	100.00[a]	97.25[a]	100.00	89.21	1.616	**	**	**
Walking, %	8.00[b]	13.25[ab]	18.25[a]	14.74	11.60	1.507	*	NS	NS
Idling, %	70.29	65.50	72.00	61.67	77.86	2.525	NS	**	NS
Investigation, %	8.29	3.50	1.25	6.83	1.86	1.554	NS	NS	*
Drinking, %	1.14	0.50	0.50	0.91	0.52	0.221	NS	NS	NS
Rumination, %	12.29	17.25	8.00	15.86	9.17	1.813	NS	NS	NS

[A] : Data are least squares means.
[a, b] : Differences between the mean values of transport groups carrying different letters in the same line are significant.
NS : Not significant (P>0.05); * P<0.05; ** P<0.01.

Table 5. Effects of transport group and breed on frequencies of eliminative, abnormal and other behavioural activities during the two-hour lairage[A].

Tablo 5. Transport grubu ve ırkın iki saatlik dinlenme periyodunda boşaltım, anormal ve diğer davranış aktivitelerinin frekansları üzerine etkisi.

Behaviours	Transport Group (TG)			Breed (B)		SEM	Significance of fixed effects		
	Ram	Ewe	Mixed	Red Karaman	Imroz		TG	B	TG × B
Defecation	0.14	0.06	0.25	0.09	0.21	0.054	NS	NS	NS
Urination	0.36[b]	1.19[ab]	1.88[a]	0.69	1.59	0.205	**	*	**
Licking or gnawing walls, feeder etc.	0.14	0.19	0.94	0.72	0.13	0.201	NS	NS	NS
Butting walls, feeder etc.	1.36	0.00	0.00	0.91	0.00	0.297	NS	NS	NS
Butting other animals	4.00 [a]	0.44[b]	1.25[ab]	2.78	1.01	0.514	**	NS	*
Selfgrooming	1.07	0.25	0.25	0.17	0.88	0.209	NS	NS	NS
Allogrooming	0.14	0.00	0.00	0.00	0.10	0.043	NS	NS	NS
Vocalisation	4.79	3.13	1.50	5.89	0.38	0.751	NS	***	NS

[A] : Data are least squares means.
[a, b] : Differences between the mean values of transport groups carrying different letters in the same line are significant.
NS : Not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001.

Aggression between animals can be result from the endeavour of animals to gain more food, to have priority for food and to get a higher rank in social hierarchy (Sevi et al., 2001). Previous studies in pigs (Ekkel et al., 1997; de Jong et al., 2000; Merlot et al., 2004) and cattle (Mounier et al., 2006) indicate that mixing unfamiliar animals can cause a competition between animals to reorganise the social hierarchy. After the competition, animals are divided into the groups of winners or losers (Merlot et al., 2004). In the current study an increase in aggression behaviour was not observed in Ram-Ewe mixed group. This result might be explained by the ewes being in lower groups in terms of social hierarchy than rams. Therefore, ewes did not struggle with rams. On the other hand, the more frequent observation of "butting other animal" behaviour in Ram group than Ewe group points out that the social hierarchy struggle was more among rams. Supporting the current results, Tölü and Savaş (2007) reported elevated frequency of 'butting other animals' behaviour in Turkish Saanen goats against its fellows with increased dominance rank in social hierarchy. Stolba et al. (1990) noted that the frequency of dominance fights within a flock was greater in single-sex groups than mixed sex group in Merino sheep. Guilhem et al. (2006) observed higher agonistic behaviours in male lambs than in female ones.

The difference between Red Karaman and Imroz sheep in terms of frequencies of defecation, abnormal and other behavioural activities during the two hours of lairage period after transport were not significant ($P>0.05$), except urination and vocalisation behaviours. Imroz sheep exhibited a higher frequency of urination behaviour ($P<0.05$) and a lower frequency of vocalisation behaviour ($P<0.001$) compared with Red Karaman sheep. Sevi et al. (2001) noted that elevated vocalisation behaviour in adult animals might be a sign of distress and arousal. Therefore, Red Karaman sheep might be mentioned to have higher stress responses to the transportation and lairage procedures than Imroz sheep. This outcome was also supported by the results for cortisol concentrations measured immediately after transport and after lairage, which were higher in Red Karaman than Imroz sheep. Similar to the present result, Ekiz et al. (2012a) found a higher frequency of vocalisation behaviour in Red Karaman rams than Karakul, Sakız and Imroz rams and the authors noted that Red Karaman rams were in more distress and arousal than other breeds during the two hours of lairage period after transport. The effect of transport group × breed interaction on frequencies of urination ($P<0.01$) and butting other animals ($P<0.05$) behaviours were significant. The differences between Ram groups of Red Karaman and Imroz breeds for frequency of urination behaviour was not significant, while significant differences between breeds were observed in Ewe groups and Mixed groups. Ram group in Red Karaman breed displayed higher frequency of butting other animals behaviour compared with other sub-groups.

Conclusion

Transportation of Red Karaman and Imroz sheep for 75 min caused a stress in animals with increasing cortisol, CK, LDH, glucose levels and N:L ratio. The results of haematological and biochemical stress parameters showed that the mixed transportation of ewes and rams did not cause an additional stress response. Moreover, the mixed lairage of rams and ewes also did not cause any increase in behavioural stress parameters such as "butting other animals", "butting walls, feeder, etc." and "vocalisation". Therefore, it was concluded that the mixed transportation of rams and ewes during the non-breeding season did not have negative effects in terms of animal welfare in Red Karaman and Imroz indigenous sheep breeds.

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