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Certain behavioral characteristics and stress responses of out-of-breeding ewes and rams during an intensive fattening program

Hülya YALÇINTAN[1,*], Elif ERGÜL EKİZ[2], Bülent EKİZ[1], Ömür KOÇAK[1], Deniz AKTARAN BALA[3], Alper YILMAZ[1]

[1]Department of Animal Breeding and Husbandry, Veterinary Faculty, İstanbul University, Avcılar, İstanbul, Turkey

[2]Department of Physiology, Veterinary Faculty, İstanbul University, Avcılar, İstanbul, Turkey

[3]Food Technology Program, Department of Food Processing, Vocational High School, Faculty of Veterinary Medicine, İstanbul University, Avcılar, İstanbul, Turkey

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Abstract: The aim was to investigate behaviors and stress responses of rams and ewes in an intensive fattening period. Out-of-breeding rams (Hemsin = 10, Karakul = 10) and ewes (Hemsin = 8, Karakul = 10) were used in the study. Sheep and rams belonging to each breed were placed into four different pens. Behavioral observations (individual, feeding, abnormal self-grooming behaviors) were performed 2 days a week for two groups a day for 1 h. Blood samples were collected at the beginning, at the 4th week, and at the end of the fattening period. Karakul ewes displayed significantly more feeding and rumination behavior than Hemsin ewes, while there was no significant difference between ram groups. Ewes displayed more lying and rumination behavior than rams in the current study. On the other hand, rams were more active than ewes and also showed more abnormal behavior (butting other animals) during the fattening period. Sheep breed had no influence on packed cell volume (PCV), hemoglobin (Hb) concentration, or cortisol level at any sampling time. PCV, Hb, and cortisol levels at the middle of the fattening period were higher in ewes than rams. In conclusion, the behavioral repertoire of Hemsin and Karakul breeds in intensive fattening does not reveal any stress responses.

Key words: Breed effect, sex effect, behavior, fattening

1. Introduction

The primary income of sheep breeders in Turkey is from lamb production. Sheep meat also constitutes 24.1% of the total red meat production in Turkey (1). As of 2016, mutton and lamb production is approximately 83,000 t in Turkey (2). Although consumers usually prefer to consume lamb, mutton is also consumed in Turkey. In the traditional sheep production system in Turkey, farmers apply a short-term fattening program for sheep that are out of breeding with the aim of reaching proper slaughter weight. After a short fattening period, farmers sell sheep at lower prices than lambs. The short fattening program is usually done intensively.

Intensive conditions cause chronic stress in animals; first certain behavioral disorders occur and then some health and physiological problems are observed. Increased plasma cortisol concentration is one of the stress responses in animals (3). The responses of animals to various stress stimuli may vary depending on numerous individual or environmental factors (3). Animals' genetic structure, such as breed, is one of the individual factors that can affect the stress responses of animals (4). Karaağaç et al. (5) observed that crossbred lambs exhibited higher percentages of licking or gnawing the feeder, stalls, and walls and eating wool and also more aggression towards other animals than Kivircik lambs. Because sheep and goats are often reared under extensive conditions, studies on the welfare of these animals are much more limited than those of other farm animals (6). However, it is necessary to investigate the behaviors, physiological indications, and production potentials of sheep as stress responses in different production systems, especially intensive systems of sheep breeding for milk production or fattening purposes.

The objective of the present study was to investigate the behavior repertoire of rams and ewes from the Hemsin and Karakul breeds and also determine their stress responses in an intensive fattening program.

2. Materials and methods

Animal rearing and handling procedures of the study were approved by the Ethics Committee of İstanbul University (No: 2012/32).

* Correspondence: hyalcint@istanbul.edu.tr

2.1. Animals, handling, and finishing period

This study was conducted at the İstanbul University Veterinary Faculty. Hemsin and Karakul rams (Hemsin = 10, Karakul = 10) and ewes (Hemsin = 8, Karakul = 10), which were 5–6 years old and out of breeding, were used in the study. Ewes and rams belonging to each breed were kept in four different pens during the 56 days of fattening. The floor area of each pen was 20 m^2 excluding the feeder and water bowl.

Sheep in all subgroups had free access to concentrate feed (89% dry matter (DM), 17% crude protein, and 2866 kcal/kg DM, ME) during the first 2 weeks of fattening, and then they were fed 600 g of concentrate per day per sheep. Sheep had continuous access to alfalfa hay and clean fresh water.

2.2. Behavioral observations

We observed animal behaviors 1.5 h after they were fed in the morning. Two experienced researchers performed the behavioral observations for 1 h for each group once a week. Observations were performed 2 days a week and for two groups a day. The observer might create differences between subgroups. In order to prevent this situation, we rotated the observers of subgroups on a weekly basis.

The time sampling method was used to observe the feeding and individual behaviors of sheep (7). In this methodology, certain feeding behaviors (feed consumption, drinking, and rumination) and individual behaviors (standing, walking, lying, idling, and investigation) of each sheep were recorded at the beginning of every 5 min during the whole observation period. The dataset for each of these behavioral characteristics included totally 3952 observations recorded from 38 animals, and 104 observations for each animal. Previously prepared behavioral marking charts included the following behaviors: i) butting walls, feeder, etc.; ii) licking or gnawing feeder, walls, etc.; iii) butting other animals; iv) self-grooming; v) allogrooming; and vi) vocalization behaviors. The last mentioned behaviors were recorded at the time when sheep displayed these behaviors during the observation period. During the study, sheep did not exhibit behaviors of butting walls, feeder, etc.; licking or gnawing feeder, walls, etc.; allogrooming; or vocalization behaviors. Hence, we did not include these parameters in the article. Description of the behaviors recorded in the current study were previously reported by Ekiz et al. (8).

2.3. Blood collection and laboratory analyses

Three blood samples were taken from each sheep from the jugular vein to determine the stress responses of animals. Sampling times were: 1) at the beginning of fattening; 2) in the middle of fattening (4th week); 3) at the end of fattening (8th week). In order to avoid additional variation caused by the technician, the same trained person took blood throughout the study.

At each blood sampling, two blood samples (heparinized and EDTA samples) were taken from animals. While EDTA samples were used to measure packed cell volume (PCV), hemoglobin concentration (Hb), and neutrophil-to-lymphocyte ratio (N:L), heparinized samples were used for determination of plasma cortisol concentration. In order to obtain plasma, heparinized tubes were centrifuged for 15 min at 3500 rpm. Plasma samples were kept at –85 °C until cortisol analysis. Standard capillary microhematocrit and oxy-hemoglobin methods were used to determine PCV and Hb, respectively. N:L ratio was determined using blood smears stained with May–Grünwald–Giemsa stain. Plasma cortisol concentration was determined with a diagnostic ELISA direct immunoenzymatic kit (DiaMetra, Italy).

2.4. Statistical analyses

The GLM procedure was used to determine the effects of breed (Karakul, Hemsin), sex (ram, ewe), and breed × sex interaction on hematological and biochemical stress parameters measured before fattening, at the 4th week of fattening, and at the end of fattening. Furthermore, repeated measurement of ANOVA was used in order to analyze data for hematological and biochemical stress parameters for each subfactor (Karakul, Hemsin, ram, and ewe). The mathematical model used for these characteristics included sampling time (before fattening, 4th week of fattening, and end of fattening) as a within-subject effect.

Before the statistical evaluation of feeding and individual behaviors, percentages of each behavioral characteristic thorough all observation periods were calculated using data recorded by the time sampling methodology. Data for abnormal behaviors, self-grooming, allogrooming, and vocalization behaviors were first transformed to logarithmic form before statistical analyses because they did not supply a normal distribution. Repeated measurement of ANOVA was used in the statistical analyses of behavioral data. The statistical model for behavioral data included breed, sex, and breed × sex interaction as between-subject effects and sampling week (1st to 8th week of fattening), breed × sampling week interaction, and sex × sampling week interaction as within-subject effects.

3. Results

The effects of sheep breed and sex on PCV, Hb concentration, N:L ratio, and plasma cortisol concentration are presented in Table 1. The N:L ratio was higher in Hemsin sheep than Karakul sheep in blood samples taken at the beginning and at the 4th week of the study, but the N:L ratio measured at the end of fattening was similar in the Hemsin and Karakul breeds ($P > 0.05$). Rams and ewes had similar PCV, Hb, and cortisol levels

Table 1. Effects of breed and sex on PCV, Hb concentration, N:L ratio, and plasma cortisol concentration[A].

Traits	Sampling time	Breed (B)		Sex (S)		SEM	Significance of fixed effects		
		Karakul	Hemsin	Ram	Ewe		Breed	Sex	B × S
PCV, %	Prefattening	27.25 [c]	27.25 [c]	26.10 [c]	28.40 [c]	0.632	NS	NS	NS
	4th week of fattening	29.95 [b]	28.90 [b]	27.70 [b]	31.15 [b]	0.426	NS	***	NS
	End of fattening	32.95 [a]	31.30 [a]	31.70 [a]	32.55 [a]	0.446	NS	NS	NS
	Significance [d]	***	***	***	***				
Hb, g/dL	Prefattening	9.66 [c]	10.15 [c]	9.62 [c]	10.19 [c]	0.224	NS	NS	NS
	4th week of fattening	11.22 [b]	10.95 [b]	10.64 [b]	11.53 [b]	0.160	NS	**	NS
	End of fattening	12.43 [a]	12.37 [a]	12.37 [a]	12.42 [a]	0.144	NS	NS	NS
	Significance [d]	***	***	***	***				
N:L ratio	Prefattening	0.85	1.25 [a]	1.05 [a]	1.05 [a]	0.077	*	NS	NS
	4th week of fattening	0.59	0.82 [b]	0.73 [b]	0.68 [b]	0.050	*	NS	NS
	End of fattening	0.62	0.68 [b]	0.65 [b]	0.65 [b]	0.041	NS	NS	NS
	Significance [d]	NS	***	**	**				
Cortisol, ng/mL	Prefattening	59.21 [b]	44.28 [b]	43.07 [ab]	60.42 [b]	6.994	NS	NS	NS
	4th week of fattening	77.88 [a]	95.24 [a]	51.18 [a]	121.94 [a]	16.738	NS	*	NS
	End of fattening	53.40 [b]	59.01 [b]	34.06 [b]	78.36 [b]	12.382	NS	NS	NS
	Significance [d]	**	*	*	*				

[A] Data are least squares means.
[a, b, c] Differences between the means of sampling times with different superscript letters following them 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 sampling times for the same breed or sex according to repeated measurements of ANOVA statistics.

in blood samples taken at the beginning of finishing. However, PCV, Hb, and cortisol levels at the middle of fattening were higher in ewes than rams. At the end of fattening, ewes and rams had similar PCV, Hb, and cortisol levels. The influence of sampling time on PCV and Hb concentration was significant (P < 0.001) in all subgroups. There were significant increases in PCV and Hb level due to sampling time in all subgroups. An increased plasma cortisol concentration was observed in Karakul, Hemsin, and ewe subgroups at the 4th week of fattening compared with the initial values. In blood samples taken at the end of fattening, plasma cortisol values of these groups returned to the values measured at the beginning of fattening. Such an increase in plasma cortisol concentration at the 4th week of fattening was not observed in the ram subgroup. However, plasma cortisol concentration at the end of fattening was significantly lower than that measured at the middle of fattening in the ram subgroup.

Results for percentages of feeding and individual behaviors, and frequencies of abnormal behavioral activities, are presented in Table 2. Breed had no significant effect on postural behaviors (lying and standing) or on walking, investigation, drinking, and abnormal behaviors. On the other hand, the Karakul breed exhibited lower idling behavior and higher feeding and rumination behavior than the Hemsin breed. Sex had a significant effect on postural behaviors during the fattening period. Rams spent much more time standing than females. Moreover, rams also exhibited higher percentages of walking behavior. On the other hand, percentage of time spent for rumination was higher in ewes. The effect of breed × sex interaction on walking, idling, feeding, and rumination behaviors was significant. Karakul rams walked less than other subgroups, whereas Hemsin ewes exhibited higher idling behavior than other subgroups. While Hemsin ewes spent less time for feeding behavior, Karakul ewes spent much more time for rumination compared with other subgroups.

Licking walls, butting walls, and butting other animals as abnormal behaviors and also vocalization, allogrooming, and self-grooming behaviors were investigated in the study. However, only butting other animals and self-grooming behaviors were exhibited by animals during the observation period. Butting other animals was observed more frequently in rams than ewes.

Table 2. Effects of breed and sex on percentages of individual and feeding behaviors, and frequencies of abnormal activities during the fattening.

Behaviors	Breed (B)		Sex (S)		SEM	Significance of fixed effects		
	Hemsin	Karakul	Ram	Ewe		Breed	Sex	B × S
Lying, %	11.83	15.82	10.34	17.31	1.627	NS	*	NS
Standing, %	88.17	84.18	89.66	82.69	1.627	NS	*	NS
Walking, %	2.66	3.16	3.80	2.01	0.238	NS	**	*
Ram	4.04 [a]	1.25 [b]						
Ewe	3.56 [a]	2.76 [a]						
Idling, %	53.41	40.06	44.47	49.00	1.773	**	NS	**
Ram	44.52 [b]	44.42 [b]						
Ewe	62.31 [a]	35.70 [b]						
Investigation, %	2.31	1.43	2.16	1.58	0.485	NS	NS	NS
Feeding, %	36.97	47.56	45.24	39.29	1,786	**	NS	*
Ram	44.52 [a]	45.96 [a]						
Ewe	29.42 [b]	49.16 [a]						
Drinking, %	0.77	1.41	1.01	1.67	0.168	NS	NS	NS
Rumination, %	3.89	6.38	3.32	6.96	0.548	*	**	*
Ram	3.37[b]	3.27 [b]						
Ewe	4.42 [b]	9.50 [a]						
Self-grooming	0.02	0.01	0.01	0.02	0.004	NS	NS	NS
Butting other animals	0.15	0.14	0.25	0.04	0.015	NS	***	NS

[NS] Not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[a, b, c] Means for B × S subgroups with different superscript letters following them are significantly different ($P < 0.05$).

4. Discussion

During the fatting period, farm animals may be subjected to numerous stressors such as restricted area, poor handling, malnutrition, and unfamiliar environment and animals. An increase in blood cortisol concentration in stressed animals is observed due to the stimulation of the hypothalamo–pituitary–adrenal axis (3). In the current study, ewes had higher PVC, Hb, and plasma cortisol concentration at the middle of the fattening period than rams. These results may indicate higher stress responses in ewes than rams. Kent (9) noted that an increase in PCV during stress might be due to mobilization of red blood cells from the spleen in response to catecholamines. Ekiz et al. (8) and Hall and Bradshaw (10) also reported increased PCV as a response to several stressors as a consequence of splenic contractions, which forces erythrocytes to move into the circulation. On the other hand, changes in PCV, Hb, and cortisol concentration during the fattening period were similar in ewes and rams. PCV and Hb levels were increased during the fattening period both in rams and ewes, while a significant decrease was observed in cortisol concentration between the 4th week and the end of fattening. One of the possible explanations for high PCV, Hb, and cortisol levels determined in ewes at the middle of the fattening period might be higher acute stress responses of ewes to blood sampling. However, the same experienced person collected the blood samples from animals in approximately 60 s and similar trends according to sampling time in terms of these parameters were observed in ewes and rams. On the other hand, although PCV and Hb levels increased during the fattening period, they were still within the normal reference ranges according to Jones and Allison (11). Although there was a significant difference between Hemsin and Karakul breeds in terms of N:L ratio, a significant breed effect for this trait could not be related to the difference between breeds for stress responses to fattening conditions, because such a difference was also obtained from prefattening measurements.

Behavioral measures are important tools in the assessment of animal welfare (12). In the current study, Karakul ewes displayed significantly more feeding and rumination behaviors than Hemsin ewes, while there were no significant differences between ram groups in terms of feeding and rumination behaviors. There is a strong

relationship between rumination behavior and feeding as a result of the digestion physiology of sheep (5). The higher rumination behavior in the Hemsin breed might be related to the higher feed consumption observed in the present study. Supporting the current results, Schirmann et al. (13) reported that following periods of high feeding times, cows spent more time for ruminating. On the other hand, Grant and Albright (14) reported that greater stocking density may cause reduced rumination time. The Hemsin breed had larger mature body size than the Karakul breed. Therefore, space allowance per sheep was quite lower for the Hemsin breed than the Karakul breed. However, space allowance for physical comfort for sheep provided in the current study was approximately 2 m^2 per animal, in the ranges acceptable by European legislation (15). Floor space allowance should be 1.0–1.2 m^2 per ewe and 1.5–2.0 m^2 per ram.

Ewes displayed more lying and rumination behavior than rams in the current study. On the other hand, rams were more active than ewes and also showed more abnormal behaviors (butting other animals) during the fattening period. Similar to the current study, Karaağaç et al. (5) reported more lying behavior for female lambs compared to male lambs in intensive conditions. Schirmann et al. (13) also reported that periods of rumination were associated with time spent lying down in cows. We know that time spent lying and rumination behavior are important indicators of animal comfort and welfare (5,16). Therefore, a higher percentage of lying behavior in ewes could be indicative of better adaptation of ewes to the fattening than rams.

Abnormal behaviors are considered the main indicator of stress and distress. Sheep may express abnormal behaviors such as mouthing bars, chewing slats or chains, biting and chewing pen fixtures, and repetitive butting, although they do not display these behaviors as frequently as other farm species (12). In the current study, rams displayed more butting of other animals compared to ewes. Guilhem et al. (17) also reported higher agonistic behaviors for male lambs compared to females. Rams also engage in butting as a playful behavior more than female ones (18). Similar to the current study, Ekiz et al. (19) found that rams exhibited more butting of other animals than ewes during transportation. Tölü and Savaş (20) reported that 'butting behavior' was frequently observed in goats with a high social hierarchy. In this study, the absence of abnormal or stereotypic behaviors such as licking or gnawing feeder, walls, etc.; butting feeder, walls, etc.; and allogrooming indicates that the intensive fattening conditions were enough to satisfy the behavioral needs of animals and also were not stressful.

The results of the current study indicate that the behavioral repertoires of Hemsin and Karakul breeds in intensive fattening do not reveal any stress responses. However, butting of other animals was observed more in rams, which indicates that rams engage more in forming a social hierarchy during intensive fattening.

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References

1. FAO. Food and Agriculture Databases. Rome, Italy: FAO; 2016.
2. TÜİK. I., II., III. ve IV. Çeyrek kırmızı et üretim istatistikleri. TÜİK Haber Bülteni. Ankara, Turkey: TÜİK; 2016 (in Turkish).
3. Ergul Ekiz E, Yalcintan H. Comparison of certain haematological and biochemical parameters regarding pre-slaughter stress in Saanen, Maltese, Gokceada and hair goat kids. Journal of the Faculty of Veterinary Medicine Istanbul University 2013; 39: 189-196.
4. Broom DM. The effects of land transport on animal welfare. Rev Sci Tech OIE 2005; 24: 683-691.
5. Karaağaç F, Özcan M, Savaş T. Some behaviour traits observed on the Kıvırcık and crossbred lambs raised in intensive conditions. Turk J Vet Anim Sci 2005; 29: 803-809.
6. Sevi A, Casamassima D, Pulina G, Pazzona A. Factors of welfare reduction in dairy sheep and goats. Ital J Anim Sci 2007; 8: 81-101.
7. Bogner H. Verhaltensbeobachtungen, versuchsanlage und auswertungen. In: Bogner H, editor. Verhalten landwirtschaftlicher Nutztiere. Stuttgart, Germany: Verlag Eugen Ulmer; 1984. pp. 61-74.
8. Ekiz B, Ergül Ekiz E, Yalçıntan H, Koçak Ö, Yılmaz A, Güneş H. The effect of transport stress on certain welfare parameters and behaviours in Red Karaman, Imroz, Sakız and Karakul rams. Journal of the Faculty of Veterinary Medicine Istanbul University 2012; 38: 15-28.
9. Kent JE. Stress in transported sheep. Comp Haematol Int 1997; 7: 163-166.
10. Hall SJG, Bradshaw RH. Welfare aspects of the transport by road of sheep and pigs. J App Anim Wel Science 1998; 1: 235-254.
11. Jones ML, Allison RW. Evaluation of the ruminant complete blood cell count. Vet Clin Food Anim 2007; 23: 377-402.

12. Nowak R, Porter RH, Blache D, Dwyer CM. Behaviour and the welfare of the sheep. In: Dwyer C, editor. The Welfare of Sheep. Berlin, Germany: Springer; 2008. pp. 81-134.

13. Schirmann K, Chapinal N, Weary DM, Heuwieser W, von Keyserlingk MAG. Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. J Dairy Sci 2012; 95: 3212-3217.

14. Grant RJ, Albright JL. Feeding behaviour. In: D'Mello JPF, editor. Farm Animal Metabolism and Nutrition. Wallingford, UK: CABI; 2006. pp. 365-382.

15. EEC. Council Directive 98/58/EC of 20 July 1998 Concerning the Protection of Animals Kept for Farming Purposes; OJ L 221, 8.8.1998. Brussels, Belgium: EEC; 1998.

16. Arney DR. Welfare of large animals in scientific research. Scand J Lab Anim Sci 2009; 36: 97-101.

17. Guilhem C, Bideau E, Gerard JF, Maublanc ML, Pépin D. Early differentiation of male and female interactive behaviour as a possible mechanism for sexual segregation in mouflon sheep (*Ovis gmelini*). Appl Anim Beh Sci 2006; 98: 54-69.

18. Dwyer C. The behaviour of sheep and goats. In: Jensen P, editor. The Ethology of Domestic Animals. 2nd ed. Wallingford, UK: CABI; 2009. p. 174.

19. Ekiz B, Ergul Ekiz E, Yalcintan H, Yilmaz A, Kocak O, Gunes H. Effect of ram-ewe mixed transportation on certain welfare parameters in Red Karaman and Imroz sheep. Journal of the Faculty of Veterinary Medicine Istanbul University 2013; 39: 155-167.

20. Tölü C, Savaş T. A brief report on intra-species aggressive biting in a goat herd. Appl Anim Beh Sci 2007; 102: 124-129.