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Influence of social rank on certain stress response variables and behavioral characteristics of adult rams

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Abstract: Dominance rank within a group can affect the stress level of an animal. However, there is no consensus on whether the dominant or subordinate animals are physiologically more stressed. It was aimed herein to determine the effect of social rank on the stress level and expression of some behaviors of adult Hemsin, Chios, and Karakul rams. According to their dominance index (DI) values, the rams were classified as low-ranked (DI < 0.33; n = 13), medium-ranked (DI: 0.33–0.66; n = 13), and high-ranked (DI > 0.66; n = 13) individuals. The low-ranked rams had lower body weight, body length, chest depth, chest circumference, cannon bone circumference, and tail width than the medium- and high-ranked rams. The high-ranked rams tended to exhibit more rumination than the low- and medium-ranked rams (P = 0.066). The rank group had no influence on the frequency of vocalization, butting other animals, and itching behaviors. The low-ranked rams had lower hematocrit, hemoglobin, and red blood cell counts than the medium- and high-ranked rams. The rank group had no influence on the white blood cell count, neutrophil:lymphocyte ratio, and cortisol level. In conclusion, under the conditions of the current study, the welfare of the low-ranked rams was not adversely affected.

Key words: Animal welfare, dominance hierarchy, body dimensions, aggressiveness, cortisol

1. Introduction

Farm animals living in groups form a well-organized hierarchical order within the group. Agonistic struggles between individuals play a major role in the formation of hierarchical order [1]. The animals that win the dyad made between animals are positioned as dominant against the losers, and those that succumb are positioned as subordinate in the hierarchical order [2]. As a result of these dual struggles that are made among all of the animals in a group, a social order is established for that group [3]. A higher social rank is achieved by not submitting to other individuals during the struggle made for dominance [4]. Establishment of the dominance relationship and social hierarchy being accepted by all of the animals within the group is important in terms of the formation of social organizations, and the prevention of aggression among animals that may cause injuries [3–5].

Rank within dominance hierarchies may greatly affect the quality of life of an animal. The social rank of animals may also determine the order of use of available resources, especially when resources are limited [6,7]. However, there is no consensus on whether the dominant or subordinate animals are physiologically more stressed or have poorer

welfare [8]. There have been reports that stress may be experienced by both high-ranked [6,8] and low-ranked [9] animals. On the other hand, while some authors have observed a significant influence of social rank on certain behaviors, such as feeding behaviors [10,11] and aggressiveness [3,7,10], Ketelaar-de Lauwere et al. [12] did not find a relationship between the dominance value and the time that dairy cows spent feeding or lying down. Differences among the studies in terms of whether dominant or subordinate animals exhibited more stress responses might have been due to use of different species, or from the design of the studies (exposure of the animals to stressful handling procedures during the study or the animals being in a situation of major hierarchical reorganization, or being in a flock where a social hierarchy was established). On the other hand, to the best of our knowledge, no studies exist investigating the effects of social rank on some stress response parameters, behaviors, and the welfare of animals in domestic sheep breeds. Many indigenous sheep breeds, which have adapted to the climate conditions and nutrition opportunities of the region where they live, are bred in Turkey. There are major differences among these sheep breeds in terms of

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temperament and morphological characteristics, such as body size, horn shape and length, and tail structure (i.e. fatty, thin, or semi-fatty tails). The Karakul breed is a small-sized and fatty-tailed breed. Karakul ewes mostly do not have horns, but rams have strong curved horns. Chios is a long-legged breed with semi-fatty tails that is known for its high milk yield and dairy body structure. Chios rams have strong spiral horns. Hemsin is a middle-sized and semi-fatty tailed breed. Hemsin rams have large helical horns. The Chois sheep is generally bred in small family herds and known as an easy-to-manage breed, and its temperament is calmer than the other Turkish breeds. Karakul and Hemsin sheep are indigenous breeds, mostly bred in large herds and graze in high pastures [13,14]. In this study, it was aimed to determine the effects of social rank on the levels of certain stress response variables, such as the hematocrit (HCT) value, red blood cell (RBC) count, hemoglobin (HGB) level, white blood cell (WBC) count, neutrophil:lymphocyte (N:L) ratio, cortisol level, and expression of certain behaviors of adult Hemsin, Chios, and Karakul rams.

2. Materials and methods
The experimental procedures of the study were approved by the Ethics Committee of İstanbul University-Cerrahpaşa (Approval No.: 2012/64).

2.1. Animals and management
The study was conducted at the sheep breeding unit of the Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa, during the months of April, May, and June. The animal material of the study consisted of 13 Hemsin rams, 12 Chios rams, and 14 Karakul rams. All of the rams used in the study were 6–7 years old.

Rams of the same breed were maintained in separate pens (4.50×4.66 m) built in the sheepfold (3 pens in total). The space allotted for lying down was 1.61, 1.74, and 1.50 m^2 for the Hemsin, Chios, and Karakul rams, respectively. Each pen contained 1 feeder (200×50 cm) for alfalfa hay and 1 feeder (300×24 cm) for commercial concentrated feed. The rams received alfalfa hay (15.85% crude protein and 2070 kcal/kg ME), clean water, and 600 g/day of commercial concentrated feed (17% crude protein, 2866 kcal/kg ME) ad libitum. Feed was given to the rams twice a day at 08:30 AM and 16:00 PM.

2.2. Determination of the social rank
Before determination of the social rank within each breed, the rams were kept separated in pens based on their breeds for 8 weeks. The food competition test described by Ungerfeld and Lacuesta [15] was used to determine the social ranks of the rams within their own breeds.

For the food competition test, a separate pen with an area of 21 m^2 was established in the sheepfold. A special manger, measuring $30 \times 20 \times 45$ cm, which only 1 ram

could insert his head into, was used during the test. All of the rams were allowed to interact with other rams within their breed as a dyad. On each test day, paired competitions were performed between 8:30 and 11:30 AM, and the rams were not fed until the competitions were completed. Each ram performed only 1 competition in a day. When a ram could prevent the other ram from reaching the feed, and could eat continuously from the manger for at least 1 min, it was considered to be dominant over its opponent [15]. After testing all of the possible pairs, the dominance index (DI) for each ram was calculated according to the formula given below [10,16,17].

DI = number of rams subdominant / (number of rams dominant + number of rams subdominant).

The rams were then classified into 3 ranking groups according to their DI values, as low-ranked (DI < 0.33; n = 13), medium-ranked (DI = 0.33 – 0.66; n = 13), and high-ranked (DI > 0.66; n = 13) rams [10,16,18]. These ranking groups were used to determine the effects of social rank on live weight, certain body measurements, testosterone levels, some hematological stress response variables, and various behavioral characteristics of the rams.

2.3. Body measurements
One week prior to the beginning of the food competition tests, the rams were weighed and the following morphological characteristics were measured: wither height, rump height, body length, chest depth, chest width, chest circumference, rump width, cannon bone circumference, tail length, and tail width [19]. Moreover, the linear horn length (linear distance between horn root and horn endpoint) and curved horn length (distance from the horn root to the horn endpoint measured by following each horn curve using a rope) were measured.

2.4. Behavioral observations
To determine possible behavioral differences among the high-, medium- and low-ranked rams, direct observations were performed regarding certain individual, feeding, and abnormal behaviors. To distinguish the animals individually during the observations, a number was painted with spray paint on each ram, on the lumbar region of the animal. In order to avoid the suppressing effect of feeding behavior over the other behaviors, behavioral observations were initiated 1 h after feeding the rams. The observations were made between 09:30 and 11:30 AM by the same experienced researcher at a distance of 1 m away from the pens. A total of 4 observations were performed for each breed. The observer was present at the observation point 15 min prior to the observation time in order to allow the rams to get used to the observer.

Lying, standing, walking, feeding, drinking, and rumination behaviors were recorded using the time-sampling method [20–22]. These behaviors were recorded every 5 min over the whole observation period. Because

vocalization, butting other animals, and itching behaviors were expressed by the animals less often, these behaviors were recorded at the time they were observed. Descriptions of the behavioral characteristics investigated in the study are given in Table 1 [21–24].

Behavioral data recorded using the time-sampling method were arranged as percentage values, which gave the proportion of each behavioral activity within the total frequency of these behavioral activities.

2.5. Blood sampling and analysis

To determine possible differences in the plasma concentrations of testosterone, cortisol, and hematological parameters among the low-, medium-, and high-ranked rams, blood samples were collected from the rams at 2 different times (at the end of the first and fourth behavioral observation periods) throughout the study. The necks of the rams were shaved prior to beginning the study to facilitate and complete the venipuncture more quickly and easily. Blood samples were collected by the same trained person, and the sampling process was completed in 1 min. Specific attention was paid to avoid excessively stressing the animals.

At each sampling time, 2 blood samples (EDTA and heparinized) were collected from each ram. The HCT and hemoglobin HGB levels, and total number of RBCs and WBCs were determined in the EDTA samples using an automated hematology analyzer. Blood smears prepared from the EDTA samples were stained with May-Grünwald Giemsa stain, and on each smear, a total of 100 leukocytes were classified under light microscopy. The N:L ratio was determined by dividing the number of neutrophils by the number of lymphocytes.

The heparinized samples were centrifuged at 3500 rpm for 15 min within 1 h of collection, and the obtained plasma samples were stored at –80 °C until further analysis. Plasma concentrations of cortisol (DiaMetra, Segrate (MI),

Italy; Reference number: DKO001; Lot number: 3100) and testosterone (DiaMetra; Reference number: DKO015; Lot number: 3076) were determined using commercial ELISA direct immunoenzymatic kits.

2.6. Statistical analysis

Normal distribution of the data was checked using the Shapiro-Wilk test. Data for the live weight, body measurements, and testosterone concentration were analyzed using general linear model (GLM) procedures. The statistical model for analysis of these characteristics included the fixed effects of the rank group (low, medium, and high), breed (Hemsin, Chios, and Karakul) and rank group × breed interaction.

Repeated measurements of ANOVA were used for analysis of lying, standing and feeding behaviors and hematological data. Statistical model included rank group, breed and rank group × breed interaction as fixed effects, sampling/observation time was fitted as within-subject factors. In the GLM and repeated measurements of the ANOVA statistics, the Tukey test, in SPSS 13.0 (IBM Corp., Armonk, NY, USA), was used as a post hoc test.

The walking, drinking, rumination, vocalization, butting other animals, and itching behavior data did not fit in the normal distribution. Therefore, the Kruskal-Wallis test was used to compare these data for the low-, medium- and high-ranked rams. In these cases, the percentage or frequency of these behaviors over all of the observation periods were evaluated. Differences were considered significant at $P \leq 0.05$. SPSS 13.0 was used for the statistical analysis [25].

3. Results

According to their DI values, the rams were classified as low-ranked (DI < 0.33; n = 13), medium-ranked (DI: 0.33–0.66; n = 13), and high-ranked (DI > 0.66; n = 13) individuals. P-values determined for the breed × rank

Table 1. Description of behavioral traits investigated in the study.

Behavior	Description
Lying	Lying in a resting position without showing rumination or any other behavior
Standing	Standing in a resting position without showing rumination or any other behavior
Walking	Moving from one place to another
Feeding	Roughage or concentrate feed consumption
Drinking	Water consumption
Rumination	Ruminating in either a lying or standing position
Vocalization	A low pitched 'rumble' or 'mmm' bleat made with the mouth closed or a louder 'baa' vocalization made with the mouth open
Butting other animals	Butting the head of another ram or other body parts using the horns or head
Itching	Scratching of any part of the body by head, legs, feeders, or walls

group interaction regarding the investigated characteristics ranged between 0.203 and 0.844, except for the RBC. The P-value for the interaction regarding the RBC was 0.106. Since the effects of the breed × rank group interaction on the investigated parameters were not significant, it was assumed that the influence of the rank group was similar in the Hemsin, Karakul, and Chios rams. Therefore, only the rank group results were given for all of the parameters.

Mean values for the body weights, certain body measurements, and testosterone concentrations of the low-, medium- and high-ranked rams are presented in Table 2. The low-ranked rams had a lower body weight (P < 0.001), body length (P = 0.003), chest depth (P = 0.034), chest circumference (P = 0.003), cannon bone circumference (P = 0.036), and tail width (P = 0.006) when compared with those of medium- and high-ranked rams. Moreover, the chest width was greater in the high-ranked rams than in the low-ranked rams (P = 0.019). There were no significant differences among the rams of the different rank groups in terms of the withers and rump heights, rump width, tail length, linear and curved horn lengths, and testosterone concentration (P = 0.367).

Percentages of the lying down, standing, walking, feeding, drinking, and rumination behaviors are presented in Figure 1. The rank group had no significant influence on the percentages of these behaviors (P > 0.05). However, the

high-ranked rams tended to exhibit more rumination than the low- and medium-ranked rams (P = 0.066).

The mean frequency of vocalization, butting other animals, and itching behaviors exhibited during the 2-h observation period are shown in Figure 2. The effect of the rank group had no significant influence on the frequency of vocalization (P = 0.508), butting other animals (P = 0.225), and itching (P = 0.980) behaviors.

The effect of the social rank on certain hematological parameters and the cortisol level are presented in Figure 3. Mean HCT, HGB, and RBC values of the low-ranked rams were lower than those of the medium- and high-ranked rams (P = 0.038, P = 0.044, and P = 0.021, respectively). On the other hand, the WBC, N:L ratio, and cortisol levels were not different in the low-, medium-, and high-ranked rams (P = 0.670, P = 0.125, and P = 0.573, respectively).

## 4. Discussion
The low-, medium- and high-ranked rams had similar mean values in terms of the height measurements (i.e. withers and rump heights), horn size measurements (linear and curved horn lengths), and testosterone levels. However, certain chest measurements (i.e. chest depth and circumference), tail width, cannon bone circumference, body length, and body weight were higher in the medium- and high-ranked rams than in the low-ranked rams.

Table 2. Body weight, certain body measurements, and testosterone level of rams in different social rank groups (mean ± standard error).

Parameter	Rank group			P-value
	Low (n = 13)	Medium (n = 13)	High (n = 13)	
Body weight (kg)	56.18[b] ± 1.60	67.39[a] ± 1.72	67.63[a] ± 1.60	<0.001
Withers height (cm)	66.13 ± 0.95	68.85 ± 1.02	68.06 ± 0.95	0.144
Rump height (cm)	66.82 ± 0.88	68.97 ± 0.95	68.00 ± 0.88	0.263
Body length (cm)	79.74[b] ± 1.01	85.33[a] ± 1.09	82.97[a] ± 1.01	0.003
Chest depth (cm)	36.95[b] ± 0.42	38.13[a] ± 0.45	38.52[a] ± 0.42	0.034
Chest width (cm)	25.72[b] ± 0.59	26.85[ab] ± 0.64	28.24[a] ± 0.59	0.019
Chest circumference (cm)	97.35[b] ± 1.35	103.66[a] ± 1.46	103.62[a] ± 1.35	0.003
Rump width (cm)	19.04 ± 0.49	19.29 ± 0.53	19.91 ± 0.49	0.441
Cannon bone circumference (cm)	8.65[b] ± 0.13	9.11[a] ± 0.14	9.09[a] ± 0.13	0.036
Tail length (cm)	39.94 ± 0.98	41.15 ± 1.06	41.63 ± 0.98	0.467
Tail width (cm)	16.91[b] ± 0.92	20.54[a] ± 0.99	21.21[a] ± 0.92	0.006
Linear horn length (cm)	23.27 ± 1.10	23.56 ± 1.18	26.37 ± 1.10	0.108
Curved horn length (cm)	48.21 ± 1.77	49.17 ± 1.91	53.39 ± 1.77	0.109
Testosterone (pg/mL)	7.22 ± 6.71	8.69 ± 7.21	19.80 ± 6.71	0.367

[a, b, c]: Means in the same row with different letters differed significantly (P ≤ 0.05).
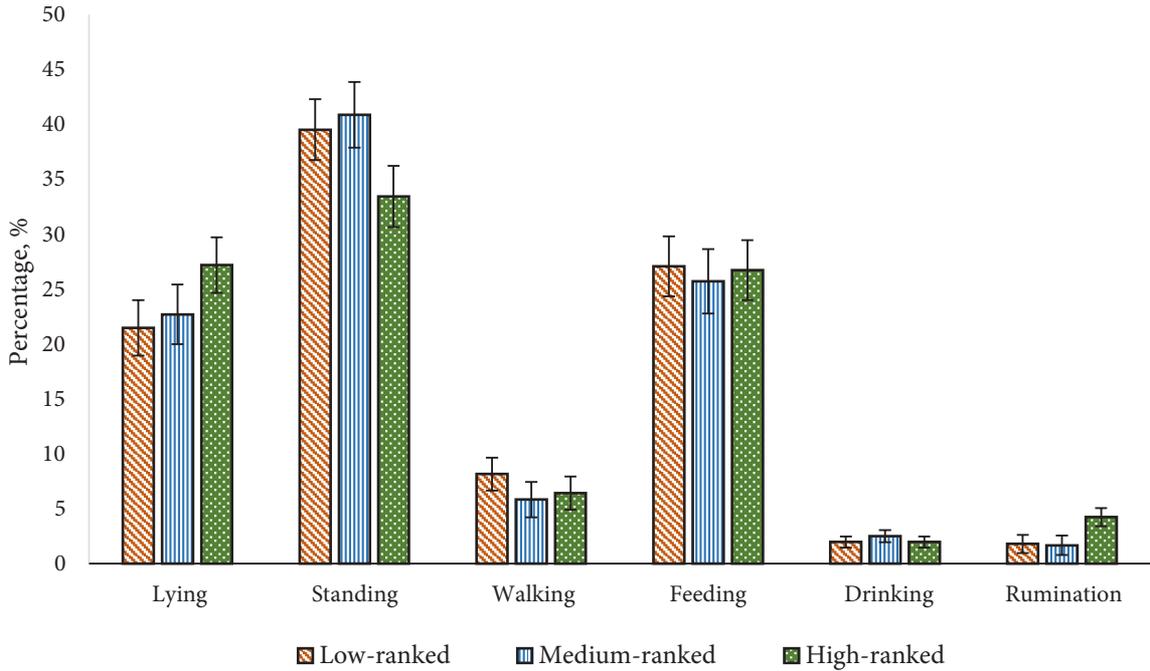
Figure 1. Percentages of the individual and feeding behaviors of rams in different social rank groups.
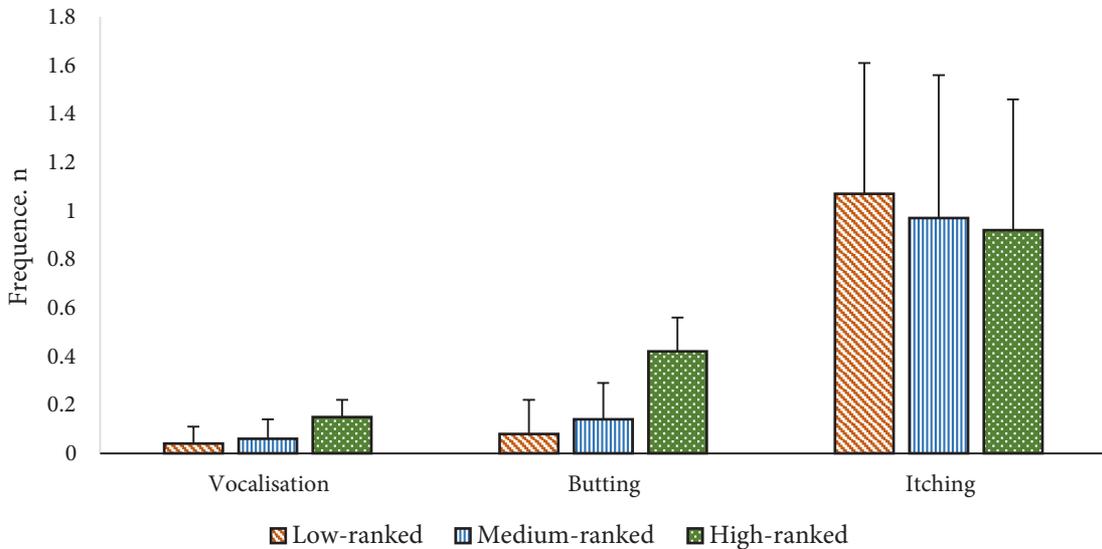
Figure 2. Frequency# of vocalization, butting other animals, and itching behaviors of rams in the different social rank groups. #: Frequency of the related behaviors over a 2-h observation period.

Supporting the current results, Maksimović et al. [26] determined higher body mass and chest circumference in dominant lambs at 12 and 18 months of age when compared to submissive individuals. In their study, there was no statistically significant difference between the dominant and submissive lambs in terms of other body measurements, such as the withers height, rump height, body length, chest width, and chest depth. Maksimović et al. [26] concluded that body mass was one of the main

determinants of social rank in rams. Ungerfeld and González-Pensado [27] observed greater and earlier growth in high-ranked rams than in low-ranked individuals and attributed this result to the high-ranked animals having greater access to food. The authors also noted that one of the reasons for the lower body weight in the low-ranked rams might have been due to their chronic stress, which causes an unfavorable physiological status in these rams when compared with high-ranked individuals. Pelletier
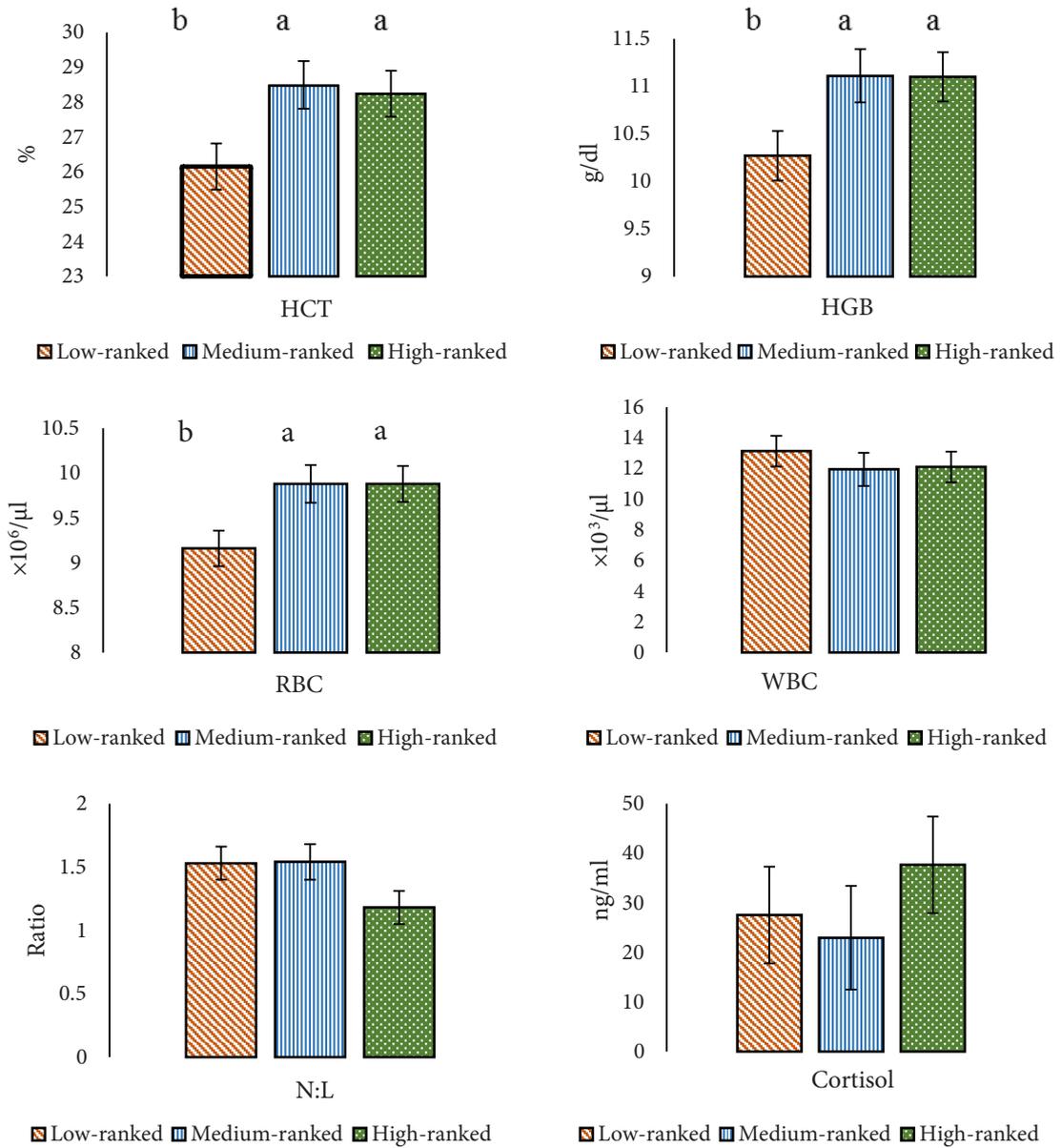
Figure 3. Certain hematological parameters and cortisol levels of rams in the different social rank groups. [a, b]: Means with different letters differed significantly (P ≤ 0.05).

and Festa-Bianchet [4] investigated the determinants of social rank in bighorn rams (*Ovis canadensis*) and found that body mass explained 68% and 37% of the variance in rank for rams between 2 and 5 years of age and those 6 years and older, respectively. On the other hand, Ungerfeld and Lacuesta [15] reported that high-ranked rams had higher body weights than low-ranked individuals, when they were 1.5 years old; however, at 2.5 years of age, there was only a tendency to be heavier for high-ranked rams.

The differences regarding testosterone concentration among the low-, medium- and high-ranked rams were not significant (P = 0.367). Similar to the current results, in a

study conducted with Corriedale × Milchscaf cross-breed rams [15], the testosterone concentrations of high- and low-ranked rams were reported to be similar. Ungerfeld and González-Pensado [27] also reported no significant influence of social rank on the serum testosterone concentration in lambs. On the other hand, Pelletier et al. [28] found a significant correlation of fecal testosterone levels with social rank in bighorn rams (*Ovis canadensis*). In their study, the correlation between the fecal testosterone and age was also significant, and it was concluded that when age was taken into account, the relationship between the fecal testosterone level and social rank was not significant.

Behavior is the interaction of an animal with its environment, that is, the response of the animal to different internal and external stimuli. Animals express their inner states through their behaviors [29]. Therefore, observing animal behavior is an indispensable element of protocols for assessing animal welfare in farm animals [30]. Furthermore, observing changes in behaviors provides important clues about handling and management for farmers, as behaviors contribute to our understanding of how well animals adapt to the conditions in which they live. Understanding the behaviors of farm animals will facilitate the management of these animals by humans, as well as reduce stress and increase both safety of the handler and animal welfare [31].

The low-, medium- and high- ranked rams had similar mean percentages in terms of the time they spent lying down (P = 0.259), standing (P = 0.161), walking (P = 0.546), feeding (P = 0.940), and drinking (P = 0.718). Moreover, the time spent for rumination tended to be higher in high-ranked rams than in the rams of the other rank groups (P = 0.066). Ketelaar-de Lauwere et al. [12] also reported that the time spent lying down, standing, and feeding were not influenced by social dominance in cows. However, in their study, low-ranked cows spent more time waiting in front of the feeding gate and automatic milking system. Moreover, low-ranked cows adapted their timing of visits to the automatic milking system by entering more often at the early hours when the high-ranked cows visited the system less frequently.

In group-living animals, the establishment of dominance hierarchy has great importance during competition for limited feeding sites, bedding sites, or mates, because the priority of individuals for accessing scarce resources is mostly determined by social rank [7]. In a stable social environment, aggressiveness in a group is usually reduced because the individuals have learned the probability of winning in a competition. This is also beneficial for reducing the cost of energy and decreasing the risk of injury caused by fighting [7,32]. In the present study, the rams were kept in the pens built for their breeds for 8 weeks before the study began, which may have been an adequate period of time for the establishment of a dominance hierarchy. Therefore, the lack of significant differences among the low-, medium- and high-ranked rams in terms of the frequency of vocalization and butting other animals might have resulted from the previously established dominance hierarchy in these groups. Moreover, the space allowance and feeding sites supplied to rams were within the ranges of values recommended by the Royal Society for the Prevention of Cruelty to Animals[1] for sheep welfare. Hence, one of the possible reasons for

the lack of differences among the rank groups in terms of aggressive behaviors may have been that the resources provided to the rams in the pens were sufficient. Barroso et al. [10] found that high-ranked goats had priority access to food both in feeding at the stall and the pasture. However, the authors determined that this priority was much more evident in the stall due to limited manger space, and they recommended increasing the number of mangers in the stall to prevent an increase in aggression during the food supplementation.

Farm animals are exposed to various physical and psychological stressors related to handling and management over their lifetime. These stressors disturb homeostasis, and consequently, an adaptive stress response, including certain endocrine, biochemical, hematological, and behavioral changes, is triggered to restore the balance. Activation of the hypothalamic-pituitary-adrenal (HPA) axis due to stressors leads to the secretion and release of glucocorticoids from the adrenal cortex, and catecholamines from the adrenal medulla. These hormones evoke a number of biochemical changes, and help the animal to adapt to stress by altering the cardiovascular, energy producing, and immune systems [33]. Therefore, alterations in some physiological parameters, such as heart rate, blood pressure, RBC and WBC counts, HCT value, HGB level, N:L ratio, plasma adrenocorticotropic hormone levels, cortisol, adrenaline, noradrenaline, glucose etc., are the most commonly used parameters to evaluate the stress level of an animal [34–37].

In the current study, the HCT value (P = 0.038), HGB level (P = 0.044), and RBC count (P = 0.021) of the medium- and high-ranked rams were higher than those of the low-ranked rams (Figure 3). Increases in HCT, HGB, and RBC are generally associated with either dehydration or splenic contraction induced by sympathetic stimulation as an initial response to stress. It is well known that the release of an increased amount of catecholamines, due to stimulation of the sympathico adrenal system, leads to the contraction of the spleen and the mobilization of stored erythrocytes into circulation [34,35]. However, the values determined in the current study regarding these parameters were within the normal ranges reported for sheep in the literature [36]. These results indicated that rams in all 3 dominance groups did not experience stress levels high enough to cause a significant increase in the HCT value, erythrocyte count, and HGB level. Furthermore, results regarding the N:L ratio and plasma cortisol levels also supported this finding.

Social interactions may significantly affect the HPA axis of animals living in groups [38]. However, there is

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[1] The Royal Society for the Prevention of Cruelty to Animals (RSPCA) (2013). Welfare standards for sheep [online]. Website https://www.berspcaassured. org.uk/media/1081/rspca-welfare-standards-sheep-jan2013.pdf [accessed 22 March 2020].

no consensus as to whether the dominant or subordinate animals are physiologically more stressed [8]. In general, it has been reported that during periods of changes in hierarchical order in a group, dominant individuals are exposed to more physical and psychological stress than the subordinate ones and therefore, the dominant individuals exhibit more physiological stress responses [9]. Bartoš et al. [9] found that during periods of increased social struggle, due to the introduction of new fallow deer to the herd, cortisol levels of the dominant individuals were higher than those of the subordinate ones. Creel et al. [39] reported that dominant individuals may exhibit higher stress responses in species where dominant individuals have to struggle constantly to maintain their position (e.g., dogs). On the other hand, it has also been reported that subordinate animals exhibited more physiological stress responses after a dominance hierarchy was established in the group [9]. Abbott et al. [40] concluded that the cortisol levels of subordinate individuals were higher because these animals were exposed to more stress than the dominant animals. Solano et al. [6] explained the lower cortisol levels that were determined in low-ranked Zebu cows exposed to stressors related to repeated handling and blood sampling, by the faster habituation of low-ranked animals to the repeated handling procedures than their medium- and high-ranked counterparts. The above mentioned statements indicated that there may also have been differences between the species as to whether the animals that experienced more stress were the dominant or recessive ones. In the current study, the differences among the rank groups in terms of the cortisol level and N:L ratio, which were used as the potential indices of stress in the animals, were not significant. Contrary to the expected results, the lack of a significant increase in the cortisol levels of subordinate rams of the Hemsin, Chios, and Karakul groups, in which a dominance hierarchy was established, might have been due to the fact that the space allowance was appropriate, and a sufficient amount of feed and clean water were provided to the rams throughout the experimental period, and therefore, the rams did not need to struggle with each other for resources. On the other hand, there are no reports in the literature comparing the stress levels, behaviors, and well-being of dominant and subordinate rams in groups where a dominance hierarchy was established. Unlike the declarations reported for goats, cattle, and deer [6,9,24], the lack of a significant difference in terms of the indicated characteristics among dominance groups in the current study might have been a normal behavior pattern due to the social structure of the ram groups. Therefore, for sheep species, there is a need for further research investigating the behaviors and welfare levels of individuals in various dominance groups when the resources are sufficient or limited for the groups in which a dominance hierarchy was established.

Under the conditions of the current study, there were no differences among the social rank groups in terms of the investigated behavioral characteristics, cortisol concentration, and N:L ratio. Furthermore, the effects of the social rank on the hematological parameters and behaviors investigated in the current study were not dependent on the breed.

As a conclusion, when adequate space allowance and feeding opportunities are provided to ram groups that have an established dominance hierarchy, the welfare of the low-ranked individuals may not be adversely affected. On the other hand, further research is needed to clarify the behaviors and welfare levels of individuals in various dominance groups under different handling and management conditions, such as sufficient or limited resources.

Acknowledgments

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. De Vries H. Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. Animal Behaviour 1998; 55 (4): 827-843. doi: 10.1006/anbe.1997.0708

2. Drews C. The concept and definition of dominance in animal behaviour. Behaviour 1993; 125: 283-313. doi: 10.1163/156853993X00290

3. Tölü C, Savaş T. Social rank and agonistic behaviour in livestock. Hayvansal Üretim 2006; 47 (2): 26-31 (in Turkish with an abstract in English).

4. Pelletier R, Festa-Bianchet M. Sexual selection and social rank in bighorn rams. Animal Behaviour 2006; 71 (3): 649-655. doi: 10.1016/j.anbehav.2005.07.008

5. Taillon J, Côté SD. The role of previous social encounters and body mass in determining social rank: an experiment with white-tailed deer. Animal Behaviour 2006; 72 (5): 1103-1110. doi: 10.1016/j.anbehav.2006.03.016

6. Solano J, Galindo F, Orihuela A, Galina CS. The effect of social rank on the physiology response during repeated stressful handling in Zebu cattle (Bos indicus). Physiology & Behavior 2004; 82 (4): 679-683. doi: 10.16/j.physbeh.2004.06.005

7. Taillon J, Côté SD. Social rank and winter forage quality affect aggressiveness in white-tailed deer fawns. Animal Behaviour 2007; 74 (2): 265-275. doi: 10.1016/j.anbehav.2006.11.018

8. Sapolski R M. The influence of social hierarchy on primate health. Science 2005; 308 (5722): 648-652. doi: 10.1126/science.1106477

9. Bartoš L, Schams D, Bubenik GA, Kotrba R, Tománek M. Relationship between rank and plasma testosterone and cortisol in red deer males (Cervus elaphus). Physiology & Behavior 2010; 101: 628-634. doi: 10.1016/j.physbeh.2010.09.011

10. Barroso FG, Alados CL, Boza J. Social hierarchy in the domestic goat: effect on food habits and production. Applied Animal Behaviour Science 2000; 69 (1): 35-53. doi: 10.1016/S0168-1591(00)00113-1

11. Hasegawa N, Nishiwaki A, Sugawara K, Ito I. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. Applied Animal Behaviour Science 1997; 51 (1-2): 15-27. doi: 10.1016/S0168-1591(96)01082-9

12. Ketelaar-de Lauwere CC, Devir S, Metz JHM. The influence of social hierarchy on the time budget of cows and their visits to an automatic milking system. Applied Animal Behaviour Science 1996; 49 (2): 199-211. doi: 10.1016/0168-1591(96)01030-1

13. Akçapınar H. Koyun Yetiştiriciliği. Medisan Yayın Serisi No: 8. Ankara, Turkey: Medisan Yayınevi; 1994. pp. 1-4.

14. Demir H. Koyun ve Keçi Yetiştiriciliği. İstanbul, Turkey: Teknik Yayınları; 1997.

15. Ungerfeld R, Lacuesta L. Social rank during pre-pubertal development and reproductive performance of adult rams. Animal Reproduction Science 2010; 121 (1-2): 101-105. doi: 10.1016/j.anireprosci.2010.05.007

16. Alvarez L, Martin GB, Galindo F, Zarco LA. Social dominance of female goats affects their response to the male effect. Applied Animal Behaviour Science 2003; 84 (2): 119-126. doi: 10.1016/j.applanim.2003.08.003

17. Langbein J, Puppe B. Analysing dominance relationships by sociometric methods-a plea for a more standardised and precise approach in farm animals. Applied Animal Behaviour Science 2004; 87 (3-4): 293-315. doi: 10.1016/j.applanim.2004.01.007

18. Ungerfeld R, González-Pensado S, Dago AL, Vilariño M, Menchaca A. Social dominance of female dairy goats and response to oestrous synchronisation and superovulatory treatments. Applied Animal Behaviour Science 2007; 105 (1-3): 115-121. doi: 10.1016/j.applanim.2006.06.008

19. Alizadehasl M, Ünal N. Some morphological traits of Kilis, Norduz and Honamlı indigenous goat breeds. Journal of Lalahan Livestock Research Institute 2011; 51 (2): 81-92 (in Turkish with an abstract in English).

20. Bogner H. Verhaltensbeobachtungen, versuchsanlage und auswertungen. In: Bogner H, Grauvogl A (editors). Verhalten landwirtschaftlicher Nutztiere. Stuttgart, Germany: Eugen Ulmer; 1984. pp. 61-74.

21. Tölü C, Göktürk S, Savaş T. Effects of weaning and spatial enrichment on behavior of Turkish Saanen Goat kids. Asian-Australasian Journal of Animal Sciences 2016; 29 (6): 879-886. doi: 10.5713/ajas.15.0597

22. Ugur F, Savas T, Dosay M, Karabayır A, Atasoglu C. Growth and behavioral traits of Turkish Saanen kids weaned at 45 and 60 days. Small Ruminant Research 2004; 52 (1-2): 179-184. doi: 10.1016/S0921-4488(03)00253-0

23. Ekiz B, Ergül Ekiz E, Yalçıntan H, Koçak Ö, Yılmaz A et al. The effect of transport stress on certain welfare parameters and behaviours in Red Karaman, İmroz, Sakız and Karakul rams. Journal of the Faculty of Veterinary Medicine İstanbul University 2012; 38: 15-28.

24. Tölü C, Savaş T. A brief report on intra-species aggressive biting in a goat herd. Applied Animal Behaviour Science 2007; 102 (1-2): 124-129. doi: 10.1016/j.applanim.2006.03.002

25. SPSS (1999). Statistical package for the social sciences, Release 13.0. Chicago, IL, SA: SPSS Inc.

26. Maksimović N, Žujović M, Hristov S, Petrović MP, Stanković B et al. Association between the social rank, body mass, testicular circumference and linear body measures of rams. Biotechnology in Animal Husbandry 2012; 28 (2): 253-261. doi: 10.2298/BAH1202253M

27. Ungerfeld R, González-Pensado SP. Social rank affects reproductive development in male lambs. Animal Reproduction Science 2008; 109 (1-4): 161-171. doi: 10.1016/j.anireprosci.2007.12.006

28. Pelletier F, Bauman J, Festa-Bianchet M. Fecal testosterone in bighorn sheep (Ovis canadensis): behavioural and endocrine correlates. Canadian Journal of Zoology 2003; 81 (10): 1678-1684. doi: 10.1139/Z03-156

29. Matthews SG, Miller AL, Plötz T, Kyriazakis I. Automated tracking to measure behavioural changes in pigs for health and welfare monitoring. Scientific Reports 2017; 7: 17582. doi: 10.1038/s41598-017-17451-6

30. Battini M, Stilwell G, Vieira A, Barbieri S, Canali E et al. On-farm welfare assessment protocol for adult dairy goats in intensive production systems. Animals 2015; 5: 934-950. doi: 10.3390/ani5040393

31. Grandin T. Behavioral principles of livestock handling. The Proffesional Animal Scientist 1989; 5 (2): 1-11. doi: 10.15232/S1080-7446(15)32304-4

32. Poisbleau M, Jenouvrier S, Fritz H. Assessing the reliability of dominance scores for assigning individual ranks in a hierarchy. Animal Behaviour 2006; 72 (4): 835-842. doi: 10.1016/j.anbehav.2006.01.024

33. Squires EJ. Applied Animal Endocrinology. Oxon, UK: CABI Publishing; 2003.

34. Hall SJG, Bradshaw RH. Welfare aspects of the transport by road of sheep and pigs. Journal of Applied Animal Welfare Science 1998; 1 (3): 235-254. doi: 10.1207/s15327604jaws0103_4

35. Liotta L, Nanni Costa L, Chiofalo B, Ravarotto L, Chiofalo V. Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. Italian Journal of Animal Science 2007; 6: 375-384. doi: 10.4081/ijas.2007.375

36. Jones ML, Allison RW. Evaluation of the ruminant complete blood cell count. The Veterinary Clinics of North America: Food Animal Practice 2007; 23 (3): 377-402. doi: 10.1016/j.cvfa.2007.07.002

37. Czech A, Kiesz M, Kiesz A, Próchniak T, Różański P et al. Influence of type of use, age and gender on haematological and biochemical blood parameters of Małopolski horses. Annals of Animal Science 2019; 19 (1): 85-96. doi: 10.2478/aoas-2018-0031

38. De Vries AC, Glasper ER, Detillion CE. Social modulation of stress responses. Physiology & Behavior 2003; 79 (3): 399-407. doi: 10.1016/S0031-9384(03)00152-5

39. Creel S, Creel NM, Monfort SL. Social stress and dominance. Nature 1996; 379: 212. doi: 10.1038/379212a0

40. Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP et al. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior 2003; 43 (1): 67-82. doi: 10.1016/S0018-506X(02)00037-5