Horn presence determines social rank in Dorper rams

La presencia de cuernos determina el rango social de carneros Dorper

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Scientific note

Received: February 07, 2024
Accepted: May 08, 2024

ABSTRACT. The aim was to determine if the presence of horns on rams defines whether they are high-ranking or low-ranking. Dorper rams (n = 20) were evaluated to determine their social rank (SR), either high (HSR) or low (LSR), under intensive management conditions. Upon SR classification, the morphometric variables horns presence (HP) or absence (HA) and distance between them, the height at the withers, body length, thoracic perimeter, and scrotal circumference, male odor, body weight, and body condition score were registered. Afterward, semen evaluations were performed, recording the ejaculated volume, sperm concentration, and mass motility. There were no differences ($p > 0.05$) for the morphometric variables between the groups. However, HP as well as semen quality were higher ($p < 0.05$) in HSR males. The presence of horns in the rams determined their social rank and the dominant males showed better semen quality than the subordinate males.
Keywords: Social hierarchy, social dominance, sheep, sexual performance, reproduction.

RESUMEN. El objetivo fue determinar si la presencia de cuernos en carneros define si son de jerarquía alta o baja. Se evaluaron 20 carneros Dorper para determinar su rango social (SR), alto (HSR) o bajo (LSR) en condiciones de manejo intensivo. Al momento de la clasificación del SR, se registraron la presencia (HP) o ausencia (HA) de cuernos y distancia entre los mismos, la altura a la cruz, largo del cuerpo, perímetro torácico, circunferencia escrotal, olor, peso corporal y condición corporal. Posteriormente, se realizó la evaluación seminal, registrando el volumen del eyaculado, la concentración espermática y la motilidad masal. No se presentaron diferencias ($p > 0.05$) para las variables morfométricas entre los grupos. Sin embargo, la HP así como la calidad seminal, fueron mayores ($p < 0.05$) en los machos HSR. La presencia de cuernos en los carneros determinó su rango social y los machos dominantes mostraron una mejor calidad seminal que los machos subordinados.
Palabras clave: Jerarquía social, dominancia social, ovinos, desempeño sexual, reproducción.

How to cite: Calderón-Leyva G, Sifuentes-Lamont PI, Contreras-Villarreal V, Ángel-Garcia O, Moreno-Ávalos S, Veliz-Deras FG, Alvarado-Espino AS (2024) Horn presence determines social rank in Dorper rams. Ecosistemas y Recursos Agropecuarios 11(2): e4004. DOI: 10.19136/era.a11n2.4004.

INTRODUCTION

A social hierarchy is defined as individuals centred on reciprocal dominance-subordination relationships often determined by a mutual evaluation, ranging from simple recognition to ritualized displays or serious fights (Barroso *et al*. 2000). Social rank is positively associated with differential access to available resources, mainly related to the feeding behaviour of group members (Sifuentes-Lamónt *et al.* 2022). Therefore, dominant males are heavier than subordinate males (Aguirre *et al.* 2007), which implies better productive and reproductive outcomes for high social rank (HSR) than low social rank (LSR) animals (Sifuentes-Lamónt *et al.* 2022). For example, HSR females have a a higher percentage and duration of estrus, with a higher ovulation rate and corpus luteum size than LSR females (Zuñiga-Garcia *et al.* 2020). Similar results were found in HSR females when evaluating udder morphometric components in dairy goats (Castillo-Zuñiga *et al.* 2022).

In males, HSR rams showed more appetitive and consummatory sexual behavior than LSR rams (González-Tavizón *et al.* 2022). This could be due to the involvement of seasonal reproduction and its endocrine mechanism, which can be influenced by behavioural stimulation provide by the social relationships an individual establishes with conspecifics (Aguirre *et al.* 2007). Social and sexual relationships between females and males are related; after exposure to sexually active males, high-ranking anestrous goats show more rapid neuroendocrine activation, being the first to ovulate and become pregnant (Alvarez *et al.* 2003).

Hierarchical structure is influenced by several factors, such as the presence of horns, live weight, and age of the animal (Barroso *et al.* 2000, Tölü *et al.* 2007). Morphometrics are among the factors that determine social rank (Sifuentes-Lamónt *et al.* 2022). Horns in ruminants play a role in social behaviour and protection (Simon *et al.* 2022). There is a relationship between the presence of horns and morphometric characteristics (Sim and Coltman 2019). The natural absence of horns in these species, also known as polledness, is surprisingly heterogeneous in nature, despite being a Mendelian trait (Simon *et al.* 2022). This study hypothesizes that the horns on rams determine whether they are high or low ranking. The study aims to determine whether the presence of horns in rams determines whether they are of high or low rank and their effect on seminal characteristics.

MATERIALS AND METHODS

Location and animals

The study was carried out in northern Mexico (Comarca Lagunera, 25° 35´ NL, 103° 17´ WL, 1120 m). This region is characterized by semi-arid weather, with an average annual temperature of 25.3 °C, with lows of -3.0 °C (winter) and highs of 41.0 °C (summer). The photoperiod varies from 13 h 41 min on the summer solstice to 10 h 19 min on the winter solstice (INEGI 2023).

The experimental animals consisted of Dorper rams (n = 20; 3 ± 1 year), both with (n = 10) and without horns (n = 10), and Dorper ewes (n = 10; 3 ± 1 year); all sheep were managed under the same intensive management and environmental conditions. Each ram received 2.8 kg of a dietary mix (maize, stubble, mineral salts, molasses, and cotton hulls), 1.4 kg in the morning (10:00

h) and 1.4 kg in the afternoon (18:00 h), designed to cover their nutritional requirements (NRC 2007), and clean water was provided ad libitum. All animals were dewormed three weeks prior to experimental period. The animals in this study were handled in accordance with international (FASS 2010) and national (SADER 2001) guidelines for the ethical use, care, and welfare of animals in research. The chronology of the main activities performed during the experimental period is shown in Figure 1.

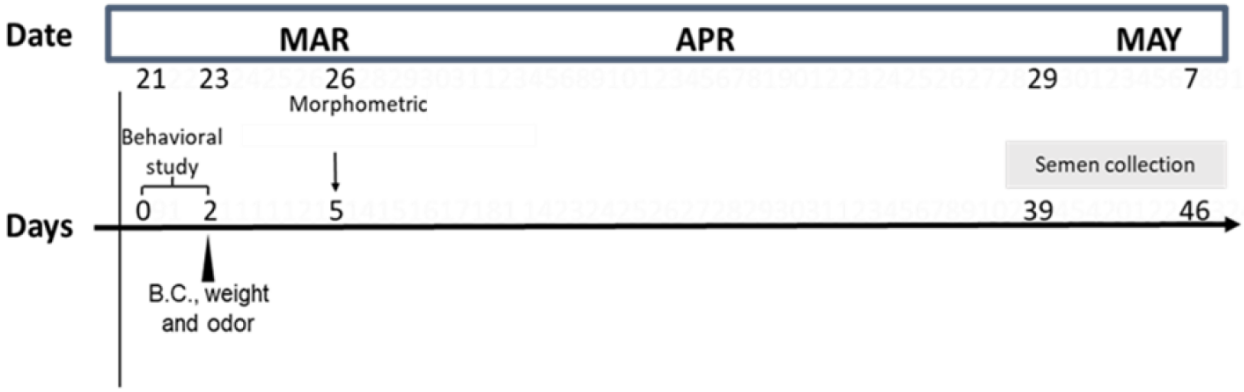

Figure 1. Timeline of the main activities completed in the experimental period. We conducted a behavioral study in all the experimental units (n = 20) to determine the Dorper males' social rank, either high or low, under intensive management conditions in northern Mexico (25° NL). In late March, the behavioral test was carried out in the morning. The rams' body condition score (BCS), body weight, odor, and morphometric measurements were also recorded. In late April and early May, semen collections were performed.

Behaviorual study

At the end of March, the behavioral study started (Figure 1). The main agonistic behavior between two rams with and without horns in the presence of one estrus female was registered as reported by Sifuentes-Lamont *et al.* (2022). Briefly, to determine the social rank of each male, rams were exposed to Dorper ewes (n=10) that were placed in individual pens (2.25 × 2.25 m). The behavioral test was carried out over 3 days. Each pair of males was observed simultaneously during 3 min trials when exposed to the estrus ewes (two males × one female × 3 min × pen). The agonistic social behaviors and the male-to-female sexual interactions were recorded by 10 people trained. At the end of the three minutes, one of the males was moved to the next female pen. In the following three minutes, the male that had remained in the pen during the previous test was moved to the next pen sequentially. Therefore, each one of the males had interaction with the other 20 males and with a different female (a total of 20 behavioral tests were performed on each male). This paired-ram arrangement allowed for competition between a male and the rest of the males under study. Considering the behavioural recording, the success rate (SI) was calculated for each male (Barroso *et al.* 2000). Using the SI obtained, the males were classified into two social ranks: rams with an SI from 0 to 0.5 were classified as of low social status, and rams with an SI greater than 0.5 and up to 1.0 were classified in the high social rank. The success index (SI) was obtained from each experimental unit using the following formula:

$$\mathrm{SI} = \frac{\text{number of won events}}{\text{number of won events} + \text{number of lost events}}$$

Male odor, body weight, and body condition

In late March, at the end of the behavioral test (Figure 1), male odor (MO, units), body weight (BW, kg) and body condition score (BC, units) were evaluated. The odor was scored (Gelez *et al.* 2004) on a scale of 1 to 4, where the odor shown by an ewe is considered 1, and 4 is a powerful odor of the ram. Live weight was determined using an electronic scale with a precision of 50 g and a capacity of 250 kg (Torrey 110v/220v, Digital Industrial Scale, Jalisco, Mexico). In addition, BC was measured by a qualified technician by palpating the transverse processes of the lumbar vertebrae, using a scale of 1 (emaciated) to 5 (obese) (Ghosh *et al.* 2019).

Morphometric measurements

The morphometric variables of the rams were determined three days after the behavioral study (Figure 1). The presence (HP) or absence of (HA) and the distance between them (DBH), the height at the withers (HEIG, cm), body length (LENG, cm), thoracic perimeter (PERI, cm), and scrotal circumference (SCRC, cm), were evaluated. A flexible plastic tape measure graduated in millimeters was used to measure morphometrics.

Semen analysis

In late April and May, the males were exposed to estrus females to collect semen using an artificial vagina at 38 °C (Figure 1). The artificial vagina was heated to 45 °C with hot water. Semen was collected in graduated tubes, which were immediately immersed in a 38 °C water bath and transported to the laboratory for analysis within 10 min of collection. Macroscopic and microscopic evaluations were then performed, recording the following semen response variables: ejaculated volume (mL), quantified directly in the conical collection tube graduated at 0.1 mL intervals; sperm concentration ($\times 10^6$/mL), by photometric analysis, using undiluted semen; and mass motility (%) was evaluated using an arbitrary scale of 0 to 5; where 0 = 0 % and 5 = 100% motile sperm. Sperm concentration was measured using the SDM 1 photometer calibrated for sheep semen (Minitube®, Tiefenbach, Germany). Mass motility was measured using an Olympus CX43 phase-contrast microscope (Minitube®, trinocular and heated stage, Tiefenbach, Germany).

Statistical analysis

Means were analyzed using the ANOVA procedure of the SAS (SAS Institute Inc, Cary, NC, USA, V9.1). As social rank was determined individually, each ram was considered an experimental unit. Least squares means and standard errors were calculated for each class of social rank status with abscence or presense of horns and used for multiple comparisons of means using Fisher's least significant difference with the LSMEANS option of SAS. Statistical differences between mean values were set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The success index recorded in all rams, with and without horns, to define the social hierarchy is shown in Table 1. The S.I. was higher in the HSR group (0.84 ± 0.05) than in the LSR group (0.24 ± 0.06) group ($p = 0.01$). The relationship between SR and the presence of horns is

shown in Table 2. Most horned males belong to the high rank (85.7%), whereas most hornless males belong to the low rank (69.2%). Male reproductive success is positively related to social rank, which is strongly associated with age, body mass, and horn presence (Barroso *et al*. 2000, Pelletier and Festa-Bianchet 2006, Tölü *et al*. 2007). In our study, the high social hierarchy Dorper rams were those that had horn presence. This results agree with Ekiz *et al*. (2024) who observed that the horn length explained 79.5% of the variation in the dominance index in Karakul rams. The length of horns and their early development is a reliable indicator of reproductive success (Willisch *et al*., 2015). Males with the highest SI are more aggressive and received less aggression, so there is a positive relationship between the individual SI and the rate of aggression and the higher social rank (Barroso *et al*. 2000). Comparative studies demonstrated consistent associations between the size of the female groups and the development of secondary sexual characters in males, as the size and elaboration of male weaponry, such as male horns (Clutton-Brock and Huchard 2013). The presence of horns encourages animals to fight, so horned animals belong in the top ranks of any herd (Tölü *et al*. 2007).

Table 1. Least square means ± standard error for success index, either low (n = 13) or high (n = 7) social rank in Dorper rams (n = 20) in northern Mexico (25° N).

		Events %		
Rank	**Animals %**	**Won**	**Lost**	**S.I.**
Low	65 [a]	92/380 (24.2%) [a]	288/380 (75.8%) [a]	0.24 ± 0.06 [a]
High	35 [b]	320/380 (84.2%) [b]	60/380 (15.8%) [b]	0.84 ± 0.05 [b]

[a,b] between files indicate a significant difference (P < 0.05).

Table 2. Least square means for social rank, either low (n = 13) or high (n = 7), and horns presence (HP) or absence (HA) in Dorper rams (n = 20) in northern Mexico (25° N).

Low rank (n = 13)		**High rank (n = 7)**	
HP	**HA**	**HP**	**HA**
4 (30.8 %) [a]	9 (69.2 %) [b]	6 (85.7 %) [c]	1 (14.3 %) [d]

[a,b] between columns indicate a significant difference (P < 0.05).

The morphometric variables BW, BC, male odour, scrotal circumference, height at the withers, body length, thoracic perimeter, presence of horns, the distance between horns, and age evaluated in male Dorper sheep are shown in Table 3. While the HSR group had the highest percentage of HP (p < 0.05), no differences were found for the other variables. Regarding the interaction of social rank with the presence of horns (Table 4), the hornless LSR males had a lower weight than the hornless LSR and the HSR. Horned HSR males exhibited greater male odor than hornless HSR and LSR. In both SRs, the presence of horns was determinant for height at the withers and body length. No significant differences were found for BC, SCRC, PERI, and AGE (P > 0.05).

Table 3. Least square means ± standard error for body weight (BW), body condition score (BC), male odor (MO), scrotal circumference (SCRC), height at the withers (HEIG), body length (LENG), thoracic perimeter (PERI), horns presence (HP), the distance between horns (DBH) and age, either low (n = 13) or high (n = 7) social rank in Dorper rams (n = 20).

Variables	Social Rank	
	LSR	HSR
BW (kg)	77.23 ± 3.91 [a]	78.71 ± 5.07 [a]
BC (u)	3.61 ± 0.15 [a]	3.64 ± 0.18 [a]
MO (u)	1.6 ± 0.28 [a]	2.0 ± 0.41 [a]
SCRC (cm)	28.69 ± 0.61 [a]	29.14 ± 0.7 [a]
HEIG (cm)	67.23 ± 1.01 [a]	68.0 ± 1.94 [a]
LENG (cm)	83.5 ± 2.02 [a]	84.29 ± 2.88 [a]
PERI (cm)	104 ± 2.34 [a]	104.5 ± 3.60 [a]
HP (%)	38.46 ± 0.28 [b]	71.42 ± 0.4 [a]
DBH (cm)	8.0 ± 0.52 [a]	7.57 ± 0.17 [a]
AGE (y)	3.31 ± 0.14 [a]	3.21 ± 0.26 [a]

[a,b] between columns indicate a significant difference ($P < 0.05$).

Table 4. Least square means ± standard error for the interaction of social rank (i.e., LSR and HSR) by horns presence (HP) or absence (HA) for body weight (BW), body condition score (BC), male odor (MO), scrotal circumference (SCRC), height at the withers (HEIG), body length (LENG), thoracic perimeter (PERI), and age, in Dorper rams (n = 20).

Variables	Low rank		High rank	
	HP	HA	HP	HA
BW (kg)	80.0 ± 3.31 [a]	74.25 ± 4.01 [b]	80.4 ± 3.36 [a]	79.5 ± 4.0 [a]
BC (u)	3.6 ± 0.15 [a]	3.56 ± 0.14 [a]	3.6 ± 0.2 [a]	3.5 ± 0.0 [a]
MO (u)	1.6 ± 0.3 [b]	1.6 ± 0.29 [b]	2.1 ± 0.49 [a]	1.75 ± 0.13 [b]
SCRC (cm)	29.2 ± 0.3 [a]	28.38 ± 0.76 [a]	29.2 ± 0.82 [a]	29.0 ± 0.53 [a]
HEIG (cm)	69.2 ± 1.08 [a]	66.0 ± 0.85 [b]	69.2 ± 2.18 [a]	65.0 ± 0.0 [b]
LENG (cm)	86.4 ± 1.2 [a]	81.69 ± 2.33 [b]	86.4 ± 3.1 [a]	80.0 ± 0.0 [b]
PERI (cm)	103 ± 1.46 [a]	104.0 ± 2.84 [a]	104 ± 4.1 [a]	105.8 ± 3.07 [a]
AGE (a)	3.6 ± 0.06 [a]	3.38 ± 0.06 [a]	3.1 ± 0.31 [a]	3.5 ± 0.0 [a]

[a,b] between columns indicate a significant difference ($P < 0.05$).

Male displays frequently emphasize male weaponry, including horns (Pelletier and Festa-Bianchet 2006). Competition leads to selection operation through individual differences in mating

success in males, and sexual selection considers the horns in males as a product of sexual selection (Clutton-Brock and Huchard 2013). The presence of horns is an essential factor in determining dominance and sometimes suppresses the effect of age (Tölü *et al*. 2007). Studies on social competition and its consequences in males show that competition between males for access to groups of individual females strengthens selection for traits that succeed in fights or attract potential mating partners (Pelletier and Festa-Bianchet 2006). It has been demonstrated that there are consistent relationships between the size of the groups of females and the development of secondary sexual characteristics in males, including body size and horns. Interestingly, unlike previous studies of our group (Zuñiga-Garcia *et al*. 2020, Castillo-Zuñiga *et al*. 2022), there were no differences in body weight between males of high or low social hierarchy.

Regarding semen quality, the HSR group exhibited the best indicators in terms of mount latency (seconds), ejaculate volume (mL), sperm concentration (×10^6/mL), and mass motility (%) (Table 5). In the interaction of social rank with semen quality, HSR males presented a lower mount latency than LSR males (Table 5). The presence of horns was decisive for ejaculate volume and mass motility. However, for sperm concentration, horned HSR males had higher values, hornless HSR males and horned LSR males had similar values, and hornless LSR males had the lowest values.

Table 5. Least square means ± standard error for the interaction of social rank (i.e., LSR and HSR) by horn presence (HP) or absence (HA) for mount latency (seconds), ejaculate volume (mL), sperm concentration (×10^6/mL), and mass motility (%), in Dorper rams (n = 20) in northern Mexico (25° N).

Variables	Low rank		High rank	
	HP	HA	HP	HA
Latency (s)	141.2 ± 16.36 ^{b}	156.3 ± 12.66 ^{b}	50.0 ± 21.51 ^{a}	7.5 ± 4.0 ^{a}
Volume (mL)	0.82 ± 0.21 ^{a}	0.31 ± 0.16 ^{b}	1.1 ± 0.31 ^{a}	0.35 ± 0.19 ^{b}
Concentration (×10^6/mL)	1635.8 ± 421.87 ^{b}	711.6 ± 369.17 ^{c}	3205.2 ± 704.58 ^{a}	1144.5 ± 611.76 ^{b}
Motility (%)	2.0 ± 0.52 ^{a}	0.88 ± 0.46 ^{b}	2.4 ± 0.91 ^{a}	1.0 ± 0.53 ^{b}

^{a,b} between columns indicate a significant difference (P < 0.05).

In our study, semen quality favored males from the HSR with the presence of horns. These results are similar to our previous study in that males with higher social rank depicted higher sexual behavior and semen quality than males from lower social status (Sifuentes-Lamont *et al.* 2022). On the other hand, a recent study by Mauleon *et al* (2023) in hair rams reported no differences in semen quality between dominant and subordinate rams during the breeding season, but without the competitive environment of the rams (ewes synchronized for oestrus). In rodents, more dominant males had higher weight testes, seminal vesicles, and coagulation glands than subordinate males (Kruczek and Stirna 2009). Although we were unable to quantify plasma testosterone, testosterone concentration is an excellent indicator of seminal production and quality. At the same time, spermatogenic activity is directly influenced by the level of testosterone released by the Leydig cells, the testicular endocrine component, modulating in turn the function of Sertoli cells, the gametogenic component of the testes (Kruczek 1997, Maurya *et al.* 2017).

The results of the present study show that horn presence determine the social rank in Dorper Rams. Dominant rams showed a higher semen volume, sperm concentration and sperm motility than subordinated males.

ACKNOWLEDGMENTS

The authors acknowledge Jaime Gilberto Quiroga Herrera for facilitating and taking care of the experimental animals used in the present study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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