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Original Research Articles

Ejaculate traits of ram lambs with different rectal temperatures under heat stress conditions

Características del eyaculado de corderos con diferente temperatura rectal bajo condiciones de estrés térmico

Características do ejaculado de cordeiros com diferentes temperaturas retais sob estresse térmico

Jorge-Humberto McCormick-Palacios[1]; Gustavo Ramírez-Valverde[2]; Canuto Muñoz-García[3]; Jorge-Alonso Maldonado-Jáquez[4]; Saúl Hernández-Aquino[1]; Rodrigo Flores-Garivay[1]; Reyna-Lucero Camacho-Morales[1]; Ernesto Avelar-Lozano[1]; Juan González-Maldonado[1] *

[1]*Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Ejido Nuevo León, 21705, Mexicali, México.*
[2]*Colegio de Postgraduados. Departamento de Estadística, Montecillo, 56230, Texcoco, México.*
[3]*Facultad de Medicina Veterinaria y Zootecnia No. 1, Universidad Autónoma de Guerrero, Ciudad Altamirano, 40660, Guerrero, México.*
[4]*Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Campo Experimental La Laguna, Matamoros, 27440, Coahuila, México.*

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***Corresponding author:** Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Ejido Nuevo León, 21705, Mexicali, México. E-mail: juan.gonzalez.maldonado@uabc.edu.mx

Abstract

Background: Heat stress reduces semen quality in rams. **Objective:** To evaluate the productive and reproductive responses of rams and ram lambs with different rectal temperatures under heat stress conditions. **Methods:** The animals (n = 12, Dorper × Katadhin × Pelibuey crossbreed, aged 235 to 730 days) were assigned to one of three experimental groups: ram lambs with low rectal temperature (RLLRT), ram lambs with high rectal temperature (RLHRT), and Rams. **Results:** Afternoon rectal temperatures were higher ($p \leq 0.05$) in the RLHRT group, and there was no difference ($p > 0.05$) between the Rams and RLHRT groups. Ejaculate volume and sperm concentration were higher ($p \leq 0.05$) in the Rams group than in the RLLRT and RLHRT groups. Mass motility and sperm concentration were lower ($p \leq 0.05$) in the RLLRT group than in the RLHRT and Rams groups. Average daily weight gain did not differ ($p > 0.05$) between the RLLRT and RLHRT groups. **Conclusion:** The selection of ram lambs with low rectal temperature under heat stress conditions did not provide any benefit in terms of average daily weight gain or ejaculate traits.

Keywords: *heat stress; motility; rams; resilience; ruminants; semen; sperm; testicle.*

Resumen

Antecedentes: El estrés calórico reduce la calidad del semen en carneros. **Objetivo:** Evaluar las respuestas productivas y reproductivas de carneros y corderos con diferente temperatura rectal bajo condiciones de estrés calórico. **Métodos:** Los animales (n = 12, cruza de Dorper × Katadhin × Pelibuey, de 235 a 730 días de edad) fueron asignados a uno de tres grupos experimentales: corderos con temperatura rectal baja (RLLRT), corderos con temperatura rectal alta (RLHRT), y Carneros. **Resultados:** Las temperaturas rectales fueron mayores ($p \leq 0.05$) en el grupo RLHRT que en el RLLRT; sin embargo, no se encontraron diferencias (p > 0.05) entre este y el grupo de Carneros. El volumen del eyaculado y la concentración espermática fueron mayores (p ≤ 0.05) en el grupo de Carneros que en los grupos RLLRT y RLHRT. La motilidad masal y la concentración espermática fueron menores (p ≤ 0.05) en el grupo RLLRT que en los grupos RLHRT y Carneros. La ganancia diaria de peso no fue diferente (p > 0.05) entre los grupos RLLRT y RLHRT. **Conclusión:** La selección de corderos con temperatura rectal baja en condiciones de estrés calórico no proporciona beneficios en su ganancia de peso ni en las características del eyaculado.

Palabras clave: *espermatozoide; estrés calórico; carnero; motilidad; resistencia; semen; testículo.*

Resumo

Antecedentes: O estresse térmico reduz a qualidade do sêmen em carneiros. **Objetivo:** Avaliar as respostas produtivas e reprodutivas de carneiros e cordeiros com diferentes temperaturas retais sob condições de estresse térmico. **Métodos:** Os animais (n = 12, cruzamento Dorper × Katadhin × Pelibuey, com idade entre 235 e 730 dias) foram distribuídos em um dos três grupos experimentais: cordeiros com baixa temperatura retal (RLLRT), cordeiros com alta temperatura retal (RLHRT), e Carneiros. **Resultados:** As temperaturas retais foram maiores (p ≤ 0,05) no grupo RLHRT do que no RLLRT, mas não foram encontradas diferenças (p > 0,05) entre este e o grupo Carneiros. O volume ejacula do e a concentração espermática foram maiores (p ≤ 0,05) no grupo Carneiros do que nos grupos RLLRT e RLHRT. A motilidade massal e a concentração espermática foram menores (p ≤ 0,05) no grupo RLLRT do que nos grupos RLHRT e Carneiros. O ganho de peso diário não foi diferente (p > 0,05) entre os grupos RLLRT e RLHRT. **Conclusão:** A seleção de cordeiros com baixa temperatura retal sob condições de estresse térmico não apresenta benefícios para o ganho de peso nem para as características da ejaculação.

Palavras-chave: *carneiro; esperma; estresse térmico; motilidade; resistência; sêmen; testículo.*

Introduction

Heat stress compromises the physiological functions and welfare of farm animals, leading to reduced reproductive and productive performance (Boni, 2019). This is a concern among animal production specialists, scientists, and world authorities because it compromises food security. Moreover, it is expected that by 2050, some animal production systems will no longer be viable in certain parts of the world due to heat stress (Thornton et al., 2021). To address this challenge, researchers emphasize the need to breed livestock with a high degree of resilience to heat stress conditions (Sejian et al., 2018). Unfortunately, selection of farm animals, such as rams, has traditionally been based on phenotypic characteristics, disregarding genetic, reproductive, and health traits (Maquivar et al., 2021).

Studies have revealed that some sheep genotypes are resilient to heat stress (McManus et al., 2020), opening a window of opportunity to select animals with natural adaptations for regions with a high incidence of solar radiation. In general, local breeds from tropical regions exhibit greater tolerance to heat stress than breeds from temperate regions (Pantoja et al., 2017), and genetic variability within breeds allows the selection of animals with robust tolerance to heat conditions (Menéndez-Buxadera et al., 2014). However, there is still abundant evidence of negative impacts of heat stress on the reproductive performance of sheep (van Wettere et al., 2021), highlighting the urgency of developing management and reproductive strategies to mitigate its effects on sheep production.

The selection of heat stress-tolerant sires is recommended to minimize the negative impacts of hot weather on the reproductive performance of farm animals (Morrell, 2020). This selection can be achieved through a genomic approach (Ramón et al., 2014) or by identifying local breed rams that perform better under heat conditions (Kahwage et al., 2018). Economic and technological limitations pose a challenge to implementing genomic selection in several regions of the world, and some local genotypes are not widely available. In addition, animals with resilience to heat stress have typically been identified by comparing different genotypes (Kahwage et al., 2017), disregarding intra-genotypic variability. Therefore, local genotypes and individual animals must be tested using traditional methodologies to identify those with natural resilience to heat stress. This study aimed to evaluate the productive and reproductive responses of ram lambs with different rectal temperatures under heat stress conditions.

Materials and Methods

Ethical statement and location

The experiment was conducted from July to November 2019 at the Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, México. The climate of the region is classified as a hot desert (Bwh); the highest and lowest temperatures recorded during the summer and winter seasons are 50 °C and -5 °C, respectively, and the average annual rainfall is 88 mm (García, 2004). The animals used were handled according to the guidelines of the Canadian Council of Animal Care (CCAC, 2009). In addition, the institutional Ethics Committee for Research Evaluation and Postgraduate Studies approved experimental procedures and animal handling (Reference number: 067/2024-2).

Animals

A trial was conducted to measure the rectal temperature of eight ram lambs and four rams for 13 consecutive days at the end of July, before feeding, in the mornings (7:00 h) and afternoons (17:00 h), using a standard digital thermometer (Neutek, MT-201C, Hangzhou Sejoy Electronics & Instruments Co. Ltd.; Accuracy: ± 0.1 °C). The tip of the thermometer was inserted 6 cm into the rectum and placed against the lateral rectal wall until the temperature reading was completed. Air temperature and relative humidity were recorded before each rectal temperature measurement to calculate the temperature-humidity index (THI) (Belhadj et al., 2019). THI values ≥ 72 were considered indicative of heat stress (Belhadj et al., 2019).

At the end of the trial, and considering that the highest daily rectal temperature is reached in the afternoon (Kahwage et al., 2018), the animals were assigned to one of three experimental groups according to age (ram lambs and rams) and average afternoon rectal temperature (low < 39.4 °C; and high ≥ 39.4 °C): ram lambs with low afternoon rectal temperature (RLLRT), ram lambs with high afternoon rectal temperature (RLHRT), and rams. The rams served as a reference group for reproductive variables.

The RLLRT animals (n = 4) were 235.50 ± 8.38 days old, had a live weight of 48.47 ± 6.74 kg, and a scrotal circumference of 32.0 ± 2.70 cm. The RLHRT animals (n = 4) were 236.0 ± 2.0 days old, had a live weight of 51.65 ± 6.27 kg, and a scrotal circumference of 31.25 ± 1.70 cm. The rams (n = 4) were 2 years old, had a live weight of 65.1 ± 8.04 kg, and a scrotal circumference of 32.12 ± 0.62 cm. All the experimental animals were Dorper × Katahdin × Pelibuey crossbreeds. The animals used were handled according to the guidelines of the Canadian Council of Animal Care (CCAC, 2009).

Experimental period

The animals in the experimental groups underwent semen collection using an artificial vagina and a non-estrous ewe twice a week (Thursday and Sunday) from August to November. Thirty semen samples were collected from each experimental unit. Rectal temperature, air temperature, and relative humidity were recorded in the mornings (7:00 h) and afternoons (17:00 h) on semen collection days to calculate THI. This index was used to classify the experimental period into two phases: heat stress (first 17 sampling days) and no heat stress (last 13 sampling days).

Animal housing and feeding

The RLLRT and RLHRT groups were housed in the same pen from weaning until the end of the experiment. The rams were kept in separate pens, but they had also been housed together for at least a year before the experiment began. The pens provided free access to shade and drinking water. Each animal was fed 2 kg/day of a ration containing 30% wheat straw, 48.5% ground wheat grain, 20.0% soybean meal, and 1.5% ground limestone (crude protein: 17.7%, ether extract: 2.62%, fiber: 14.4%, neutral detergent fiber: 22.14%, calcium: 2.0%).

Response variables

The response variables were morning and afternoon rectal temperatures recorded during the evaluation and experimental periods. Sperm volume, concentration, and mass motility were evaluated in semen samples following established methodologies (Maurya et al., 2016). Ram lambs were weighed weekly for 15 weeks to calculate average daily weight gain.

Statistical analysis

Rectal temperatures, ejaculate volume, sperm concentration, and mass motility were analyzed using a mixed model. The fixed effects included a factorial design with two factors:

- Stress period: two levels (heat stress and no heat stress).
- Experimental group: three levels (rams, RLLRT, and RLHRT).

The random factor was the day of measurement.

Weight gain was analyzed as repeated measures using mixed models, considering the experimental group as a fixed factor with two levels (RLLRT and RLHRT) and week (15 weeks) as a second fixed factor. The random factor was the individual ram lamb, and the identity matrix was used as the covariance matrix. Means were compared using Fisher's least significant difference test, with $P \leq 0.05$ considered statistically significant. Statistical analyses were performed using INFOSTAT (INFOSTAT, 2020).

The statistical model used was:

$$y_{ijk} = \mu + S_i + G_j + SG_{ij} + D_k + \varepsilon_{ijk}$$

Where:

- y_{ijk} = response variable value in the sample taken on day *k*, under stress level *i* in experimental group j.
- μ = overall mean.
- S_i = effect of the stress period *i*.
- G_j = effect of the experimental group *j*.
- SG_{ij} = interaction effect between stress period i and experimental group *j*.
- D_k = random effect of day *k*.
- ε_{ijk} = random error in the sample taken on day *k*, under stress period *i* in experimental group *j*.

Results

Mean morning rectal temperatures (38.94 ± 0.06 °C vs. 38.85 ± 0.07 °C) during the trial were not significantly different ($P > 0.05$) between RLHRT and RLLRT. The lowest ($P \leq 0.05$) rectal temperature was recorded in the ram group (38.52 ± 0.06 °C). However, the afternoon rectal temperature was higher in RLHRT than in RLLRT (39.62 ± 0.08 °C vs. 39.23 ± 0.08 °C) and was not significantly different from that observed in the ram group (39.34 ± 0.08 °C) (Figures 1 and 2). The mean THI during the trial was 82.09 (range: 76.99 to 87.67) in the mornings, and 87.06 (range: 84.31 to 90.24) in the afternoons.

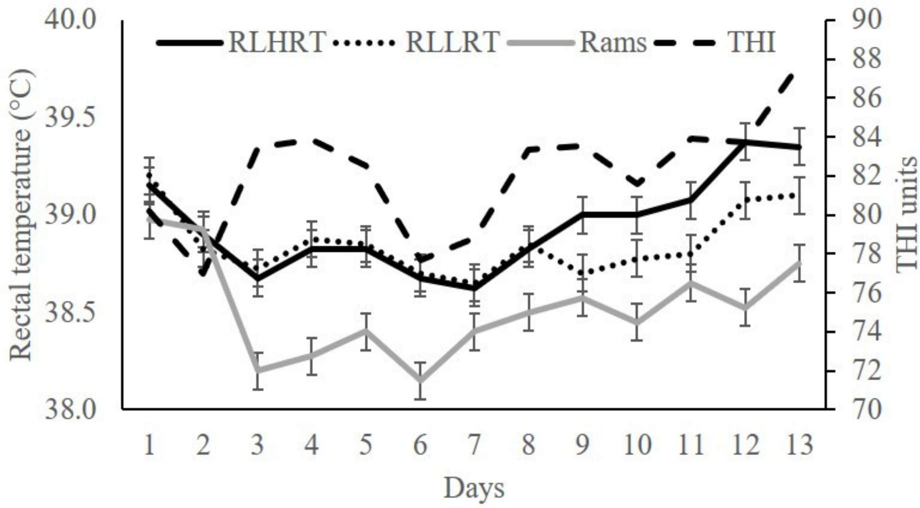

Figure 1. Temperature-humidity index (THI) recorded during morning rectal temperature measurements in rams and ram lambs classified as having low (RLLRT) or high (RLHRT) rectal temperature.

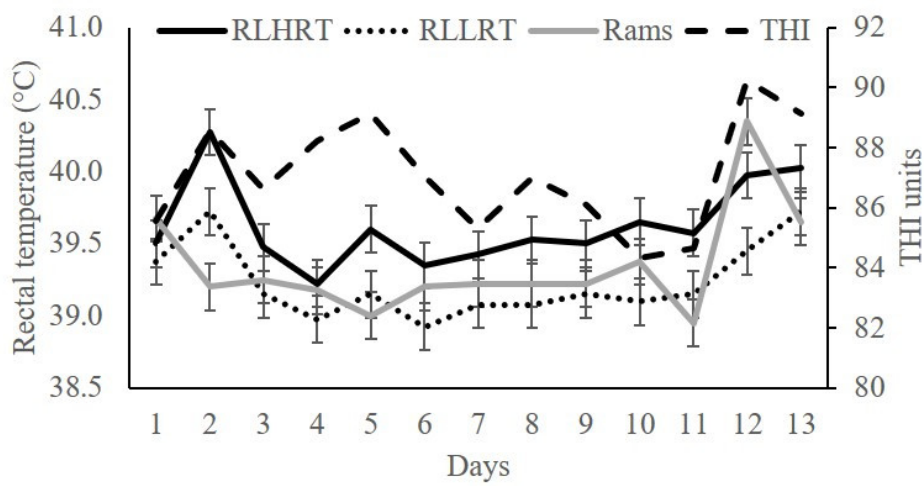

Figure 2. Temperature-humidity index (THI) recorded during afternoon rectal temperature measurements in rams and ram lambs classified as low (RLLRT) or high (RLHRT) rectal temperature.

Morning rectal temperatures during the experimental period were lower ($P \leq 0.05$) in the group of rams, with no significant difference ($P > 0.05$) observed between the RLLRT and RLHRT groups (38.79 ± 0.05 °C, 38.88 ± 0.05 °C, and 38.37 ± 0.05 °C for RLLRT, RLHRT, and rams, respectively). In addition, rectal temperatures during the no-heat-stress period were lower ($P \leq 0.05$) than those during the heat stress period (38.45 ± 0.06 °C vs. 38.92 ± 0.06 °C). The interaction between the experimental group and the period was also significant ($P \leq 0.05$) (Figure 3). The mean THI during the experimental periods—heat stress and no-heat-stress—was 83.83 (range: 73.52 to 87.40) and 64.21 (range: 53.75 to 69.81), respectively.

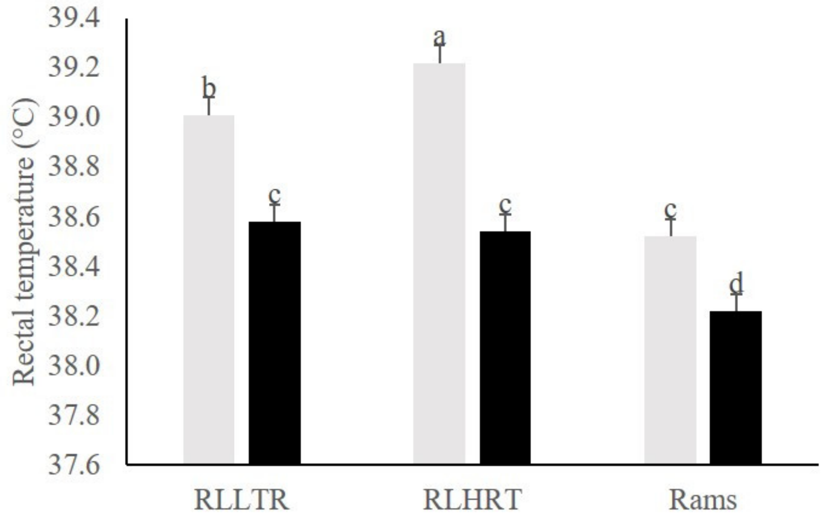

Figure 3. Morning rectal temperatures of rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperatures during heat stress (gray bars) and no-heat-stress conditions (black bars). Treatments with different superscripts are significantly different ($P \leq 0.05$).

Afternoon rectal temperatures during the experimental periods were higher ($P \leq 0.05$) in the RLHRT group, and there was no difference ($P > 0.05$) between rams and ram lambs in the RLHRT group (39.23 ± 0.06 °C, 39.54 ± 0.06 °C, and 39.20 ± 0.06 °C for RLLRT, RLHRT, and rams, respectively). In addition, rectal temperatures were lower ($P \leq 0.05$) during the no-heat-stress period than during the heat-stress period (39.14 ± 0.08 °C vs. 39.51 ± 0.07 °C). The interaction between treatment and experimental period was also significant ($P \leq 0.05$) (Figure 4).

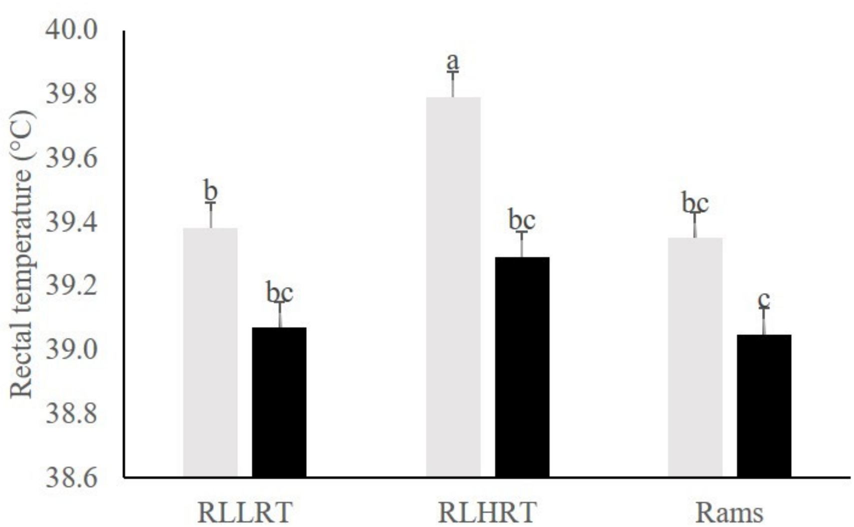

Figure 4. Afternoon rectal temperatures of Rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature during heat stress (gray bars) and no-heat-stress conditions (black bars). Treatments with different superscripts are significantly different ($P \leq 0.05$).

Seminal traits of the experimental groups were affected (P ≤ 0.05) (Table 1). The effect of the experimental period on motility (2.59 ± 0.19 vs. 1.65 ± 0.19 for the periods with and without heat stress, respectively) and on sperm concentration (10^6) (2857 ± 95.80 vs. 2038 ± 89.31 for the periods with and without heat stress, respectively) was significant. The interaction between the experimental group and the period was not significant (P > 0.05). Average daily weight gain was not different between lambs in groups RLLRT and RLHRT (Figure 5).

Table 1. Seminal traits (mean ± SE) in rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature under heat stress conditions.

Experimental group	n	Ejaculate volume (mL)	Mass motility	Sperm concentration (10^6)
RLLRT	4	0.90 ± 0.03[a]	1.72 ± 0.18[a]	1876 ± 91.70[a]
RLHRT	4	0.97 ± 0.03[a]	2.29 ± 0.18[b]	2444 ± 91.70[b]
Rams	4	1.29 ± 0.03[b]	2.36 ± 0.18[b]	3024 ± 91.98[c]

Different superscript letters within the same column indicate difference (P ≤ 0.05).

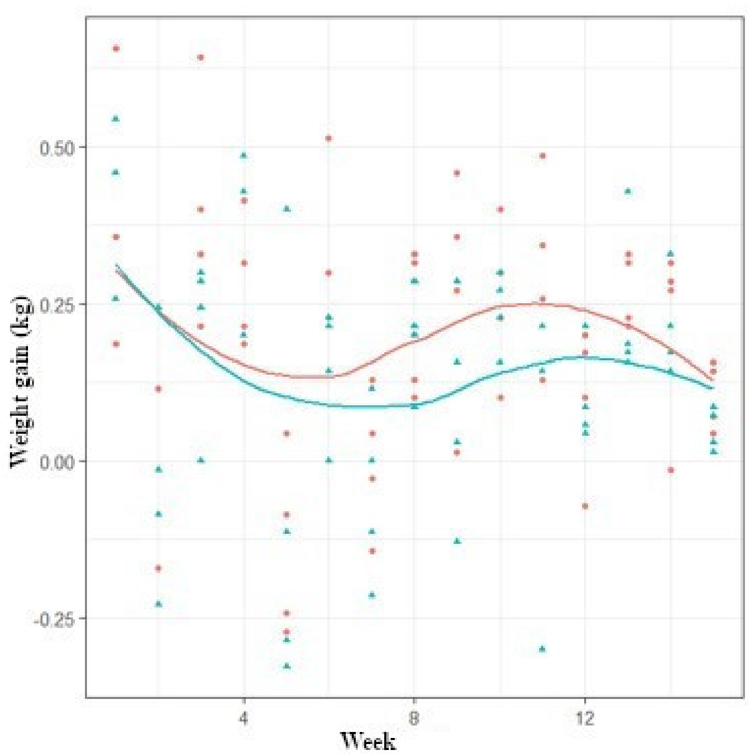

Figure 5. Average daily weight gain in ram lambs with low (blue line) and high (red line) rectal temperature.

Discussion

Rams experience seasonal variations in reproductive traits. Scientific research has shown that reproductive performance is lowest during the season when they suffer heat stress (van Wettere et al., 2021). Rams suffering heat stress display abnormal physiological functions such as increased rectal temperature and respiration rate, reduced testicular blood flow, and oxidative stress damage (Hedia et al., 2020; Joy et al., 2020a), which reduces semen quality by increasing the number of dead and abnormal sperm and by decreasing sperm motility and concentration (El-Zeftawy et al., 2020). Moreover, sexual behavior is altered (Maurya et al., 2016). Such effects may last even after overcoming the heat stress challenge (Hamilton et al., 2016). These impacts of heat stress can compromise ram fertility; it has been reported that oocyte fertilization with semen collected from males suffering heat stress yields a lower blastocyst formation rate (Seifi-Jamadi et al., 2020). Rectal temperatures and THI recorded in our study revealed that the experimental units were suffering heat stress (Kahwage et al., 2018). However, regarding rectal temperature and semen traits, the experimental groups responded differently to heat stress. This is worth noting because it creates a window of opportunity to select animals that are resilient to heat stress.

Hair sheep breeds are considered to be resilient to heat stress (McManus et al., 2020), and among these breeds, some have better performance than others when exposed to heat stress. Researchers have deemed Saint Croix rams more resilient to heat stress than Dorper and Katahdin rams due to their ability to maintain lower rectal temperature under heat stress conditions (Tadesse et al., 2019). Similarly, Dorper lambs were classified as more resilient to heat stress than second-cross lambs because they showed lower rectal and skin temperature under heat stress conditions (Joy et al., 2020b). In our study, the group of rams and the RLLRT group maintained lower afternoon rectal temperatures than the ram lambs in the RLHRT group during the heat stress period, suggesting that they were resilient to heat stress. However, no benefit to seminal traits in RLLRT animals was found. On the other hand, the group of rams had the highest ejaculate volume and sperm concentration. This, however, may not be associated with a heat stress resilience factor but rather with an age factor, since it is known that the values of these variables naturally increase as rams mature (Salhab et al., 2003).

The group of rams showed the lowest morning rectal temperatures during the heat stress period, and similar temperatures (38.4-38.7 °C) were recorded in the summer in adult Dorper, Morada Nova, and Santa Inês rams (Pantoja et al., 2017). Afternoon rectal temperatures increased in all experimental groups, but the highest value was recorded in the RLHRT group during the heat stress period. An increase in body temperature is associated with higher scrotal temperature (Shahat et al., 2021), which compromises sperm quality (Alves et al., 2016). Morada Nova and Santa Inês rams are considered resilient to heat stress because they have efficient testicular thermoregulation (Kahwage et al., 2018), which allows them to maintain seminal characteristics within an acceptable range despite hot weather conditions (Kahwage et al., 2017). Although they had lower rectal temperatures during the heat stress period, the lambs in the RLLRT group had the lowest values of mass motility and semen concentration of the three groups. These results were unexpected, since we had assumed that a lower body temperature during heat stress conditions would result in higher sperm quality. It is possible that the lambs in the RLHRT group had efficient testicular thermoregulation (Kahwage et al., 2018), allowing them to maintain seminal traits like those in the group of rams despite their higher rectal temperatures.

The values of the seminal traits of the RLLRT group were similar to those reported for rams suffering heat stress (Maurya et al., 2016), indicating that this group was the most sensitive to heat stress in terms of seminal traits. Selection of domestic animals to farm under hot weather conditions is challenging because of the antagonism between heat resistance and productivity (Carabaño et al., 2019). Therefore, animal selection should consider a balance of adaptation, health, production, and reproduction (Joy et al., 2020a). The ram lamb groups showed no differences in average daily weight gain. Thus, the ram and RLHRT groups might be regarded as more suitable for reproduction because of their ability to achieve higher seminal motility and concentration under heat stress conditions compared to lambs in the RLLRT group, which might be considered less suitable for reproduction under heat stress conditions because seminal traits are known to impact ewe fertility (Abecia et al., 2020).

Conclusion

Low rectal temperatures of ram lambs under heat stress conditions did not provide benefits regarding average daily weight gain, sperm concentration, and ejaculate mass motility.

Declarations

Funding

The authors did not receive financial support to conduct this study.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

JHMP collected the data. GRV performed the statistical analysis. CGM, JAMJ, SHA, RFG, RLCM, and EAL designed the experiment, wrote the manuscript draft, and edited the final version. JGM designed the experiment, collected the data, and wrote the manuscript draft.

Use of artificial intelligence (AI).

No AI or AI-assisted technologies were used during the preparation of this work.

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