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SHORT COMMUNICATION

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 calórico

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

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Abstract

Background: heat stress diminishes 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, 235 to 730 days old) 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. The ejaculated volume and sperm concentration were higher ($p \leq 0.05$) in the group of Rams 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 was not different ($p > 0.05$) between the RLLRT and RLHRT groups. **Conclusion**: the selection of ram lambs with low rectal temperature under heat stress conditions showed no benefit in terms of average daily weight gain or ejaculate traits.

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

Resumen

Antecendentes: el estrés calorico disminuye la calidad del semen en carneros. **Objetivo**: evaluar las respuestas productivas y reproductivas de carneros y corderos con diferente temperatura rectal bajo condicones de estrés calórico. **Metodos**: los animales (n = 12, cruza de Dorper × Katadhin × Pelibuey, con 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, pero no se encontraron diferencias ($p > 0.05$) entre este y el grupo de Carneros. El volumen del eyaculado y la concentración espermatica fueron mayores ($p \leq 0.05$) en el grupo de Carneros que en los grupos RLLRT y RLHRT. La motilidad masal y la concentración espermatica fueron menores ($p \leq 0.05$) 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 beneficia su ganancia de peso y las caracteristicas del eyaculado.

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

Resumo

Antecedentes: o estresse térmico diminui 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, 235 a 730 dias de idade) 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 ($p \leq 0{,}05$) no grupo RLHRT do que no RLLRT, mas não foram encontradas diferenças ($p > 0{,}05$) entre este e o grupo Carneiros. O volume ejaculado e a concentração espermática foram maiores ($p \leq 0{,}05$) no grupo Carneriros do que nos grupos RLLRT e RLHRT. A motilidade da massa e a concentração espermática foram menores ($p \leq 0{,}05$) no grupo RLLRT do que nos grupos RLHRT e Carneros. 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 traz benefícios para o ganho de peso e as características da ejaculação.

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

Introduction

Heat stress compromises farm animal physiological functions and welfare and results in low 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 some parts of the world due to heat stress (Thornton et al., 2021). To face this scenario, researchers have pointed out the need to farm livestock breeds with a high degree of resilience to heat stress conditions (Sejian et al., 2018). Unfortunately, the 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 have shown better 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 stress conditions (Menéndez-Buxadera et al., 2014). However, there is still abundant evidence of the negative impacts of heat stress on the reproductive performance of sheep (van Wettere et al., 2021), making it urgent to develop management and reproductive strategies to overcome its effects on sheep's productive performance.

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). Selection can use the genomic approach (Ramón et al., 2014) or target local breed rams that perform better under heat stress conditions (Kahwage et al., 2018). Economic and technological limitations are an obstacle to using the genomic approach in several areas of the world, and some local genotypes are not widely available. In addition, animals with resilience to heat stress have been identified only by comparing different genotypes (Kahwage et al., 2017), disregarding variability within genotypes. Therefore, local genotypes and subjects must be tested using traditional methodologies to identify animals 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 hot desert (Bwh); the highest and the lowest temperatures recorded during summer and winter seasons are 50 and -5 °C, 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 Ethical Research Evaluation and Postgrad Committee approved experimental procedures and animal handling (Reference number: 067/2024-2).

Animals

A trial was carried out measuring the rectal temperature of eight ram lambs and four rams for 13 days at the end of July before feeding in the mornings (7:00 h) and afternoons (17:00 h) with 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 rectum lateral wall until the temperature reading was completed. Air temperature and relative humidity were registered before each rectal temperature recording to calculate the temperature humidity index (THI) (Belhadj Slimen et al., 2019). THI values ≥ 72 were considered heat stress conditions (Belhadj Slimen et al., 2019). At the end of the trial, and considering that animal's daily rectal temperature is at its highest in the afternoon (Kahwage et al., 2018), the animals were assigned to one of three experimental groups, according to their 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 group or rams served to obtain reference values for reproductive variables. The RLLRT animals (n = 4) were 235.50 ± 8.38 days old, live weight 48.47 ± 6.74 kg and scrotal circumference 32.0 ± 2.70 cm. The RLHRT animals (n = 4) were 236.0 ± 2.0 days old, live weight 51.65 ± 6.27 kg and scrotal circumference 31.25 ± 1.70 cm. The rams (n = 4) were 2 years old, live weight 65.1 ± 8.04 kg and scrotal circumference 32.12 ± 0.62 cm. All the experimental units were Dorper × Katadhin × Pelibuey crossbreed. 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 were subjected to semen extraction by an artificial vagina and an ewe that was not in estrus twice a week (Thursday and Sunday) from August to November. Thirty 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 the days of semen collection to calculate THI, which was then used to identify the period when animals were exposed to heat stress (first 17 sampling days of the experimental period) or no heat stress (last 13 sampling days of the experimental period).

Animal housing and feeding

The RLLRT and RLHRT were housed in the same pen from when they were weaned and during the entire experiment. The group of rams were kept in separate pens, but they also had 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^{-1} 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 the morning and afternoon rectal temperatures during the evaluation and experimental period. Sperm volume, concentration, and mass motility were evaluated in semen samples following established methodologies (Maurya et al., 2016). The ram lambs were weighed every week for 15 weeks to calculate average daily weight gain.

Statistical analysis

Rectal temperatures and ejaculate volume, sperm concentration, and mass motility were analyzed using a mixed model: the fixed part was a factorial design with two factors, a stress period with two levels (heat stress and no heat stress), and an experimental group with three levels (Rams, RLLRT, and RLHRT), considering the day as the random factor. The variable weight gain was analyzed as repeated measures using mixed models, considering as the fixed part a factorial design with two factors, experimental group with just two levels (RLLRT and RLHRT), and week as the second factor (fifteen weeks). The random factor was the ram lamb, and the identity matrix was used as the covariance matrix. The means were compared using Fisher´s least significant difference test. A $P \leq 0.05$ was considered as significant. INFOSTAT was used to perform the statistical analyses (INFOSTAT, 2020). The statistical model was $y_{ijk} = \mu + S_i + G_j + SG_{ij} + D_k + \varepsilon_{ijk}$.

In which y_{ijk} is the response variable value in the sample taken on the day *k*, with the stress level *i* in the experimental group *j*. μ is the overall mean. S_i is the effect of the stress period *i*. G_j is the experimental group *j*. SG_{ij} is the iteraction effect between heat stress period *i* and the experimental group *j*. D_k is the random effect of the day *k*. ε_{ijk} is the random error in the sample taken on day *k*, with the stress period *i* in the experimental group *j*.

Results

Mean morning rectal temperatures (38.94 ± 0.06 °C vs. 38.85 ± 0.068 °C) during the trial were not different ($P > 0.05$) between RLHRT and RLLRT. The lowest ($P \leq 0.05$) rectal temperature was recorded in the group of rams (38.52 ± 0.06 °C). However, afternoon rectal temperature was higher in RLHRT than in RLLRT (39.62 ± 0.084 °C vs. 39.23 ± 0.084 °C) and was not different from that observed in the group of Rams (39.34 ± 0.084 °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.

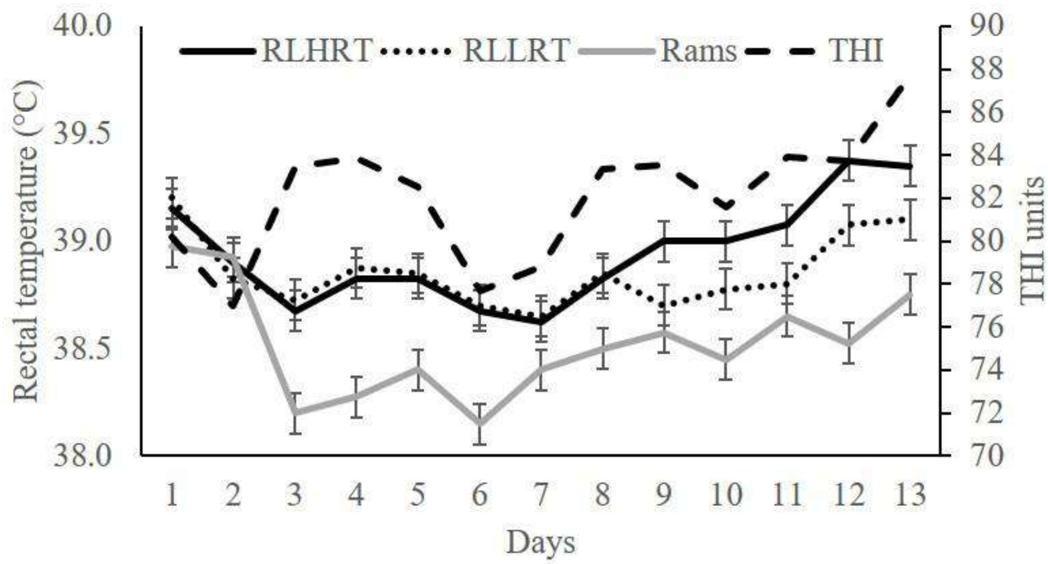

Figure 1. Temperature-humidity index (THI) while recording morning rectal temperatures in rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature.

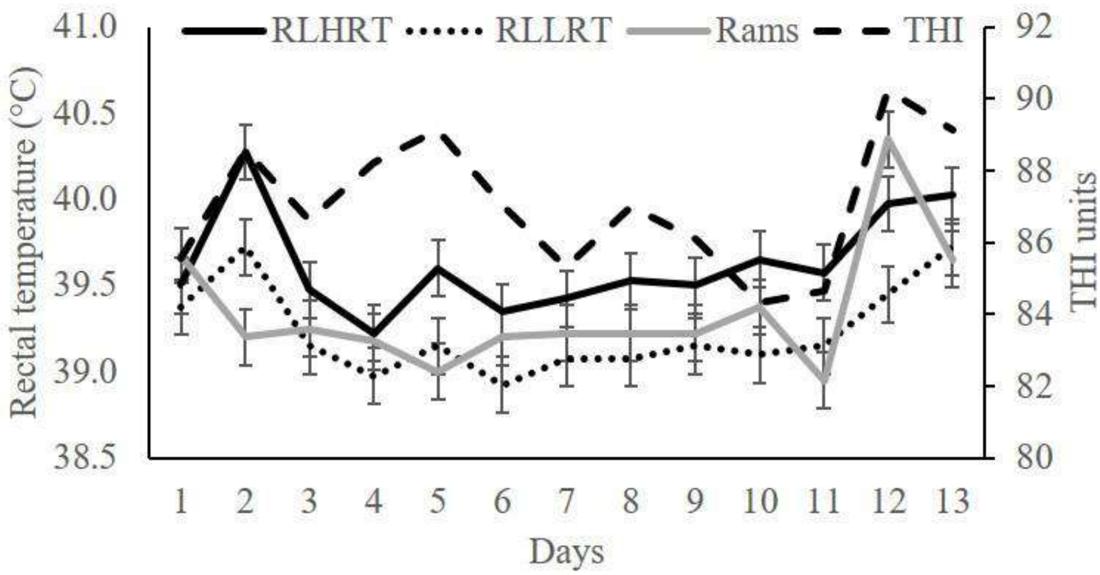

Figure 2. Temperature-humidity index (THI) while recording afternoon rectal temperatures in rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature.

Morning rectal temperatures during the experimental period were lower (P ≤ 0.05) in the group of rams, with no significant difference (P > 0.05) observed between RLLRT and RLHRT groups (38.79 ± 0.05 °C, 38.88 ± 0.05 °C and 38.37 ± 0.05 °C for RHLRT, RLHRT and rams, respectively). In addition, rectal temperatures during the period with no heat stress were lower (P ≤ 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 ≤ 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).

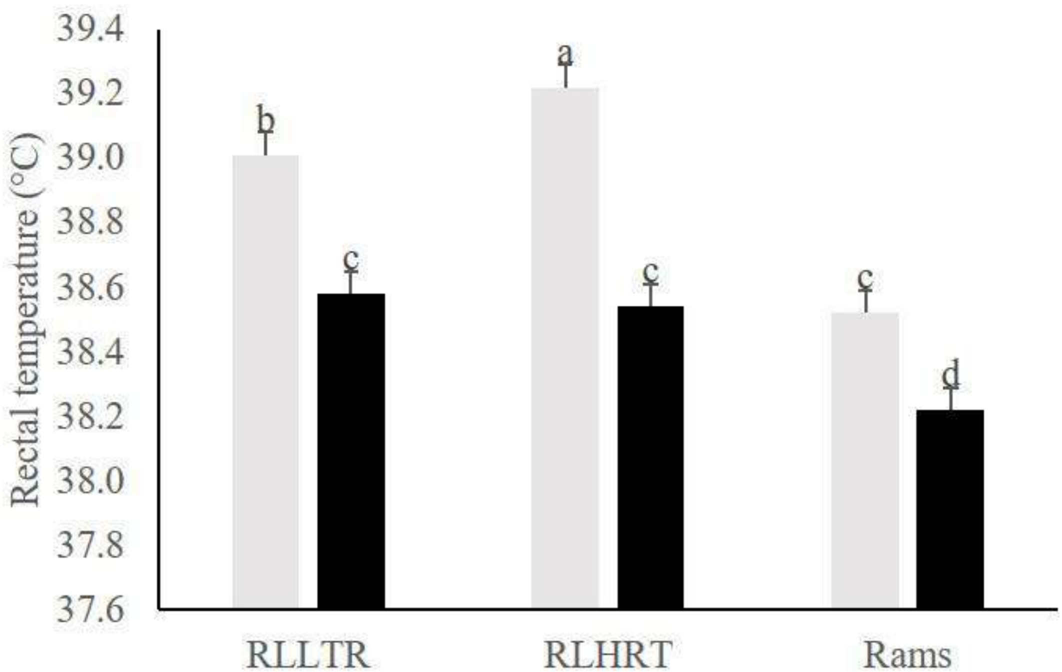

Figure 3. Morning rectal temperatures of rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature during heat stress (gray bars) and no heat stress conditions (black bars). Treatments with different superscript are significant different ($P \leq 0.05$).

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 RLHRT (39.23 ± 0.06 °C, 39.54 ± 0.06 °C and 39.20 ± 0.06 °C for RHLRT, RLHRT and rams, respectively). In addition, rectal temperatures were lower ($P \leq 0.05$) during the period of no heat stress 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).

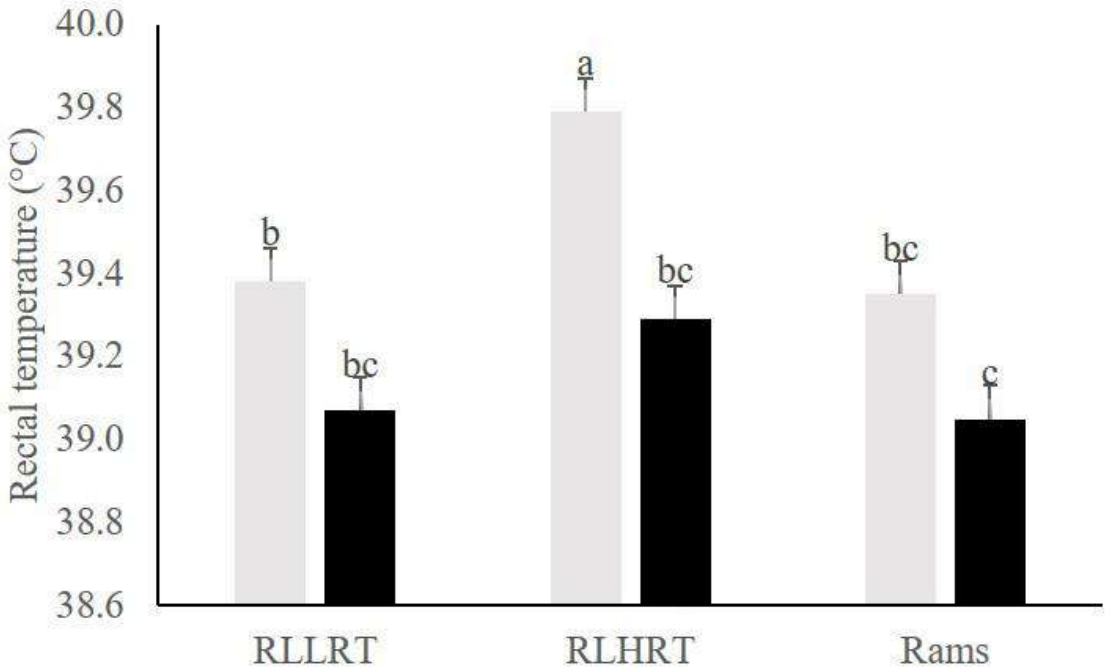

Figure 4. Afternoon rectal temperatures of rams and ram lambs classified as having low (RLLRT) and high (RLHRT) rectal temperature during heat stress (gray bars) and no heat stress conditions (black bars). Treatments with different superscript are significant different ($P \leq 0.05$).

Seminal traits of the experimental groups were affected ($P \leq 0.05$) (Table 1). The effect of the experimental period on motility (2.59 ± 0.19 vs. 1.65 ± 0.19 for the period with and without heat stress) and on sperm concentration (10^6) (2857 ± 95.80 vs. 2038 ± 89.31 for the period with and without heat stress) 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 lambs (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 ($P \leq 0.05$).

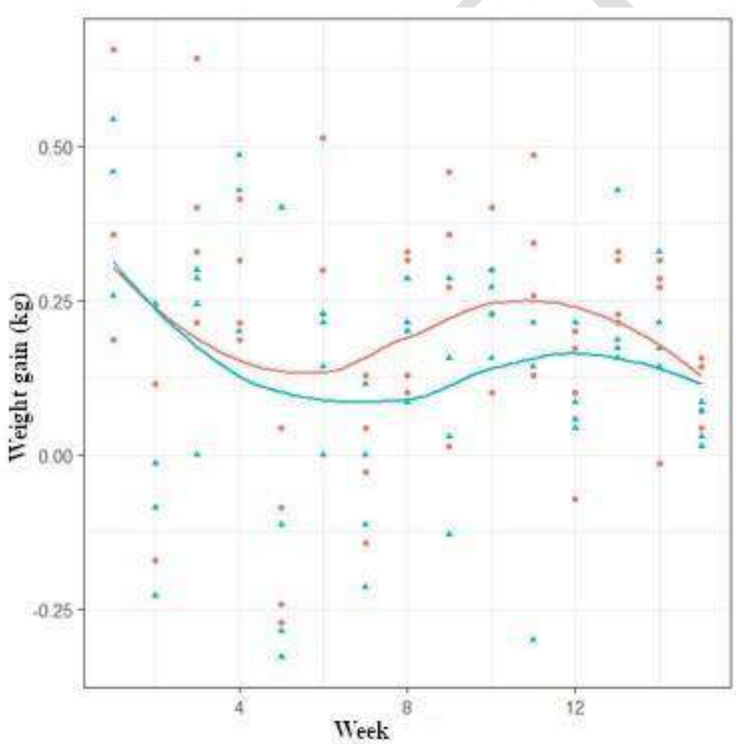

Figure 5. Average daily weight gain in ram lambs with low (blue line) and high (red line) rectal temperature.

Discussion

Rams experience seasonal variations in reproductive traits. Scientific research has shown that reproductive performance is lowest during the season in which 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) that reduces semen quality by increasing the number of dead and abnormal sperms 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 with resilience to heat stress.

Hair sheep breeds are considered to be resilient to heat stress (McManus et al., 2020), and among these breeds are those that have better performance than others during heat stress challenge. Researchers have deemed the Saint Croix rams as more resilient to heat stress than Dorper and Kathadin 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 temperature than the ram lambs in the RLHRT group during the heat stress period, suggesting that they are 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 value for ejaculate volume and sperm concentration. This, however, may not be associated with a heat stress resilient 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 Ines 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 Ines 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 temperature 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 could 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 similar to the group of rams despite higher rectal temperatures.

The values of the seminal traits of the RLLRT group are similar to those reported for rams suffering heat stress (Maurya et al., 2016), indicating that, of the three groups, this group is the most sensitive to heat stress in terms of seminal traits. Selection of domestic animals to farm in 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 reproductive practices because of their ability to achieve higher seminal motility and concentration under heat stress conditions than the 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).

Conclusions

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

Declarations

Funding

The authors did not receive financial support.

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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