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Energy, Hormones, and Performance: How Do Dairy Rams Cope with the Breeding Season?

Francesca D. Sotgiu [1,*], Claudia Caporali [1,2], Antonio Spezzigu [2], Matteo Sini [1], Chiara C. Costantino [1], Andrea Mattu [1], Valeria Pasciu [1], Christopher Odey [1], Francesca Mossa [1] and Fiammetta Berlinguer [1]

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Simple Summary

Traditional breeding management on dairy sheep farms in Mediterranean countries relies almost exclusively on natural mating. This requires rams to withstand a long breeding season (BS), usually extending from late spring to late autumn. Despite their vital role in flock productivity, ram management is typically prioritised only before the BS, with limited welfare monitoring during this period. To assess how rams cope with the reproductive effort, this study evaluated fluctuations in selected metabolites (NEFA, urea, triglycerides, cholesterol), hormones (testosterone, fecal thyroid – FTMs - and corticosteroid metabolites - FCMs), and body condition score (BCS) in Sarda rams (n = 14) raised under a semi-extensive system during the BS. Rams' reproductive efficiency was assessed by measuring pregnancy rates (PR) via transrectal ultrasound scanning and retrospective estimation of conception dates. Results showed a progressive decline in BCS and a metabolic shift in November in response to increased metabolic demand, while overall PR remained stable. Our results show that Sarda rams maintain reproductive performance by mobilising body reserves and adjusting metabolic effort and suggest the need for targeted management during and after the breeding season to support welfare, recovery, and long-term efficiency.

Abstract

This study evaluated reproductive performance, metabolic and hormonal fluctuations in Sarda rams raised under semi-extensive management conditions during the breeding season. Fourteen rams were isolated from ewes, subjected to nutritional flushing, and treated with melatonin implants (3X18 mg) before joining the flock. From June to December, body condition score (BCS), NEFA, urea, triglycerides, cholesterol, testosterone, fecal thyroid hormone metabolites (FTMs), and fecal corticosteroid metabolites (FCMs) were measured every 45 days. Ewes' pregnancy rates (PR) and conception dates were determined by reproductive ultrasound scanning to estimate rams' reproductive performance. BCS declined ($p<0.05$) from June (3.11 ± 0.06) to November (2.80 ± 0.06). In November, NEFA, cholesterol and FCMs concentrations peaked ($p<0.05$), whereas triglycerides and urea reached the lowest levels ($p<0.05$). FTMs peaked in November and June ($p<0.05$). Testosterone concentrations were three-fold higher in June than the rest of BS ($p<0.05$), while overall PR was stable during the BS. Despite metabolic and endocrine changes, rams maintained reproductive efficiency, indicating an interaction between metabolic status, stress response, and reproduction, and supporting the need for targeted management strategies to sustain welfare and long-term performance.

Keywords: dairy rams; reproductive efficiency; metabolic status; FTMs; FCMs; breeding season

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1. Introduction

Dairy sheep production represents a major economic, environmental, and socio-cultural component of the livestock sector in Mediterranean countries [1]. It is predominantly based on extensive and semi-extensive farming systems, which rely largely on natural pastures and on-farm feed resources[2,3] In many marginal and inland areas, sheep farming is often a main viable economic activity, sustaining rural livelihoods, preserving traditions, and providing environmental benefits such as reducing soil erosion, desertification, and the risk of forest fires [3].

Under semi-extensive traditional management systems, breeding is predominantly based on natural mating, typically with one lambing per year. The breeding system is designed to match the grass growth cycle [4] as well as market demands for fresh lamb meat, but it also imposes key management strategies. The mating season starts in late spring for adult ewes, resulting in lambing between October and December, whereas nulliparous ewe lambs start mating in late summer-early autumn and lamb [2] between January and March. Induced mating protocols, including the 'ram effect', hormonal treatments, and dietary supplementation [5–10] are sometimes applied to stimulate both male and female reproductive activity [2,11]. Reproductive effort is particularly demanding for rams, as they undergo an energetically taxing and prolonged breeding season, typically extending from late April/early May to early November, during which they must mate with both adult ewes and ewe lambs. During these months, rams are exposed to a range of fluctuating environmental factors (air temperature, humidity, photoperiod, and/or pasture availability) and social conditions, mostly linked to the shift in flock dynamics throughout the season. This dynamic is driven by the introduction of ewe lambs to the initial adult flock, which temporarily increases the ewe-to-ram ratio. This is followed by the progressive removal of pregnant ewes, ultimately leading to a scarce availability of females that can intensify male-male interactions toward the end of the season. It is well established that environmental stressors, such as extreme temperatures and housing conditions, alongside social stressors, like mating competition among males [12,13], can significantly influence the rams' physiological status and reproductive performance [11,14,15]. Consequently, these combined challenges can temporarily disrupt the animals' homeostatic balance, triggering an array of adaptive responses [16] that ultimately impacts male welfare and breeding efficiency.

General health, libido, and mating ability are typically assessed in rams shortly before the start of the breeding season [17,18]. However, the comprehensiveness of these evaluations is often limited by economic and time constraints. Furthermore, while several long-term studies to date have explored variations in different hormonal and/or metabolic markers in rams, mainly in relation to semen quality or testicular size [19–23], limited knowledge exists regarding the fluctuations of these markers in response to the aforementioned metabolic, environmental, and social challenges that occur during the breeding season [24]. It is plausible that the rams' responses to these challenges may vary across different phases of the breeding season, potentially influencing their reproductive performance and highlighting the need for targeted managerial interventions.

Building on these premises, this study aimed to characterize the fluctuations in metabolic profiles (circulating NEFA, cholesterol, triglycerides, and urea), hormonal status (circulating testosterone, fecal thyroid [FTMs] and corticosteroid [FCMs] metabolites), and body condition in Sarda rams during the breeding season. Managed under a traditional semi-extensive system, the animals were monitored to identify critical periods where physiological shifts might compromise reproductive efficiency. Finally, pregnancy and conception rates were assessed to determine the actual impact of these fluctuations on flock productivity and the potential need for targeted management interventions.

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2. Materials and Methods

2.1. Animals and Reproductive Management

The study was conducted on a commercial farm located in northern Sardinia, Italy (40°46′14.5″N 8°24′10.2″ E). Fourteen fertile Sarda rams, aged between 1 and 6 years, were enrolled.

From March to mid-May (approximately two months before the onset of the breeding season), all rams were housed together in an indoor pen, in complete visual, olfactory, and auditory isolation from the ewes. During this period, the daily diet consisted of 2 kg/head of a commercial pelleted feed divided into two rations, with grass hay and water provided ad libitum. In early April, each ram received three subcutaneous slow-release melatonin implants (18 mg MELOVINE®, Ceva Salute Animale SPA, Milan, Italy) to advance the onset of the breeding season [25].

In mid-May, the rams were introduced to a flock of 784 adult ewes managed in a single paddock. The animals were fed a unifeed diet supplemented with 2 kg/head of a commercial pellet (16–17% CP), hay, and water ad libitum, and had access to pasture for 3 to 6 hours per day depending on the season. Subsequently, in late June, 218 ewe lambs were added to the flock. The rams remained with the females until mid-November, when they were finally separated.

Ewe pregnancy rates were monitored from June to December through five consecutive transrectal ultrasound examinations, conducted every 45 days. Scanning was conducted using a SonoScape S8 scanner (SonoScape Europe Ltd., Rome, Italy) equipped with a 10 MHz rigid linear transducer. Pregnancy was confirmed by the presence of a corpus luteum, an enlarged uterine horn filled with anechoic fluid, and a detectable embryonic heartbeat [8]. Concurrently, fetal age was estimated by measuring the crown-rump length, thoracic diameter, and biparietal diameter [26].

The timeline of the experimental design is shown in Figure 1.

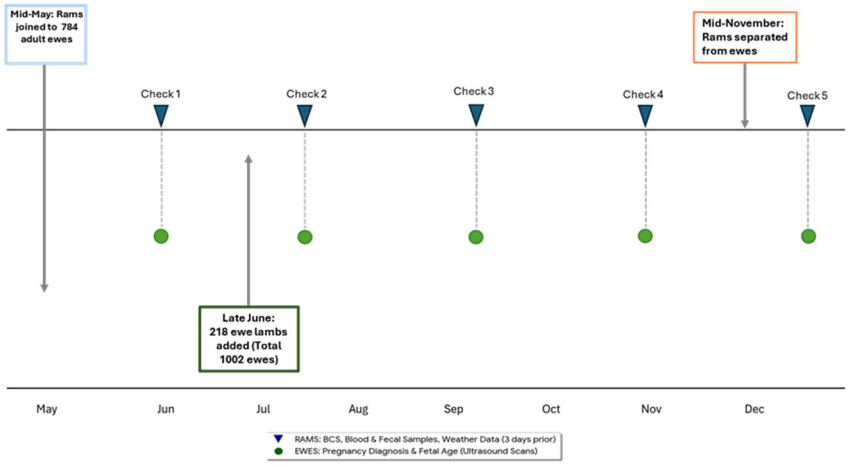

Figure 1. Timeline of the experimental design. Sampling points (triangles) indicate collection of BCS, blood and fecal samples, and weather data (3 days prior). Circles represent ultrasound assessments for pregnancy diagnosis and fetal age estimation. Rectangles indicate management phases.

2.2. Determination of Body Condition Score, Endocrine and Endometabolic Status of Rams

From June to December, all rams underwent five visits at approximately 45-day intervals. Average, maximum, and minimum air temperatures (T_mean; T_max; T_min) for the three days preceding each visit were retrieved from a nearby weather station (40°38′22.74″N 8°17′33.12″E) and processed in R using the GSODR package. This time window was chosen because fecal metabolites provide an integrated measure of hormonal activity, reflecting physiological responses to environmental conditions over the preceding 2–3 days [27,28]. During these visits, the Body Condition Score (BCS) was assessed by the same operator on a scale from 0 (extremely emaciated) to 5 (obese), according to the method described by Russel et al. [29] Blood and fecal samples were concurrently collected from all rams. Fasting blood samples were drawn from the jugular vein into

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9.0-mL vacuum collection tubes spray-coated with K2EDTA (Vacutainer Systems Europe; Becton Dickinson, Le Pont-de-Claix, France). Immediately after collection, the samples were chilled to 4 °C. Blood was then centrifuged at 1500 × g for 20 min at 4 °C; the plasma was separated and stored in vials at −20 °C until assayed. Fecal samples were collected directly from the rectum using a gloved hand, immediately placed on ice, and subsequently stored at −20 °C. All plasma and fecal samples were analysed in duplicate.

2.2.1. Biochemical Analyses

Circulating concentrations of non-esterified fatty acids (NEFA) (DiaSyS, Diagnostic Systems, Germany), total cholesterol, urea, and triglycerides (Hagen Diagnostica Srl, Italy) were measured using the enzyme endpoint method at 550 nm, 510nm, 340 nm, and 546 nm, respectively. Normal Serum I and Abnormal Serum II (FUJIFILM Wako Chemicals Europe, GmbH, Germany) were used as quality control for NEFA analysis. Clinical Chemistry Level 2 and Level 3 controls (Randox Laboratories Ltd., United Kingdom) were used for total cholesterol, urea and triglycerides. Calibration was performed using the specific standards provided by the manufacturer for each analyte: 1 mmol/L for NEFA, 50 mg/dL for urea, 200 mg/dL for total cholesterol, and 2.28 mmol/L for triglycerides. The intra-assay and inter-assay CV values and detection limits were: 1.07%, 0.98% and 0.01 mmol/L (for NEFA), 1.7%, 1.6% and 4.9 mg/dL (for UREA), 0.95%, 1.24% and 5 mg/dL (for total cholesterol), and 0.99%, 1.05% and 3 mg/dL (for triglycerides), respectively.

2.2.2. Hormonal Analyses

Testosterone circulating levels were quantified with a commercial kit (Ria Testosterone IM1087-01, Beckman Coulter, Czech Republic). Blood plasma samples were thawed and extracted according to the following procedure: A 200 µL of sample was added with 2 mL of ethyl ether, shaken vigorously and frozen at -20° C. Subsequently, the organic phase was separated from the aqueous phase and evaporated at 37° C, then re-dissolved in 200 µL of extraction buffer. Assay sensitivity was 0.05 ng/mL, and the intra- and interassay CVs were 11.6 and 13.5%, respectively.

Fecal samples were processed to determine fecal thyroid hormone metabolites (FTMs) and fecal corticosteroids metabolites (FCMs). At the time of analysis, all samples were thawed at room temperature and manually mixed using a spatula to ensure uniformity before extraction. The ensuing extraction procedure and analytical methods for determining (FTMs) and (FCMs) followed different steps.

2.3. FTMs Analysis

From each homogenised sample, 0.2 g of feces were lyophilised in 15-mL plastic tubes. Then, FTMs were extracted following a protocol previously described by Pasciu et al. [30]. FTM levels were determined using an ELISA kit supplied by DiaMetra Srl (REF DK0044, PerkinElmer company, UK).

2.4. FCMs Analysis

For the assay of FCMs, 0.5 g of fecal samples were homogenised with 80% aqueous methanol according to a procedure described by Palme, followed by centrifugation. The resulting supernatant was then analysed using a specific EIA for fecal glucocorticoid metabolites [31].

All 96-well flat-bottom ELISA plates were read at 450 nm using a microplate reader (FLUOstar Omega; BMG Labtech, Germany) suitable for standard SBS-format multiwell plates and equipped with BMG Labtech software.

2.5. Statistical Analysis

Statistical analysis was performed with R-Software (R 4.4.0).

Differences in the serially measured biological variables were analysed using linear mixed-effects models including time as a fixed effect and animal as a random effect to account for repeated

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measures. Model assumptions were assessed through graphical inspection of residuals (QQ plots and residuals vs fitted plots). Variables not satisfying normality and homoscedasticity assumptions were log-transformed prior to analysis. For these variables, results are presented as back-transformed estimated marginal means on the original scale. Pairwise comparisons were performed using Tukey-adjusted tests based on estimated marginal means. Statistical significance was set at $p < 0.05$. Results are expressed as Estimated Marginal mean ± standard error of the mean (SEM) unless otherwise stated. Relationships among variables were assessed using Pearson's correlation coefficient.

Rams' reproductive efficiency was indirectly estimated by analysing the findings obtained from the five consecutive reproductive ultrasound examinations of the ewes. At each flock visit, the pregnancy rate (PR = number of ewes diagnosed as pregnant / number of ewes exposed to the rams) and the reproductive load (number of available females per ram) were calculated. To ensure this load accurately reflected the actual mating opportunities, the number of available ewes was dynamically updated by excluding individuals that had been diagnosed as pregnant during the previous visit. In the event of an abortion, the affected ewes were immediately removed from the flock and temporarily considered unavailable; they re-entered the mating pool after a 21-day interval, which corresponded to the period required for post-abortion treatment.

Because PR is inherently influenced by the number of ewes a ram must serve, the varying reproductive load across checks made the observed PRs not directly comparable over time. To isolate ram efficiency from these demographic fluctuations, PRs were standardised using a generalised linear binomial model. By applying the average ewe-to-ram ratio calculated across all checks, a standard load of 27 ewes per ram was established, allowing for a fair comparison of ram efficiency throughout the entire experimental period.

3. Results

3.1. Rams' Reproductive Efficiency

The daily distribution of pregnancies (Figure 2) revealed distinct patterns between adult ewes and ewe lambs. In ewes, conceptions were concentrated at the onset of the breeding season, followed by a progressive decline. In contrast, ewe lambs exhibited a more dispersed distribution of conceptions over time, with a slight peak between July and August. This trend resulted in a sharp initial reduction in the reproductive load, which then decreased more gradually over time.

The evaluation of ram reproductive efficiency, conducted under a constant standardised load of 27 ewes per ram, revealed a distinct temporal trend compared to the non-standardised data (Figure 3). In this standardised scenario, the pregnancy rate (PR) peaked at the first check (85%), followed by a decline during the second (56%) and third checks (53%). Subsequently, the PR recovered at the fourth (71%) and fifth checks (85%), returning to levels comparable to the onset of the breeding season.

The observed PR values at the first and second checks showed negative deviations of –34% and –8% from the standardised rates, respectively. In contrast, the subsequent three checks yielded positive deviations of +8%, +13%, and +9%.

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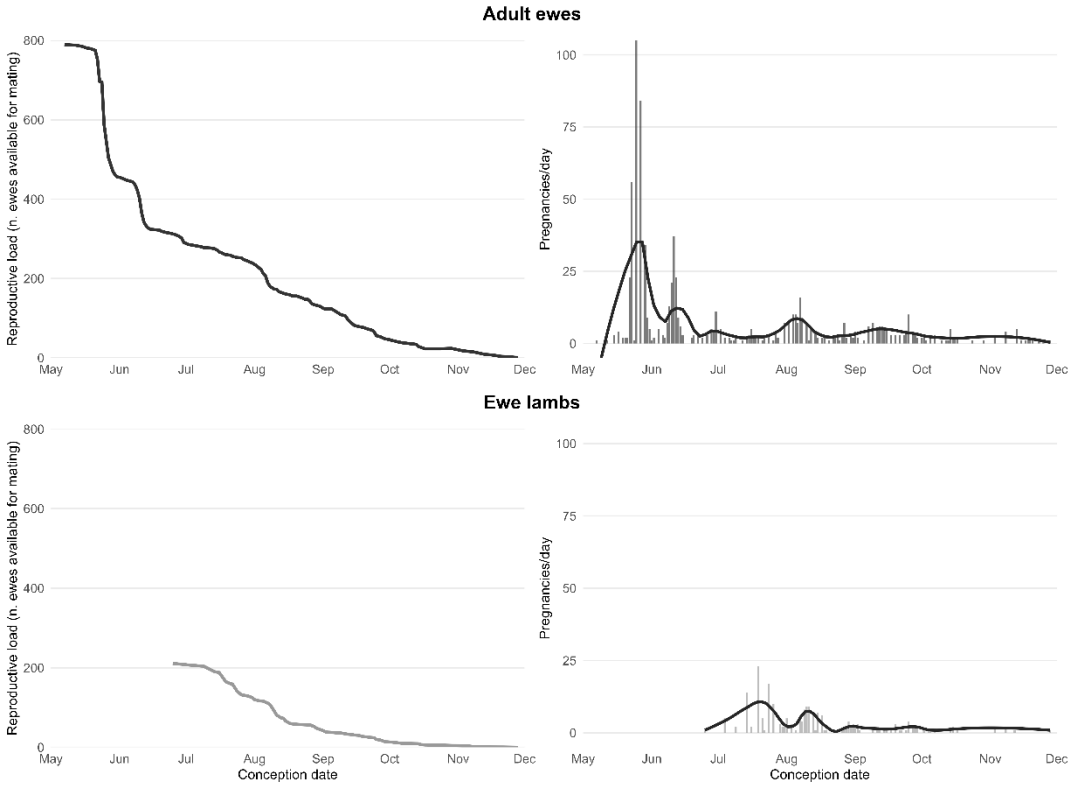

Figure 2. Daily number of conceptions and the number of females available for mating across the breeding season in adult ewes (n = 784, upper panels) and ewe lambs (n = 218, lower panels). Left panels show the temporal decline in the number of adult and ewe lambs available for mating, reproductive load, while right panels display the daily number of pregnancies (bars), with a LOESS-smoothed trend (black line) highlighting temporal patterns in conceptions.

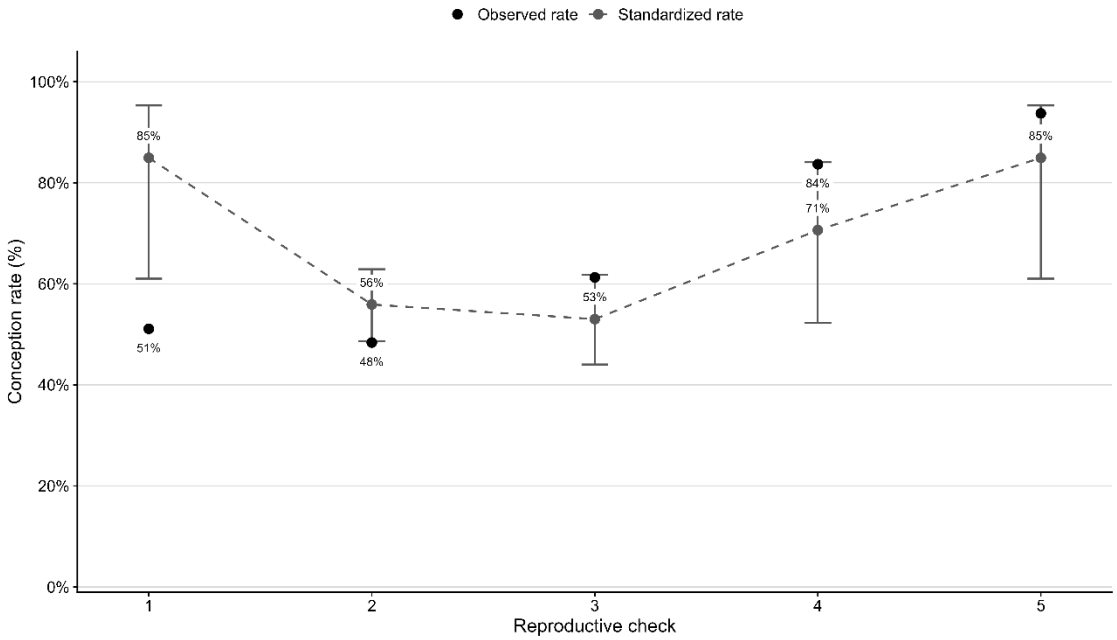

Figure 3. Rams' reproductive efficiency at the standard load (27 ewes/ram) across 5 distinct checks performed throughout the breeding season. Blue dots represent the standardised pregnancy rates; red dots represent the observed pregnancy rates.

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3.2. Biochemical, Hormonal, and Body Condition Changes During the Breeding Season

The mean external temperature was 19.98 °C in June, 23.45 °C in July, 24.13 °C in September, 17.34 °C in November, and 14.53 °C in December. The differences between maximum and minimum mean temperatures were 9.45 °C in June, 8.75 °C in July, 15.30 °C in September, 8.18 °C in November, and 6.00 °C in December. The mean BCS during the breeding season was 2.94, ranging from 3.11 to 2.77. BCS varied over time ($p<0.001$) with higher values ($p<0.05$) observed in June and July (3.11 ± 0.06 and 3.12 ± 0.06, respectively) compared to September, November and December (2.77 ± 0.06; 2.80 ± 0.06, and 2.86 ± 0.06, respectively).

Circulating NEFA and cholesterol varied during the breeding season ($p<0.001$), with mean concentrations of 0.167 mmol/L and 47.38 mg/dL, and ranges of 0.09–0.36 mmol/L and 43.5–54.8 mg/dL, respectively. NEFA concentrations were higher in November (0.36 ± 0.02 mmol/L) than in June (0.16 ± 0.02), July (0.11 ± 0.02), September (0.11 ± 0.02) and December (0.09 ± 0.02 mmol/L; $p<0.05$). Similarly, cholesterol was higher in November (54.8 ± 2.15 mg/dL) than in July (46.6 ± 2.15), September (43.6 ± 2.15) and December (43.5 ± 2.15 mg/dL; $p<0.05$).

Urea concentrations varied over time ($p<0.001$), with a mean of 20.9 mg/dL (range: 13.2–29.1 mg/dL), and were higher in June (24.0 ± 1.42), July (23.6 ± 1.42) and December (29.1 ± 1.42 mg/dL) than in September (14.4 ± 1.42) and November (13.2 ± 1.42 mg/dL; $p<0.05$). Triglycerides also varied ($p=0.001$), with a mean of 24.6 mg/dL (range: 16.9–27.8 mg/dL), and were lower in November (16.9 ± 2.15 mg/dL) than in July (26.9 ± 2.15) and December (27.8 ± 2.15 mg/dL; $p<0.05$). Testosterone concentrations varied over time ($p<0.001$), ranging from 1.18 to 5.96 ng/mL (mean: 3.21 ng/mL), and were higher in June (5.96 ± 0.82 ng/mL) than in July (1.76 ± 0.82), September (1.18 ± 0.82), November (1.44 ± 0.85) and December (1.49 ± 0.82 ng/mL; $p<0.05$).

FTMs varied during the breeding season ($p<0.001$), with a mean of 65.7 ng/g feces (range: 48.6–77.1 ng/g) and were higher in June (77.1 ± 5.24) and November (70.8 ± 5.24 ng/g) than in December (48.6 ± 5.24 ng/g; $p<0.05$). FCMs also varied ($p<0.05$), with a mean of 307 ng/g feces (range: 183.9–354.3 ng/g) and were higher in November (354.3 ± 35.2 ng/g) than in September (183.9 ± 35.2 ng/g; $p<0.05$). Overall variations in biochemical, hormonal, and body condition parameters during the breeding season are shown in Figures 4 and 5.

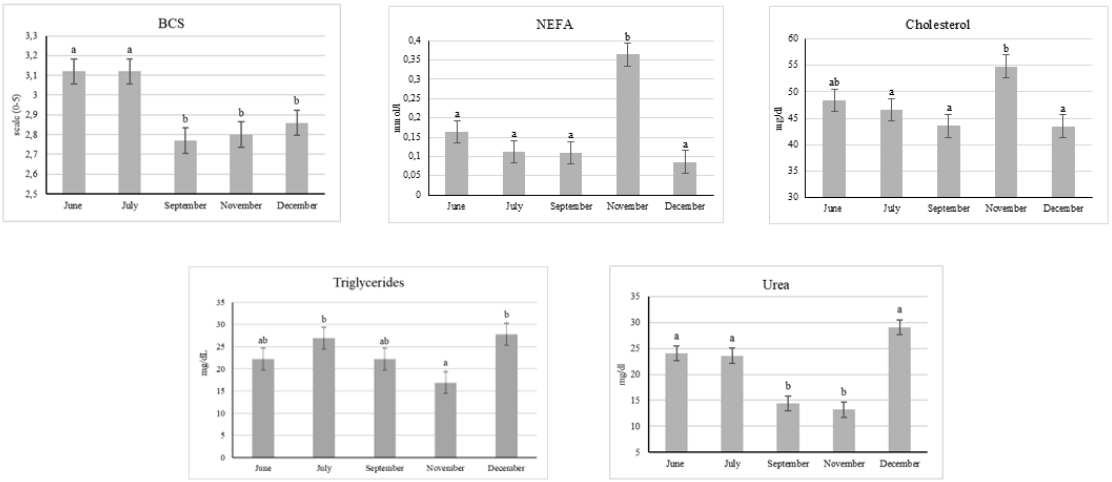

Figure 4. Mean (± SEM) body condition score (BCS) and circulating concentrations of NEFA, cholesterol, triglycerides, and urea in Sarda rams (n = 14) during the breeding season. Differences in serially measured variables were analysed using linear mixed-effects models including time as a fixed effect and animal as a random effect to account for repeated measures. The effect of time was significant for BCS, NEFA, urea, cholesterol, and triglycerides (all $p<0.001$). Different letters above bars indicate statistically significant differences between time points ($p<0.05$).

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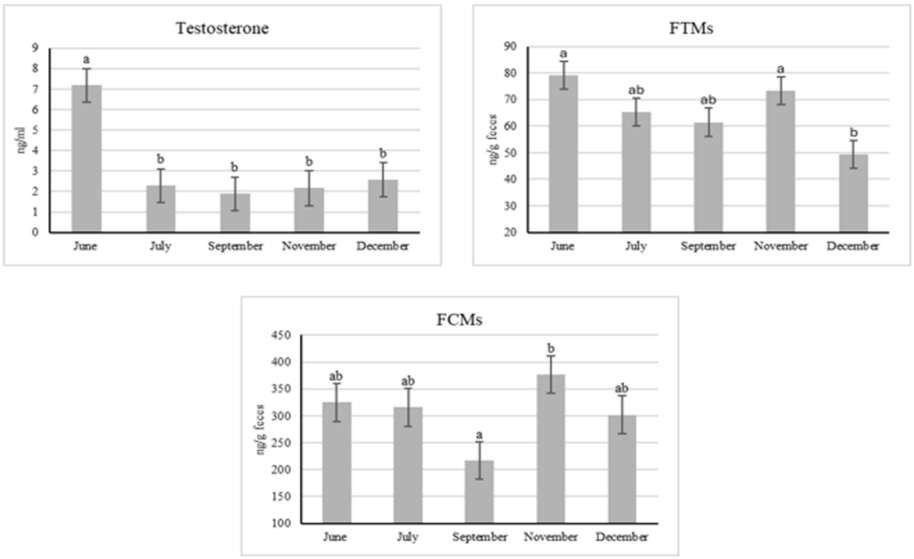

Figure 5. Mean (± SEM) levels of circulating testosterone, fecal thyroid hormone metabolites (FTMs) and fecal corticosteroid hormone metabolites (FCMs) in Sarda rams (n=14) during the breeding season. Differences in serially measured variables were analysed using linear mixed-effects models including time as a fixed effect and animal as a random effect to account for repeated measures. The effect of time was significant for testosterone and FTMs (both $p<0.001$) and for FCMs ($p<0.05$). Different letters above the bars indicate statistically significant differences ($p<0.05$).

The results of correlation analyses among hormones, metabolic indicators and environmental temperatures are presented in a correlation heat map (Figure 6) reporting correlation coefficients (r) and their respective p-values. NEFA concentration was positively correlated with FTM ($r = 0.29$; $p<0.05$) and FCMs ($r = 0.32$; $p<0.01$), and a negatively correlated with urea ($r = -0.28$; $p<0.05$) and triglycerides ($r = -0.33$; $p<0.01$). Urea was negatively correlated with maximum (T-max; $r = -0.37$, $p<0.01$) and mean temperature (T-mean; $r = -0.29$, $p<0.05$). FTMs were positively correlated with cholesterol ($r = 0.36$; $p<0.01$) and with NEFA ($r = 0.29$; $p<0.05$). Testosterone was positively correlated with FCMs ($r = 0.26$; $p<0.05$).

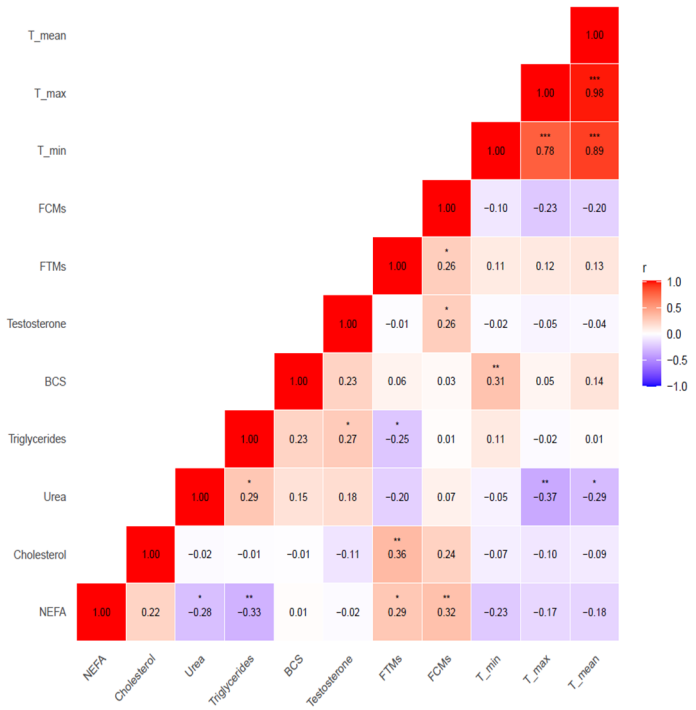

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Figure 6. Correlation matrix showing the pairwise Pearson's correlation coefficients (r) among all measured variables. The color gradient indicates both the strength and direction of correlations: blue represents negative correlations, while red indicates positive ones. Asterisks indicate significance levels (* P<0.05, ** P<0.01, *** P<0.001).

4. Discussion

This study evaluated the seasonal fluctuations in metabolic parameters, key hormones, and body condition in Sarda rams during the breeding season, specifically in relation to their reproductive efficiency. In line with our primary aim, we identified the specific periods within the traditional semi-extensive system where these physiological shifts became more pronounced, potentially necessitating targeted management interventions.

The analysis of the rams' breeding activity revealed a dynamic pattern, with high activity concentrated at the start of the season. This was inferred from the number of conceptions registered between May/June and July/August for adult ewes and ewe lambs, respectively. These results can be interpreted in light of routine reproductive management in dairy ewes; the introduction of rams typically triggers the "ram effect," increasing tonic LH secretion and inducing ovulation [32]. The resulting oestrus peaks observed around 17–25 days after introduction [33] are consistent with the conception distribution found in May and June. However, the reproductive workload evolved mid-season with the introduction of ewe lambs in late June. The lower PR observed in the intermediate checks can be explained by their lower responsiveness to the ram effect compared to adult ewes [2], likely contributed to the less synchronised and more dispersed breeding pattern observed between July and August. By the final checks (4–5), pregnancy rates returned to early-season levels, likely supported by a photoperiod more naturally favourable to sheep reproductive activity.

This sustained mating activity, despite fluctuations in female availability, suggests that rams maintained high performance throughout the season, albeit at an increasing physiological cost. A key finding to support this hypothesis was the significant decrease in BCS over time, confirming that rams mobilised body reserves to sustain reproductive effort. Notably, BCS remained above the critical threshold (BCS < 2.5) [18] suggesting that the energetic taxing did not reach a level capable of impairing overall success.

The metabolic profiles further elucidate this adaptive strategy. NEFA concentrations remained low during the early months and rose significantly only in November. This trend suggests that while the rams were losing body condition throughout the season, they were not in a state of acute negative energy balance (NEB) initially. The late-season spike in NEFA likely reflects a combined effect of accumulated reproductive fatigue, decreased intake and poor-quality autumn pastures.

The coordination of this metabolic response is further evidenced by the correlations between different parameters. A significant negative correlation between NEFA and triglycerides presumably reflects the limited hepatic capacity of ruminants to export triglycerides via VLDL [34,35]. Similarly, the lower urea levels observed between September and November support the hypothesis that protein intake was insufficient during this period. In response, the body may have reduced urea synthesis to conserve energy and prioritise nitrogen retention [36]. The negative correlation between NEFA and urea, as well as a positive correlation between triglycerides and urea, further suggests a coordinated metabolic response to the continuous energy requests. Plasma urea levels also showed a negative correlation with ambient temperature, as reported in a previous study [37]. In the literature, a positive correlation between circulating urea concentration and air temperatures is generally reported; however, dietary and metabolic factors appear to outweigh any direct effect of temperature on urea concentrations, in line with the mixed evidence in the literature [37–39].

This metabolic adaptation was closely mirrored by hormonal shifts, particularly regarding the adrenal and thyroid axes. Cholesterol levels peaked in November, which may be attributed to both enhanced lipid mobilisation and serving as a precursor for the increased demand in steroidogenesis [40]. This interpretation is supported by the concomitant peak in FCMs, indicating heightened Hypothalamic-Pituitary-Adrenal (HPA) axis activity at the end of the season. During this period, the

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low number of ewes available for mating likely intensified reproductive competition, coinciding with inadequate intake. Moreover, FCMs were positively correlated with NEFA, further supporting the hypothesis of a metabolic contribution to the observed allostatic load [41].

Interestingly, thyroid regulation appeared to prioritise these metabolic needs over environmental cues. FTMs remained stable until a subtle modulation in December, despite cold temperatures that typically stimulate thermogenesis [42,43]. Adaptation to cold causes deiodination of thyroxine (T4) and thus promotes an increase in triiodothyronine (T3) levels in the blood in humans and animals [44]. In this study, nutritional and metabolic status, rather than ambient temperature, appears to be the primary driver of thyroid regulation in rams, mirroring the adaptation patterns where energy balance overrides environmental cues to ensure long-term metabolic homeostasis [45]. The positive correlation observed between FTMs and NEFA, and between FTMs and cholesterol, further suggests a targeted metabolic response to face the high energy demands of the breeding season. Unlike the metabolic stress response observed in animals under negative energy balance (NEB), where increased lipolysis is typically associated with a suppression of the hypothalamic-pituitary-thyroid axis [45], our findings indicate that thyroid activity is maintained to orchestrate active lipid mobilisation and energy utilisation [43]. This aligns with evidence that thyroid hormones remain elevated when metabolic demands are high, provided the animal does not enter a critical state of energy deficit [45].

Finally, the gonadal axis showed a coordinated response to both management and stress. Testosterone levels showed a seasonal decline after the June peak. The early peak aligns with melatonin implants that were used from one month prior to the start of breeding season to advance the rams' breeding season and upregulate testicular function [46] Moreover, close contact with oestrous ewes rapidly elevates LH and testosterone in rams [47], thus it is plausible that such contact contributed to the high circulating testosterone concentrations measured in June. The subsequent stabilisation at lower levels, yet still sufficient to sustain reproductive activity in the rams [48], could be attributed to reduced sexual stimulation as the reproductive load decreased.

It is interesting to notice the positive correlation between testosterone and FCM highlighted in this study. It was expected that testosterone and FCMs levels would be negatively correlated, as the scientific literature supports the hypothesis of suppression of the hypothalamic pituitary gonadal axis (HPG) when the hypothalamic pituitary adrenal axis (HPA) is activated [12,49]. Conversely, the positive correlation found between testosterone and cortisol is consistent with the study by Harden et al., [50] which identifies a functional 'coupling' between the reproductive and stress axes, suggesting that the coordinated activation of both systems represents an adaptive response necessary to cope with the high energy expenditure and 'mating preparedness' required during the breeding season.

Taken together, these findings suggest that while the breeding season imposes a significant energetic tax on Sarda rams, they exhibit remarkable physiological plasticity. By mobilising reserves and coordinating metabolic and hormonal responses, they successfully maintained stable reproductive efficiency throughout the season.

5. Conclusions

In conclusion, this study demonstrates that Sarda rams exhibit significant physiological plasticity during the breeding season under semi-extensive management. While the reproductive effort leads to a progressive decline in body condition and a late-season increase in lipolysis (NEFA), the rams successfully maintain metabolic homeostasis without reaching a critical state of energy deficit. The positive correlation between testosterone and corticosteroid metabolites (FCMs) suggests a functional "coupling" of the stress and reproductive axes, enabling the animals to sustain high mating preparedness despite the energetic taxing of the season. Furthermore, the dominance of energy balance over environmental cues in regulating thyroid function (FTMs) highlights a prioritised metabolic adaptation to ensure reproductive success. These findings underscore the importance of monitoring BCS and metabolic markers during the final stages of the breeding season

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(October–November), suggesting that targeted nutritional support during this period could mitigate physiological strain and optimise long-term ram productivity.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to a temporary lack of a public accessible repository.

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