

Breeding soundness evaluation (Rams)



Ram introduction

Ram removal

35 Days – mating monitoring

Ewes randomization

Grazing period (70 days)

Wellbeing follow-up : comfort score, flock score, locomotion score

Before Grazing

Parcels :

- Rising plate meter and qualitative analysis of forage

Ewes :

- Weight
- Body condition score
- Blood work
- Shaving the lumbar area



Conventional Grazing - parcel 1
10 ewes + 1 ram



Conventional Grazing - parcel 2
10 ewes + 1 ram



Solar Grazing parcel
20 ewes + 1 ram



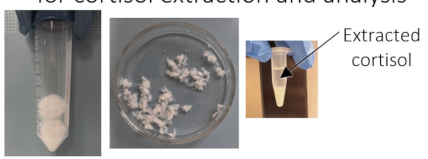
After Grazing

Parcels :

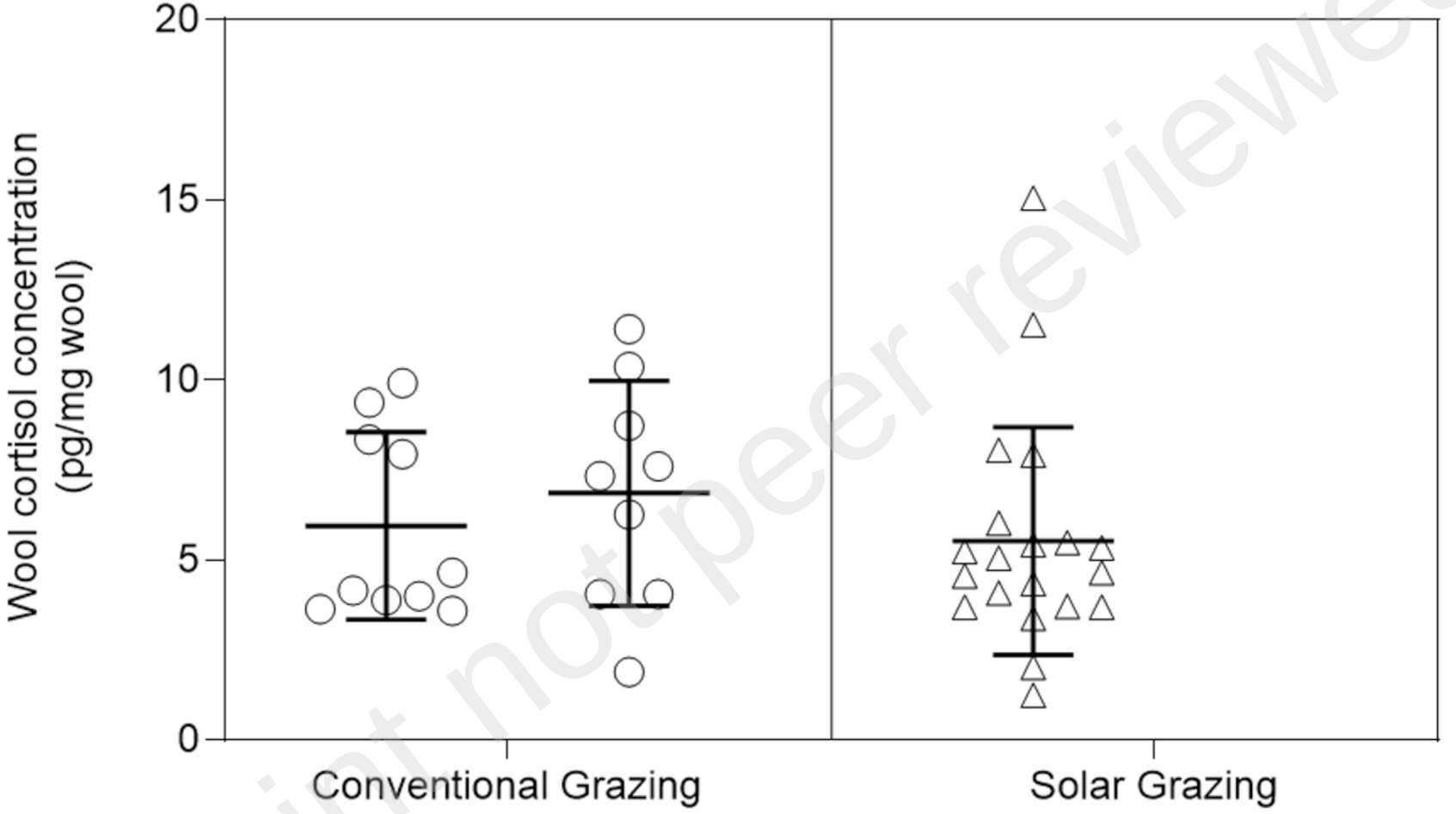
- Rising plate meter and qualitative analysis of forage

Ewes :

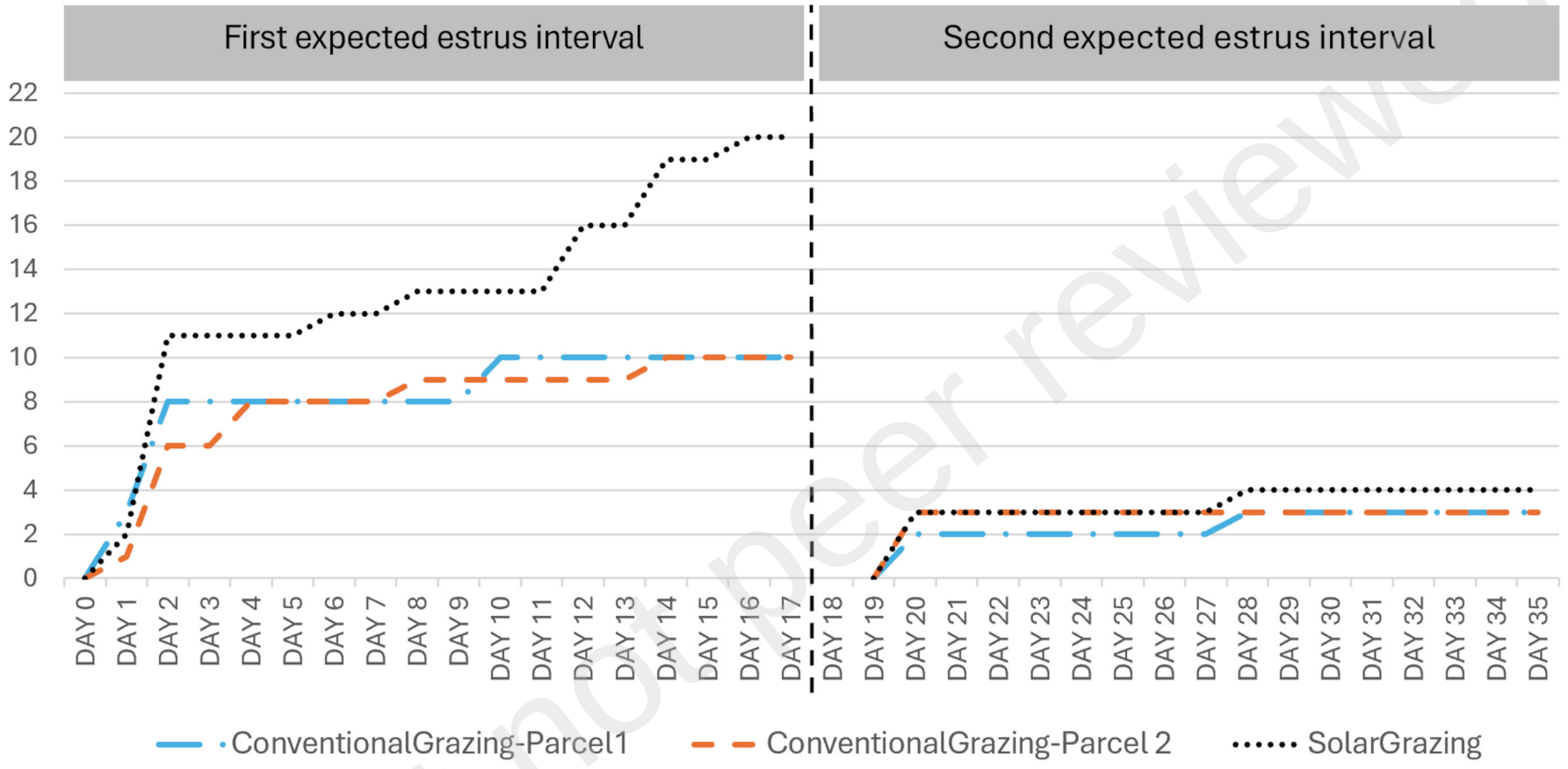
- Body condition score
- Blood work
- Ultrasonography - pregnancy
- Re-shaving the same anatomical site for cortisol extraction and analysis



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Table 1. Characteristics of the ewes from solar panels parcel and conventional grazing parcels. Values are given as Mean+/SD, otherwise stated.

	Conventional grazing Parcel 1 (N=10 ewes)	Conventional grazing Parcel 2 (N=10 ewes)	Solar grazing (N=20 ewes)	Comparison between groups (Nested)
Age (months)	33.5 +/- 15.9	33.5 +/- 13.7	33.3 +/- 13.7	P= 0.97 P (between sub columns)= >0.99
Weight (Kg)	71.20 +/- 9.9	68.9 +/- 8.1	71.4 +/- 12.1	P= 0.76 P (between sub columns)= >0.99
Body condition score (before grazing) Median (minimum-maximum)	4 (2-5)	4 (3-6)	4 (3-6)	P= 0.62 P (between sub columns)= >0.99
Body condition score (after grazing) Median (minimum-maximum)	5 (4-6)	6 (5-7)	5 (4-6)	P= 0.41 P (between sub columns)= 0.81
Wool Cortisol Concentration	6.0 +/- 2.6 pg/mg wool	6.8 +/- 3.1 pg/mg wool	5.5 +/- 3.1 pg/mg wool	P = 0.54 P (between sub columns)= 0.97
Cortisolemia BeforeGrazing	29+/-4 ng/mL	25+/-3 ng/mL	22+/-2 ng/mL	P = 0.33 P (between sub columns)= 0.97
Cortisolemia AfterGrazing	27+/-3 ng/mL	23+/-4 ng/mL	21+/-2 ng/mL	P= 0.40 P (between sub columns)= >0.99

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Wool cortisol concentration as a measure of chronic stress in grazing sheep

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Ruminants – Agrivoltaics – Pasture – Wellbeing – Wool

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Abstract

Dual use of lands for renewable energy and animals grazing raises concerns regarding animal welfare. The visual environment is modified by the presence of solar panels rows, which could contribute to impaired visual contact, social stress and reduced expression of normal behaviors. Wool cortisol concentration has been described as biomarker of chronic stress as cortisol accumulates in growing wool fibers overtime. We hypothesized that wool cortisol concentration would differ in sheep grazing on solar parcel compared to those grazing on control parcel. Forty French Texel ewes were randomly allocated to either solar grazing (N= 20) or conventional grazing parcels (N=20) for 70 days during Fall 2023. Rams were introduced for a 35-day period. The primary outcome was wool cortisol concentration, measured in regrown wool during grazing period. Reproductive performance at ultrasound (35 days after ram removal), mating monitoring, blood work, cortisolemia and body condition score were secondary outcomes. Wool cortisol concentration did not differ between grazing conditions. Secondary outcomes were within physiological reference ranges, and supported the results of wool cortisol measurements. Limitations of the study include the modest number of animals (limited by parcels size and stocking density constraints) and the short-term follow-up. Wool cortisol concentration was low in both grazing types and did not differ between groups.

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Introduction

Animal welfare is essential in research as well as in intensive or extensive production. Stress may arise from changes in social interactions between animals, chronic lameness, parasitism, or environmental factors (Destrez et al., 2013; Dwyer and Bornett, 2004). Nowadays, it is mandatory to assess the impact on stress of new interactions between human activities and animals. Over the last decades, dual-use of lands for energy production (with solar panels) and agricultural activity has developed in North and South America (Andrew et al., 2021; Faria et al., 2023; Fonseca et al., 2023; Kampherbeek et al., 2023; Kochendoerfer et al., 2019; Maia et al., 2015; Maia et al., 2020) as well as in European countries such as France, Spain, and Belgium (Tomasi et al., 2025). The presence of solar panels modifies the environment and may induce stress through partial isolation of animals between panel rows, impaired visual contact or reduced expression of sexual and social behaviors.

Acute stress results in activation of the hypothalamic-pituitary-adrenal axis leading to the adrenal release of cortisol into the bloodstream (Matteri et al., 2000). Cortisol is responsible for physiological responses such as mobilization of muscle glycogen storage or stimulation of lipolysis thereby providing energy to adapt to acute stress (Sapolsky et al., 2000). Hypercortisolemia, in the acute phase, is also responsible for negative effects such as inhibition of the immune system and suppression of the inflammatory response. This is achieved by inhibition of arachidonic pathway and by reduction of lymphocytes proliferation, resulting in lymphopenia (Sgorlon et al., 2012; Webster, 2002).

Chronic stress exerts other effects such as alterations of reproductive function (Papargiris et al., 2011), growth, and meat production (Dwyer and Bornett, 2004; Etim et al., 2013; Kumar, 2012), as well as behavioral deficit that affects feeding activity (Destrez et al., 2013). Lambs may exhibit mild (0-15%) to severe (up to 50%) reduction in feeding behaviour (Bell et al., 1989; De et al., 2018; Lee et al., 2014), that may be detrimental for farm production. Chronic

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stress-related alteration of the immune system (Rhind et al., 1999; Webster et al., 2002) makes sheep more susceptible to parasite burdens (Fleming, 1997; Sutherland et al., 2019). It also impairs reproductive performance, via decreased follicular growth and ovulation rate, dissociation between behavioral estrus and ovulation, increased silent ovulations, decreased oocyte and embryo viability, and decrease spermatogenesis and semen quality (Hargreaves, 1990; Tada et al., 2025; Van Wettere, et al., 2021).

The impact of chronic stress on animal welfare and productivity motivates the development of tools to detect long-term stress and evaluate the beneficial effects of different management systems by using objective markers. Cortisol concentration can be measured in body fluids (blood, saliva) or feces (Ghassemi Nejad et al., 2022). However, cortisol level in those matrices reflects acute stress response and does not capture chronic stress exposure (Bozovic et al., 2013; Chmelikova et al., 2020; Fell et al., 1985; Saco et al., 2008; Sheriff et al., 2010). Moreover, it is important to develop minimally invasive techniques that limit animal handling, as handling may induce acute stress by itself and introduce a measurement bias (Andanson et al., 2020).

Interestingly, hair or wool cortisol concentration measurement has previously been described in other species as a biomarker of chronic stress. In Rhesus monkeys, hair cortisol concentration is higher in abusive mothers than in nurturing mothers (Maestripieri et al., 2005), it is also negatively associated to social affiliations, mother-infant mutual gazing and social play, meaning higher cortisol concentration when poor or rare social interactions (Woodell et al., 2017). Long-term stress experienced by free-ranging grizzly bears forced to move because of human activities is also detected through hair cortisol concentration (MacBeth et al., 2010). Wool cortisol has already been measured in sheep affected by footrot and experiencing pain-related stress (Stubsjoen, et al., 2015), or in sheep experiencing heat stress with high temperature and humidity indexes (Fox et al., 2024; Nejad et al., 2014).

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Due to its low invasiveness measurement and its ability to capture long-term stress, wool cortisol appears as a promising tool to assess chronic stress related to environmental modifications such as the impact of solar panels. Studies on solar panels grazing are scarce and have documented the growing rate of Polypay lambs in Oregon (Andrew et al., 2021), the grazing time in Dorper ewes in California (Kampherbeek et al., 2023), and the time spent under the shade from solar panels over 5 days, in Corriedale sheep in Sao Paulo (Maia et al., 2020). These studies assessed outcomes mainly related to the productivity of animals but did not assess the chronic stress itself.

Our study aimed to use wool cortisol concentration (as marker of chronic stress) to compare two grazing conditions in reproductive ewes during one breeding season, under solar panels versus control parcels without panels. We hypothesized that the presence of solar panels could affect sheep wellbeing, leading to differences in wool cortisol concentration (primary outcome) that would corroborate other surrogates of stress (secondary outcomes) such as wellbeing scores, blood biochemistry and hematology, mating rate and reproductive performance.

Material and methods

Design

Primary and secondary outcomes were measured in forty French Texel ewes, randomly allocated to grazing on lands with solar panels (n = 20) and grazing on lands without solar panels (n = 20). The study was approved by the local ethical committee for animal experimentation (REF #23/418). The overall study design is summarized in Figure 1.

Population

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Ewes came from a local farmer who has been involved in sheep breeding and selection for more than 20 years, and who has recorded and saved sheep data since sheep birth (reproductive history, deworming, medications). All ewes were seronegative for Maedi-Visna and registered in the national database. Origin, history and birthdate were known. Of the forty ewes included in the study, twenty-seven ewes had already been pregnant and had lambed on previous breeding season (primiparous or multiparous at inclusion), and thirteen ewes were maiden ewes (18-19 months old and nulliparous at inclusion).

Grazing parcels

For solar grazing, the test parcel with ground-mounted solar panels was in Wierde (Belgium). The solar panels' structure was a fixed-tilt South-facing system, with a 20° inclination and 4-m interspace between rows. The solar panels were elevated above ground level (with a mean lowest height of 90 cm). The 2.2-ha (hectare) test parcel was 34-% covered by solar panels. For conventional grazing, two control parcels (1 ha and 1.2 ha, respectively) without solar panels) were available.

Both grazing systems were at short distance (3 km) to minimize potential variability in geology and meteorology. Grass characteristics were assessed to confirm baseline similarity between parcels before animal allocation. Evaluation included rising plate meter measurements (30 measures per parcel, walking in W-shape through parcels (Klootwijk et al., 2019)) and basic qualitative analysis (20 grass samples per ha, collected in absence of morning dew or rain, cut with a scythe at ground level, walking in W-shape (Murphy et al., 2021)). Grass samples were stored chilled in sealed bags, transported and analyzed within 12 hours by local certified laboratory (Office Provinciale Agricole, Belgium) for dry matter proportion, metabolizable energy, crude proteins, fibers composition, and minerals concentration according to ISO standards (ISO 6496, ISO12099, ISO5984, ISO 16634, ISO 6865, ISO12099, ISO 15510) (Dale et al., 2017). In the parcel with solar panels, samples were obtained from the portion not covered

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by panels. Detailed analysis of forage and grass was not a measure outcome in this study. However, ensuring similarities between parcels before grazing was essential. Data are provided in Supplementary materials.

Sample size and sheep allocation

Sample size required to detect chronic stress by wool cortisol concentrations was calculated from previously published data from grazing trials (comparison between open pasture and silvopasture (Poudel et al., 2022)) and from chronic stimulation of the hypothalamic-pituitary-adrenal axis (Weaver et al., 2021). Assuming a minimal effect size of 1.61, α error = 5%, power (1-β) = 95%, the minimum total sample size was 20 ewes, meaning 10 ewes for solar grazing and 10 ewes for conventional grazing. In this study, 40 animals (n = 40) were randomized in three parcels: 20 animals in the solar parcel (2.2 ha), and two groups of 10 in conventional parcels (1ha + 1.2ha) without solar panels. Distribution of sheep in two conventional parcels was due to the restricted availability of large grass surfaces for sheep grazing. The sheep density complied with national stocking recommendations (< 13 sheep/ha) (Biowallonie, 2018). Stratified randomization based on age ensured the equal distribution of young nulliparous ewes over the parcels.

Breeding management

The study took place between October and December 2023 (Fall), corresponding to the natural breeding season for French Texel sheep in North Hemisphere (Dawson et al., 2002; Janssens et al., 2004). Ewes were allowed to graze for 70 days with rams. Rams came from the same local farmer, with similar characteristics (seronegative for Maedi-Visna, encoded in the national database, with known history and origins). They were selected to avoid consanguinity with enrolled ewes. They had already serviced for reproduction in previous seasons and were confirmed to be sound for breeding before enrolment. Breeding soundness evaluation followed

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standardized procedure and included examination of the external genital tract and semen evaluation (Gouletsou and Fthenakis, 2010; Swanson and Bearden, 1951), and was performed in the veterinary department of the University of Namur.

One ram was introduced in each group of females for two consecutive expected estrus intervals (2 x 17 days) and were removed from the flocks after 35 days. The ewes-to-ram allocation ratio (maximum 20:1) was largely below the 100:1 recommendation for mature experienced ram (Allison, 1975; Kenyon et al., 2007).

Wool cortisol concentration measurement

The assessment of cortisol accumulation in wool over the grazing period followed the shave-reshave method (Poudel et al., 2022; Zoratti et al., 2023). On day 0, a 15cmx15cm area of wool was shaved at the lumbar region of ewes, using an electric clipper (Saphir cordless battery clipper with a #10 clipping blade, Heiniger/Terwagne, Belgium). Animals were subsequently allowed to graze in their allocated parcels. During the grazing period, newly grown wool accumulated cortisol diffusing from the capillaries to the hair follicles (Keckeis et al., 2012; Meyer and Novak, 2012). At the end of the grazing period, the regrown wool was shaved (at the anatomical site), wrapped in a foil sheet, protected from light and stored at room temperature until processed.

For cortisol extraction, 250mg of wool was washed 3 times in 10mL isopropanol (2-propanol 100% #20922364, VWR chemicals, France) and allowed to dry for 72h under a fume hood. Dried wool was cut in 1-mm chunk with scalpel blade. Cortisol was extracted by incubation of 50mg wool in 1mL methanol (99.9% Methanol #M/4058/17, Fischer Chemical, Belgium) overnight on an orbital shaker. After centrifugation (2150g for 15 minutes), 600µL of supernatant (containing the extracted cortisol) was transferred to a new tube and methanol was dried off under a fume hood overnight (MacBeth et al., 2010). Dried cortisol was resuspended

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in 400µL ELISA buffer and cortisol concentration was measured by competitive ELISA (Cayman Cortisol ELISA kit, #500360, Ann Arbor, MI, USA) following manufacturer instructions. Standard curve ranged from 6.6 to 4000pg/mL. Absorbance of each solution (standards and samples) was read at 405nm wavelength with a spectrophotometer (Thermo Scientific Multiskan EX #51118170 with Ascent Software, Thermo China). Measures were performed in duplicates. Concentration of solubilized cortisol (in 400µL ELISA buffer), extracted from 50mg of wool, was expressed in pg/mg of wool.

Follow-up

Body condition and wellbeing

Body weight (kg) and body condition score (from 1/9 emaciated to 9/9 overfat) were assessed at the start of the study in ewes and rams. Assessment of animal wellbeing was performed every other day following scoring scales adapted from (pre-)clinical research (Kaler et al., 2009; Shafford et al., 2004; Hontoir, 2018) and evaluating a comfort score (0-4), a lameness score (0-10) and a flock behavior score (0-3). The comfort score assessed general habitus of the animals (ruminating/eating, standing/resting, awake, calm) and the flock behavior score evaluated the social interactions between animals (acting in group, without isolated specimens). Lameness scoring aimed to detect signs of dermatitis or footrot (common painful conditions in sheep, that may interfere with male/female interactions when breeding). Details of scores are provided in Supplementary materials.

Mating monitoring

To detect mating events, rams were fitted with a breeding harness and marking crayons (Underwood et al., 2015). Each mating attempt deposited a color mark on the back of the ewe. The number of marked ewes was counted every other day. Seventeen days after ram introduction, the color of the crayon was changed, and mating was monitored with the same

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frequency during a second expected estrus cycle. Changing the crayon color allowed to differentiate any additional mating during the second half of mating monitoring.

Reproductive performance

Thirty-five days after rams removal, pregnancy diagnosis was performed by transabdominal (inguinal) ultrasonography conducted by an experienced operator from SAGPA SAS (Société d'aide à la gestation en production animale, Chalons-en-Champagne, France).

Ewes were handled in sitting position and the uterus was imaged by an inguinal approach (MyLab, 8-1 MHz Variable-Band Convex Array Transducer, used at 6.6MHz, Esaote). Pregnancy was confirmed by visualization of placentomes. Lambs were counted, and gestational age was determined by their size (Jones and Reed, 2017). Reproductive values (prolificacy, fertility, fecundity) were estimated based on ultrasonography, but not on the lambing. Fertility is the percentage of pregnant ewes in the flock (= number of pregnant ewes detected at ultrasonography, divided by total number of ewes in the group, in %). Prolificacy is generally considered as the mean number of lambs per litter. In our case, as the value was estimated by ultrasonography, it was calculated by dividing the total number of expected lambs by the number of pregnant ewes in each group. Fecundity was calculated by dividing the total number of expected lambs in the group by the total number of ewes (pregnant or not).

Blood work

Blood samples were collected before and after the grazing period, via jugular puncture, and transferred to collection tubes (one BD Vacutainer 10-ml Blood Collection Tube with K2EDTA, Becton Dickinson Company, Temse, Belgium). Samples were transferred to the laboratory for analysis within 4 hours of collection. Blood cells count (erythrocytes count and indices, total and differential leucocyte counts) and cortisolemia were conducted by referenced

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laboratory (Laboratoire Olivier - LabForVet, Fernelmont, Belgium) accredited for blood analysis (ISO15189).

Statistical analysis

Data were encoded in an excel file for each parcel. Comparison between solar grazing (sheep in a single large parcel) and conventional grazing (sheep split in two separated small parcels) required nested statistics and was done for following parameters: wool cortisol concentration, sheep age, weight, body condition score, cortisolemia, blood cell count) (GraphPad Prism 10.2.2 for Windows, GraphPad Software, USA.

Mixed models were used to analyze repeated rising plate measures, blood work and body condition score, before and after grazing. Linear mixed model (for rising plate measurements, and for blood work) and cumulative link mixed model (for body condition scores) were used to assess the effects of grazing system (SolarGrazing vs ConventionalGrazing), time (BeforeGrazing vs AfterGrazing), and their interaction. This was followed by type III ANOVA and post-hoc pairwise comparisons of estimated marginal means. Mixed model analysis was performed with R version 4.5.2. using packages for linear mixed models (lmm_model with RisingPlate ~ time * group + (1 | parcel)) and for cumulative link mixed model (clmm_model with score ~ time * group + (1 | sheep) + (1 | parcel)).

Reproductive performance was compared between grazing type (fixed effect) taking into consideration the parcels (random effect) using Generalized Linear Mixed Model for binomial data (fertility), and for discrete count and negative binomial data distribution (prolificacy). These analyses were performed with R version 4.5.2. using glmmTMB package (with prolificacy (or fertility) ~ grazing type + (1 | parcel)).

Statistical significance was considered if P-value < 0.05.

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Results

Baseline characteristics of animals and parcels

Randomization resulted in three groups of ewes without statistically significant differences in weight (P=0.76), body condition score (P=0.62), age (P=0.96) and proportion of nulliparous (P=0.76). Average age, weight and body condition score are summarized in Table 1. Baseline grass height (P=0.24) and estimated dry matter (P=0.26) did not differ between parcels. No differences were observed between grazing conditions for baseline blood work (including erythrocytes count and indices, white blood cell count, cortisolemia) (P=0.28 to 0.68 depending on parameter).

Table 1. Characteristics of the ewes from solar panels parcel and conventional grazing parcels. Values are given as Mean+/SD, otherwise stated.

	Conventional grazing Parcel 1 (N=10 ewes)	Conventional grazing Parcel 2 (N=10 ewes)	Solar grazing (N=20 ewes)	Comparison between groups (Nested)
Age (months)	33.5 +/- 15.9	33.5 +/- 13.7	33.3 +/- 13.7	P= 0.97 P (between sub columns)= >0.99
Weight (Kg)	71.20 +/- 9.9	68.9 +/- 8.1	71.4 +/- 12.1	P= 0.76 P (between sub columns)= >0.99
Body condition score (before grazing) Median (minimum-maximum)	4 (2-5)	4 (3-6)	4 (3-6)	P= 0.62 P (between sub columns)= >0.99
Body condition score (after grazing) Median (minimum-maximum)	5 (4-6)	6 (5-7)	5 (4-6)	P= 0.41 P (between sub columns)= 0.81
Wool Cortisol Concentration	6.0 +/- 2.6 pg/mg wool	6.8 +/- 3.1 pg/mg wool	5.5 +/- 3.1 pg/mg wool	P = 0.54 P (between sub columns)= 0.97
Cortisolemia BeforeGrazing	29+/-4 ng/mL	25+/-3 ng/mL	22+/-2 ng/mL	P = 0.33 P (between sub columns)= 0.97
Cortisolemia AfterGrazing	27+/-3 ng/mL	23+/-4 ng/mL	21+/-2 ng/mL	P= 0.40

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				P (between sub columns)= >0.99

Wool cortisol concentration

Wool cortisol concentration measured on regrown wool did not significantly differ between groups (P=0.54) and was 6.0 +/- 2.6 pg/mg for Conventional grazing - parcel 1, 6.8 +/- 3.1 pg/mg for Conventional grazing - parcel 2, and 6.8 +/- 3.1 pg/mg for Solar Grazing (Figure 2). There was no effect of parcels in the conventional grazing group (P >0.99).

Follow-up

Body condition and wellbeing scores

The cumulative link mixed model revealed a significant effect of time (BeforeGrazing versus AfterGrazing; P=0.000) on body condition score, but no significant effect of grazing type (ConventionalGrazing versus SolarGrazing) or interaction between both. Random effects of sheep and parcels negligibly contributed to the variance components of the body condition score. Body condition score increased by 1 unit in average (+0 to +3 depending on sheep) by the end of grazing time.

Comfort scores and flock behavior were normal during the entire study (score 0 for both). One ram showed a slight lameness of the right forelimb, due to interdigital dermatitis, a frequent pathology in wet seasons (stimulating the growth of pathogens such as *Fusobacterium necrophorum*). This short-duration lameness did not affect the reproduction behavior of the ram since the observer encoded new mating (freshly marked ewes with colored back) the following days.

Mating monitoring

Rams started to mate from the first day of introduction into the group (in the solar panel parcel and in the conventional grazing parcels). All ewes were mated at least once within the first 15

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days (Figure 3). After changing the color of the breeding harness, 6 ewes in the conventional grazing group and 4 ewes in the solar panel group get mated.

Reproductive performance

At ultrasonography, nine and seven ewes were detected as pregnant in the conventional grazing groups, and twenty (20/20) in the solar panel group, meaning a fertility at scanning of 90%, 70% and 100% respectively. All primiparous were detected as pregnant. Open ewe (non-pregnant at scanning) had already bred during previous season and were 2.5-year-old primiparous ewes (N=1 in ConventionalGrazing Parcel 1, and N=2 in ConventionalGrazing Parcel 2) and one 4-year-old multiparous ewe (ConventionalGrazing Parcel 2). Prolificacy was 1.9+/-0.3 in ConventionalGrazing Parcel1, 1.9+/-0.7 in ConventionalGrazing Parcel 2, and 2.1+/-0.7 in SolarGrazing Parcel. Generalized Linear Mixed Model did not highlight relevant effect of grazing type or parcel on prolificacy, fertility and fecundity.

Blood work

Blood work, including erythrocytes count and indices, white blood cell count, was within normal ranges (Adjou and Autef, 2013; Underwood et al., 2015), and did not show stress-related leukogram alterations (neutrophilia and lymphopenia) (Mormède et al., 2007; Cook, 2012). Linear mixed models did not detect relevant effects of time, grazing type or parcel for those parameters.

Cortisolemia before and after grazing was inferior to cortisol level associated to transport (72.5 ng/ml) and close to values reported during handling (25ng/ml) (Andanson et al., 2020; Caroprese et al., 2010; Fell et al., 1985; Weaver et al., 2021) (Table 1).

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Discussion

This study showed the feasibility of assessment of wool cortisol concentration. Comparison between wool cortisol concentration for ewes grazing in two different grazing systems (conventional versus solar grazing) did not show relevant differences, suggesting the absence of chronic stress in the solar grazing system.

Standardization of sampling for hair/wool cortisol concentration is required. In our study, wool was systematically collected at the level of the lumbar spine. Indeed, hair cortisol concentration may vary between anatomical regions in cattle (Burnett et al., 2014; Carlitz et al., 2015), and higher values of cortisol concentration are found in wool of the shoulder compared to back wool in Welsh mountain barren ewes (Fürtbauer et al., 2019). External contaminations could arise from the ground, feces, urine or saliva (Heimbürge, et al., 2020; Salaberger et al., 2016). It was therefore essential to collect wool at the same anatomical site for all sheep, to select an area free of urine, feces or ground contaminations, and to proceed for isopropanol washes before performing cortisol extraction. Moreover, collection method should be the same between groups as including the hair follicle increases the final hair cortisol concentration measure (Sergiel et al., 2020). It is therefore essential to decide if the wool is shaved or sheared, and perform the same procedure for all samplings (overtime) and all animals (included in the study). Comparison should be made between contemporaneous groups as the season may influence wool cortisol levels (Heimbürge, et al., 2019). After collection, samples should be stored at room temperature, in a dry environment, protected from light. Indeed, UV are responsible for decrease in cortisol concentration (Grass et al., 2016; Wester et al., 2016). However, if storage is adequate, values are stable even in case of prolonged laboratory storage (> 1 year) (MacBeth et al., 2010).

The shave-reshave technique allowed to capture the accumulation of cortisol in the wool that grew during the grazing period (Zoratti et al., 2023; Poudel et al., 2022). Thus, wool cortisol

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concentration should be considered as a retrospective objective measure of chronic stress over an experimental procedure (Kirschbaum, 2024). It should be combined with other parameters such as regular observation of animals to detect signs of wellbeing alteration or stress during experimental protocol.

In this study, secondary outcomes (body condition and wellbeing scores, mating monitoring, reproductive performance, blood biochemistry and hematology) corroborated the results of wool cortisol measurement, with all results being within normal ranges and no difference between groups. This is interesting since solar grazing raises questions about the animals' wellbeing and behavior (Pavie et al., 2022). In the pasture, the solar panels transform the visual environment of animals, it may restrict the visual contact between animals, or prevent them from moving freely as they should avoid supporting structures and walk between rows. Sheep are gregarious animals and their social behavior is influenced by activity (spreading when grazing, and clustering when resting), environment and weather (Doyle et al., 2016; Dwyer and Lawrence, 1999; Lawrence, 1990; Michelena et al., 2009). Sheep isolation results in systemic stress response (increased heart rate and increased cortisolemia) as well as increased agitation and vocalizations (da Costa et al., 2004; Nowak et al., 2008; Price and Thos, 1980). Behavioral responses (agitation and vocalization) mainly aim to restore bonds with conspecifics (visual, acoustic, olfactory and social contacts) (Siebert et al., 2011). Solar panels rows could reduce visual contact between animals, inducing partial isolation and alteration of wellbeing. In the present study, we did not find alteration of wellbeing as expressed by the score-0 for comfort and flock behavior, and the absence of difference of wool cortisol concentration between conventional and solar grazing.

Chronic stress is associated with alteration of blood parameters (Sgorlon et al., 2012; Webster, 2002), alteration of reproductive function (Papargiris et al., 2011) and performance (Hargreaves, 1990; Van Wettere, et al., 2021; Tada et al., 2025), low food intake (Bell et al.,

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1989; De et al., 2018; Lee et al., 2014). In the current study, blood work was within normal ranges before and after the grazing period. We did not observe any stress-related lymphopenia and neutrophilia (Sgorlon et al., 2012; Webster, 2002). Cortisolemia values measured at the beginning and the end of the study were in the range of normal values reported during sheep handling (Fell et al., 1985; Andanson et al., 2020). Values were considered low compared to literature references (Caroprese et al., 2010) and below chronic stress values (Weaver et al., 2021).

Stress is also a concern of reproduction as it can be responsible for sexual behavior abnormalities such as lower receptivity of ewes to ram attempts (Karsch and Breen, 2009; Dobson et al., 2012; Papargiris et al, 2011). Reproductive follow-up revealed that normal mating occurred as soon as the rams introduction in parcels. All ewes got mated during the first 15 days. This is expected regarding the reproductive cycle of ewes (17 days) and supports the idea that every ewe showed estrus during those first 15 days, with no silent estrus (ovulation without heat behavior, so without mating). The presence of normal mating is reassuring regarding the visual and behavioral impact solar panels could have had on sheep. Indeed, sexual behavior of ram is first stimulated by vision of ewes. The ram approaches the ewes and makes a peri-genital exploration of the ewe (sniffing), this is called the 'chasing behavior'. The peri-genital exploration of the ewe is made by flehmen and provides an olfactory stimulus for sexual behavior of the ram that kicks, licks and bites the ewe's wool. Those physical contacts enable to confirm receptivity of the ewe. Urination of the ewe even stimulates olfactorily the ram. Immobility of the ewe (in response to physical contact) signs the ewe's receptivity and stimulates the mating (the ram rises and serves the ewe) (Gaillard, 1979a and 1979b; Odagiri et al., 1995). Solar panels rows could have prevented visual stimulus for rams or have hindered chasing behavior or mating. This was not the case in the current study as we observed mating at the introduction of the rams in the flocks.

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Prolificacy and fertility at scanning represented typical reproductive performance for Texel ewes in Belgium (Dawson et al., 2002; Janssens et al., 2004). Consideration about reproductive seasonality is required when performing research with ovine. Sheep are seasonal breeders, showing polyoestrus during decreasing day-length period (fall and winter). Although some breeds (e.g. Ile-De-France) can reproduce out-of-season, French Texel cannot naturally breed outside the breeding season and is well-known seasonal breeder (Quirke and Hanrahan, 1985). It was therefore important to perform this study during fall to evaluate animals during their physiological breeding season. Reproduction parameters were either discrete counts (prolificacy) or binary outcomes summarized as proportions (fecundity, fertility). The calculated sample size for this study focused on a continuous variable (wool cortisol concentration) and was therefore smaller than the sample size required to detect differences in binomial responses (Zlowodzki and Bhandari, 2009). It means that differences in reproduction parameters should have been huge (and potentially nonphysiological) to be statistically significant with our sample size.

The modest number of animals included in the study and the short-term duration of sheep and parcels follow-up constitute a weakness of the study. Nevertheless, the sample size was calculated from previous studies hypothesizing solar panels could induce social stress in sheep and/or alter (reproduction) behavior (Pavie et al., 2022). This sample size was in the range of other ovine agrivoltaics research studies, ranging from 9 specimens (Andrew et al., 2021; Fonseca et al., 2023) to 20 specimens per group (Kampherbeek et al., 2023). However, to prove solar grazing is similar to conventional grazing, future study should include a higher number of animals since main hypothesize would be equivalence or non-inferiority (Flight and Julious, 2016). Duration of the follow-up for sheep and parcel was complying with the expected parcels ability to fulfil animals' nutritional needs in Fall, the limited grass renewal in December, and the farmers ovine management.

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This study could also have included other outcomes to document sheep social behavior in solar grazing, such as using GPS collars with accelerometers (like in precision livestock farming) to record sheep preferential distribution (under solar panels or in between), sheep activity (walking, resting, grazing), and the flock behavior. However, it should be kept in mind that the geolocation accuracy may range from 2m to 100m (Hansen and Riggs, 2008; Mendes et al., 2025; Tangorra et al., 2013; Vidal-Cardos et al., 2025). This accuracy is sufficient for farmers to locate their animals in permanent grassland from Alpine region, but it is insufficient to accurately discriminate between sheep under solar panels and sheep in the 4-m interspace between rows. Moreover, the metal structure of solar panels may introduce errors in animals' geolocation by interfering with GPS transmission. The ability to define a location varies between 43% to 99%, with daily variation (Herlin et al., 2021), suggesting this system would have limited application for solar grazing documentation.

To conclude, wool cortisol concentration may be a useful, easy and non-invasive technique that can be used to measure the hypothalamic-pituitary-adrenal axis activity and assess the impact of stress factors in sheep. In the example of the current study, wool cortisol concentration was low and not different between grazing types, meaning we did not find evidence of chronic stress in animals grazing under solar panels compared to conventional grazing.

CRediT author statement

All co-authors substantially contributed to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work AND drafted or critically revised the work for important intellectual content AND approved the final version to be published AND agreed to be accountable for all aspects of the work.

Conceptualization: FH, JMV, VS, VC, AH, FP, MLV, LP

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Supervision: FH

Project administration: FH

Writing - original draft and review & editing: LP, VS, JMV, VC, AH, FP, MLV, FH

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Figure Captions

Figure 1. Study design describing intervention on sheep before enrolment, follow up of animals during mating and grazing periods, and intervention at the end of the grazing period. A 15cm x 15cm square of wool was shaved at the lumbar site, at day 0, before starting grazing. Wool regrew during grazing period. At the end of grazing period, wool was re-shaved at the same anatomical. After washing and drying (large tube), wool was cut is small pieces (Petri dish) and cortisol was extracted in methanol (1.5-mL tube).

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Figure 2. Cortisol concentration in the wool collected at the end of grazing period and corresponding to regrown wool over the grazing period, for ewes in Conventional parcels (Parcel 1, left; Parcel 2, right) and Solar parcel.

Figure 3. Cumulative sum of mated ewes (color mark) at mating monitoring over to expected estrus intervals. Black round-dotted line is ewes in solar grazing. Blue long dash-dotted line (Parcel 1) and orange long dashed line (Parcel 2) are ewes in conventional grazing.

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