



# A long-day light program accelerates seasonal coat changes but is without effect on semen and metabolic parameters in Shetland pony stallions



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## ABSTRACT

Horses are seasonal breeders, and robust breeds may exhibit a winter hypometabolism when kept under semiferrous conditions. In this study, we analyzed the effects of artificial long days on rectal temperature, heart rate, heart rate variability, hematology, coat changes, semen parameters, and plasma testosterone concentrations in Shetland stallions stabled overnight and assigned to a control group (CON,  $n = 9$ ) kept under natural photoperiod, and a treatment group exposed to a long-day light program from 15 December to 20 March (AL,  $n = 9$ ). During the 8-month study, rectal temperature, heart rate, and heart rate variability at no time differed between groups. Plasma total protein ( $P < 0.05$ ), hematocrit, leukocyte, and lymphocyte counts ( $P < 0.001$ ) first increased and then decreased during the study period but did not differ between groups. Length of the guard hair decreased over time ( $P < 0.001$ ) and this decrease occurred earlier in AL than in CON stallions (time  $\times$  group  $P < 0.001$ ). Hair regrowth was faster in CON than in AL stallions (over time  $P < 0.001$ , time  $\times$  group  $P < 0.001$ ). Total sperm count increased from January to April (AL) and May (CON;  $P < 0.001$ ) but did not differ between groups. Sperm motility and percentage of membrane-intact spermatozoa showed no clear seasonal changes and semen parameters did not differ between groups. In conclusion, Shetland stallions showed seasonal variations in hair coat and total sperm count but only changes in hair coat but not semen parameters were advanced by a long-day light program.

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## 1. Introduction

Horses adapt to seasonal environmental changes in several ways. They are long-day breeders and increasing day length in spring stimulates reproductive activity [1]. The genetically fixed rhythm governing reproductive seasonality is synchronized with the geophysical year by changes in photoperiod but, as demonstrated in mares, is also influenced by ambient temperature [2] and energy intake [3]. Reproductive seasonality is more evident in less

domesticated breeds such as ponies compared to Thoroughbred or Warmblood horses [4]. While in Shetland pony mares kept outdoors a strict anovulatory season is observed from October to March (unpublished own observations), between 20% and 30% of Warmblood mares continue to cycle throughout the year [5]. In stallions, reproductive seasonality is less pronounced than in mares as they remain fertile throughout the year. However, release of gonadotropins in stallions is reduced in winter [6] and responds to long-term changes in photoperiod [7,8].

In addition to reduced reproductive functions, Shetland ponies kept under semiferrous conditions go through a phase of hypometabolism in winter [9] as has been described earlier for a nondomesticated equine species, the Przewalski

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horse [10]. In the Przewalski, changes in energy expenditure prepare the organism in advance for the decreased nutrient availability in winter [10]. It is not known if a winter hypometabolism exists in adequately fed, stabled horses and to what extent it is affected by changes in day length.

Artificial light programs are well established for advancing the onset of the breeding season in seasonally acyclic mares [11,12]. Exposure of stallions to artificial short days from July to December followed by 16 hours of light daily until March resulted in a higher total sperm count in February compared to control stallions kept under natural photoperiod [13]. Shorter and easy to apply light programs might thus be of interest for sires that are breeding large numbers of mares early in the breeding season. Whether exposure to prolonged day length starting in December, a protocol effective in advancing the breeding season in mares [11,12], improves semen parameters in stallions to the best of our knowledge has not been tested.

In this study, we have analyzed the effects of artificial long days on body weight, rectal temperature, heart rate, heart rate variability, hematology, coat changes, and semen parameters in Shetland stallions kept outdoors during the day and stabled overnight. We hypothesized that seasonal changes in these parameters are regulated by photoperiod and therefore respond to a long-day light program.

## 2. Materials and methods

### 2.1. Animals

A total of 18 fertile Shetland pony stallions were included into the study. At the beginning of the experiment, stallions were aged between 3 and 22 years (mean  $9.3 \pm 1.7$ ) and weighed between 116 and 199 kg (mean  $153.8 \pm 6.5$  kg). They were kept in one outdoor paddock between 8 AM and 4 PM and were separated by group in two spacious indoor stables for the remainder of the day. Hay was fed twice daily and water was freely available. The stallions were neither trained nor exercised during the study period and were used to regular semen collections.

### 2.2. Experimental design

The study was carried out in Vienna, Austria (longitude  $16.4^\circ$ , northern latitude  $48.3^\circ$ ) between 1 December and 31 July. For the study, stallions were ranked by age and assigned in alternating order to two groups. The control group (CON,  $n = 9$ ) was kept under natural light only, whereas the treatment group (AL,  $n = 9$ ) received additional light from 15 December to 20 March (spring equinox). Body weight, rectal temperature, heart rate, heart rate variability (HRV), hematologic parameters, quality and length of the coat, and semen parameters were determined. The study was approved by the competent authority for animal experimentation (Austrian Federal Ministry for Science and Research, license number BMWF-68.205/0194-II/3 b/2013).

### 2.3. Light program

All stallions were exposed to natural light during daylight hours. Stallions of group AL were exposed to

artificial light from 4 PM to 16 hours after sunrise each day from 15 December to 20 March. Light was provided by two standard lamps (Ritos R7s, 500W, Ritter Leuchten, Mömbris, Germany), and size of the stable was  $12 \times 7$  meters. Light was dispersed by reflecting screens to provide a near-homogenous light distribution in the stable. Group CON stallions were kept in a separate, identical barn compartment without artificial light. After termination of artificial illumination in group AL on 20 March, all stallions were kept under natural light only until the end of the experiment on 31 July.

### 2.4. Body weight, rectal temperature, hematology, and testosterone

Body weight was measured on a weigh-bridge once a month. Rectal temperature was determined with a clinical thermometer (resolution  $0.1^\circ\text{C}$ , Microlife, Widnau, Switzerland) at 1-month intervals between 8 and 10 AM. For a complete blood count and analysis of plasma testosterone concentration, blood was collected once monthly from one jugular vein into polystyrene tubes containing EDTA or lithium heparin, respectively (Vacuette, Becton Dickinson, Schwechat, Austria). A complete blood count was performed with routine hematology techniques. Blood for testosterone analysis was centrifuged immediately for 10 minutes at 3000 g. The supernatant was aspirated and frozen at  $-20^\circ\text{C}$  until testosterone analysis by direct enzyme immunoassay (Testosterone ELISA, Demeditec Diagnostics, Kiel, Germany) without extraction. The assay has been validated for equine plasma in our laboratory. The intra-assay coefficient of variation was 7.4%, interassay variation was 8.4%, and the minimal detectable concentration 0.01 ng/mL.

### 2.5. Heart rate and heart rate variability

Cardiac beat-to-beat (RR) intervals were recorded with mobile heart rate monitors (S810i, Polar, Kempele, Finland) as described [14]. In brief, an elastic girth containing two electrodes was fixed around the stallion's thorax, the positive electrode located on the left side of the withers and the negative on the left side of the thorax next to the heart base. Water and ultrasound transmission gel were used liberally to optimize contact between the electrodes and the skin. A second girth, containing a small pouch for the recording watch, was fixed over the elastic girth. It also prevented the stallions from removing the heart rate monitors by social interactions, when playing or grooming. Cardiac recordings of 60 minutes duration were made at monthly intervals starting always at 7 AM. Recordings were made on two consecutive days with four or five stallions from each group recorded simultaneously on each day. The stallions remained in their groups during cardiac recordings.

At the end of each recording period, data from the watches were retrieved via infrared transmission. For HRV analysis, the Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was applied. From the 60-min recordings, largely artifact-free 5-minute intervals were

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