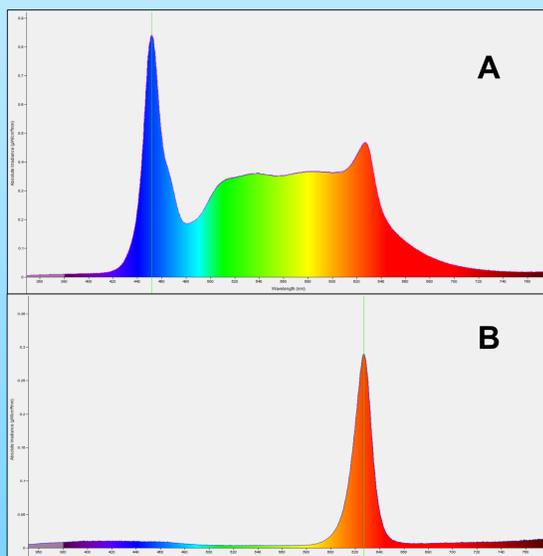


## Introduction

Racehorses spend much of the 24-h day indoors and unexposed to optimal daily light intensities or to the natural fluctuations in the environmental LD cycle. Frequent interactions with horses during night-time hours result in erratic exposure to light at night. In order to investigate whether a customized lighting system that better mimics the natural environmental LD cycles could influence body composition and peripheral rhythmicity in clock gene expression in horses in training, a 'smart' lighting (SL) system with the following features was developed:

1. Daytime polychromatic light was provided by white LEDs (peak wavelength approx. 555 nm at absolute irradiance of  $<0.84 \mu\text{W}/\text{cm}^2/\text{nm}$ , Fig. 1A).
2. Dawn and dusk were simulated by gradual increases in light intensity from 5->200 lux and decreases from >200-5 lux, respectively, over a 20 min interval.
3. An automatic timer controlled the duration of daily exposure to light such that dawn and dusk occurred at one hour prior to and two hours after natural environmental dawn and dusk, respectively. Daylength duration ranged from 10.5 – 14.5 hours during the study periods.
4. The SL system dimmed to red light at night ( $<5 \text{ lux}$ , peak wavelength approx. 625 nm at absolute irradiance of  $<0.3 \mu\text{W}/\text{cm}^2/\text{nm}$ , Fig. 1B)

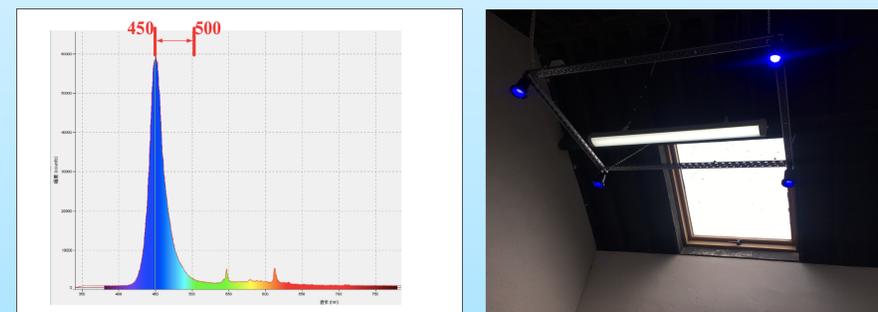


**Figure 1.** Customised 'smart' lighting system for horses comprising A) polychromatic white LED light by day and B) red LED light at night

## Methods

In study 1, 54 2-yr-old Thoroughbreds in training, housed in individual stalls at a yard in Ireland, were divided into two groups and blocked for gender. Beginning Jan 2016, Group 1 received light treatment via an SL lamp hung centrally in each stall at 3.5 m from the ground. Group 2 were maintained under traditional lighting consisting of a single fluorescent tube. Management was instructed to use the stable lights as normal for the control group. Body composition, evaluated as rump fat thickness (RFT), body weight (BW) and calculated fat free mass (FFM), was determined at three-week intervals for 15 weeks. All horses experienced an exercise regime consisting of morning exercise 6 days/week that gradually increased in intensity per week as fitness improved.

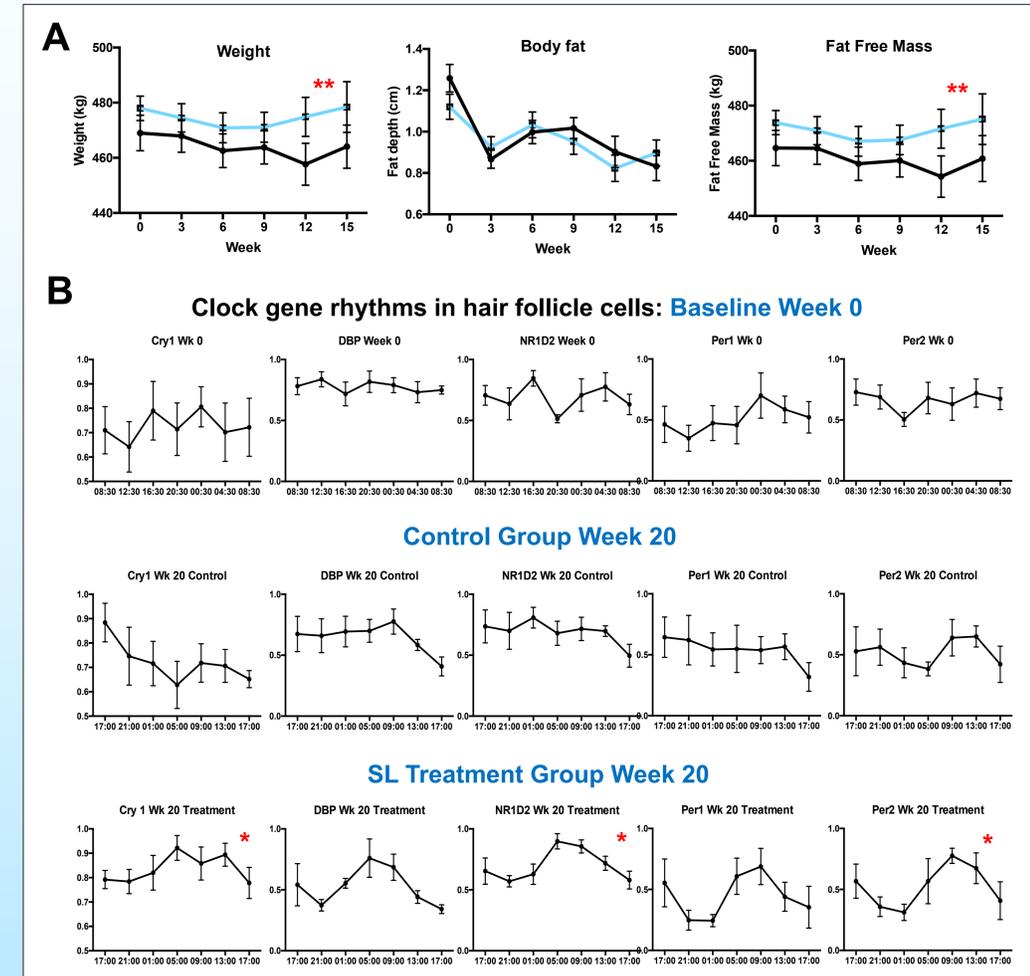
Study 2 employed a similar experimental design but used an SL lamp with supplemental short wavelength blue light provided by additional LED bulbs (Fig. 2) and began Jan 2017. On weeks 0 and 20, hair follicle samples were collected at 4-h intervals over 24-h from 5 horses per group and RNA interrogated for *Arntl*, *Cry 1*, *Dbp*, *Nr1d2*, *Per1* and *Per2* transcripts. Statistical analyses were conducted using Graph Pad Prism Version 5.0 or the Cosinor programme (Refinetti et al. 2007)



**Figure 2.** Customised 'smart' lighting system used in Study 2. A) Spectrum provided by supplemental blue light LED bulbs. B) Assembly of white LED tube and supplemental blue LED bulbs



## Results



**Figure 3**

**A)** Study 1 results: Two-way ANOVA revealed no time x treatment interaction for FFM, BW or RFT. An effect of treatment for FFM and BW ( $p<0.01$  and  $p<0.01$ ; respectively) and an effect of time for RFT ( $p<0.0001$ ) was observed. Blue lines indicate SL treated horses and black lines indicate control horses  
**B)** Study 2 results: 24-h rhythmicity was detected for *Nr1d2*, *Per2* and was close to significant for *Cry 1* ( $p=0.013$ ,  $p=0.013$  and  $p=0.051$ , respectively) in the treatment group (Group 1) on week 20 only.

## Conclusions

We conclude that an optimised LD cycle provided by a novel 'smart' lighting system influences body composition in young Thoroughbreds in training. Increases in FFM, considered an important indicator of athletic ability, may contribute to improved performance in equine athletes.

It is proposed that these improvements may occur as a result of strengthened circadian rhythmicity of peripheral clocks and better internal synchronisation. Future studies will evaluate improvements in feed efficiency, behaviour (activity rhythms) and performance parameters.