**Application**

This work is expected to improve understanding of exercise-induced inflammation and environmental conditions to optimize exercise adaptation and welfare in performance horses.

**Introduction**

Understanding daily fluctuations in physiological parameters can optimize livestock management by aligning practices with animals' circadian rhythms. Performance and cardiorespiratory responses (Kang et al., 2023) are known to vary based on the time of day of exercise, but the equine inflammatory response to exercise at different times of day is unknown. Additionally, the impacts of disrupted circadian rhythms due to unnatural barn lighting on inflammation is unexplored in horses. We hypothesized that exercise-induced inflammation would be greater following exercise in the morning compared to the evening regardless of barn light conditions.

**Materials and Methods**

Experimental protocols were reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee (2022-0268). Quarter Horses (14 fillies, 14 geldings; mean±SD 18±1mo; 360±31kg) were blocked by sex, age, and bodyweight and assigned to one of three treatments for 45 days: 1) Control (CON) – 166 lux sunlight by day, no artificial light at night; 2) Competition (COMP) – 25 lux soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with a 50-lux Equilume blue light mask by day and red-tinted visor by night to filter blue light. On d36, half of the horses performed a 2-h submaximal exercise test (SET) at 0600 h (AM) while the other half performed the same SET at 1800 h (PM). On d43, horses repeated the SET but at the opposite time of their d36 SET. Blood was collected pre-, 0, 4, 10, 22, 28, and 46h post-SET (T-2, T0, T4, T10, T22, T28, and T46, respectively) and analyzed for concentrations of anti-inflammatory cytokines, interleukin (IL)-4 and IL-10, and pro-inflammatory, IL-8 and tumor necrosis factor (TNF)α, in plasma and serum amyloid A (SAA). Data were analyzed using PROC MIXED in SASv9.4 with repeated measures (time); horse within treatment (trt) was a random effect and sex, trt, time pre/post-SET, time of exercise (AM v PM), and all interactions were fixed effects. Data presented as least squared means ± SEM.

**Results**

Regardless of trt or AM/PM, IL-10 increased from T-2 to T0 (92±8 pg/mL to 99±8 pg/mL, *P*=0.02) but returned to T-2 levels by T4 (91±8 pg/mL). Regardless of trt, IL-8 tended to increase from T-2 to T0 (982±109 pg/mL to 1,150±108 pg/mL) in PM SET (*P=*0.06) but decreased during that time in the AM SET (1,140±108 pg/mL to 965±110 pg/mL, *P*=0.05)resulting in IL-8 being greater at T0 in the PM compared to the AM SET (*P=*0.04). In the AM, IL-8 continued to decrease to T4 (857±109 pg/mL, *P*=0.002) but returned to T-2 levels by T10 (1,070±109 pg/mL). In the PM, IL-8 returned to T-2 levels (982±109 pg/mL) by T4 (1,025±109 pg/mL), tended to increase to T10 (1,191±109 pg/mL, *P=*0.06) to be above T-2 (*P=*0.02), then returned to T-2 levels by T22 (1,068±108 pg/mL). IL-10 and TNFα were greater in COMP during PM SET than AM SET (*P*<0.05; Table 1). For CON, IL-4 was greater (*P*<0.0001) and TNFα tended to be lesser (*P*=0.07) in thePM SET than AM SET. Conversely, TNFα tended to be greater in CIRC horses during the PM SET than AM SET (*P*=0.09). During the PM SET, CON IL-4 was greater than CIRC (*P*=0.05)and tended to be greater than COMP (*P*=0.08) while IL-10 and TNFα tended to be greater in COMP than CON (*P*=0.09). In all horses at AM and PM, SAA increased from T-2 to T22 (*P*<0.001) and remained elevated at T28 (*P*<0.0001; Figure 1). In CON and COMP, SAA then decreased at T46 (*P*<0.05) to be similar to T4 but above T-2 (*P*<0.05); in CIRC, SAA remained elevated at T46 in AM SET (*P*<0.05) butdecreased at T46 in PM SET (*P*=0.04)to levels above T-2 (*P*<0.0001).

**Table 1.** Mean cytokine concentrations surrounding a 2-h standardized exercise test (SET) performed at 0600 h (AM) or 1800 h (PM) in horses under three different light treatments for 45 days: 1) Control (CON) – sunlight by day, no artificial light at night; 2) Competition (COMP) – soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with an Equilume blue light mask by day and red-tinted visor by night.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Cytokine, pg/mL | Trt | AM SET | PM SET | Pooled SE | *P*-Value | | |
| **Trt** | **Time** | **Trt × Time** |
| IL-10 | CON  COMP  CIRC | 84.9a  107.7a  85.5a | 81.1a  112.0a\*  83.3a | 0.23 | 0.3 | 0.001 | 0.02 |
| IL-4 | CON  COMP  CIRC | 508.2a  354.2a  350.1a | 605.2a\*  386.3ab  345.3b | 1.61 | 0.2 | 0.9 | 0.004 |
| IL-8 | CON  COMP  CIRC | 1155.5a  962.1a  924.9a | 1233.9a  924.0a  1023.9a | 3.22 | 0.5 | 0.06 | 0.2 |
| TNFα | CON  COMP  CIRC | 32.9a  47.0a  45.7a | 30.1a  51.5a\*  48.4a | 0.16 | 0.3 | 0.7 | 0.002 |

\*Within treatment, PM differs from AM (*P*<0.05); abcWithin time of SET, treatments with different letters differ (*P*<0.05)



**Figure 1.** Serum Amyloid A concentrationspre-, 4, 22, 28, and 46h post- (T-2, T4, T22, T28, and T46, respectively) a 2-h standardized exercise test (SET) performed at 0600 h (AM) or 1800 h (PM) in horses following 45 days of one of three different light treatments: 1) Control (CON) – sunlight by day, no artificial light at night; 2) Competition (COMP) – soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with an Equilume blue light mask by day and red-tinted visor by night.

**Conclusions**

These data suggest that PM exercise is less inflammatory than AM exercise for horses under ideal circadian lighting (blue light/sunlight by day and absence of blue light at night), but this is not consistent in horses under weak barn lights 24 h/d. Optimizing time of day of exercise and barn lighting conditions could improve exercise-induced inflammatory responses in young horses.

**Acknowledgements**

The authors acknowledge funding from Equilume and Texas A&M Institute for Equine Sciences.

**References**

Kang, J., Ratamess, N.A., Faigenbaum, A.D., Bush, J.A., Finnerty, C., DiFiore, M., Garcia, A., Beller, N., 2023. Time-of-day effects of exercise on cardiorespiratory responses and endurance performance—A systematic review and meta-analysis. Journal of Strength and Conditioning Research. 37(10), 2080-2090.