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# Leucocyte coping capacity, serum cortisol and neutrophil to lymphocyte ratio in ponies exposed to repetitive handling during a research project

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## Introduction

- Stress is an umbrella term indicating increasing allostatic load and triggers a stress response to challenges.
- The ability to recognize and objectively measure the stress response is crucial for optimizing horses' wellbeing.
- Traditionally used stress makers such as cortisol concentrations or the neutrophil granulocytes to lymphocyte (NEU:LYM) ratios are expected to increase with stress.
- Stress reduces the oxidative burst capacity of NEU and therefore the leucocyte coping capacity (LCC) is impaired with increasing stress load.

## Hypothesis

- Increased stress load induced by repetitive handling and sample collection during a research project protocol would increase the cortisol concentrations and NEU:LYM ratios and decrease the LCC levels.
- There would be a negative correlation between cortisol concentrations, or NEU:LYM ratios and LCC levels.

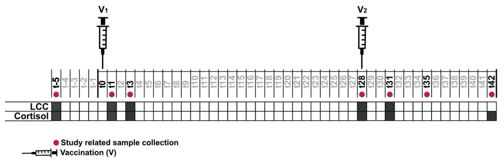


Fig. 1. Timeline of the study. Highlighted days represent study related handling, red dots represent days with study related blood/sample collection, filled boxes represent analysis of cortisol or LCC measurement at the respective day.

## Material and Methods

- Animals: Nine ponies (mean age 6.5 ± 5.3 years, mean body weight 135.2 ± 22.2 kg).
- Serum cortisol concentrations were measured by an established and validated EIA.[1,2,3]
- Hematologic analyses were performed by a commercial automated cell counter (ProCyte Dx, IDEXX Vet Med Labor GmbH, Vienna).
- LCC was measured by a portable chemiluminometer (by Oxford MediStress Ltd, Oxford).
- The study protocol included at two-dose vaccination course 28 days (d) apart and an observation period of 42 d including repetitive handling and blood sampling.
- Values were measured the day of vaccination or booster administration (d1, d28), 3 days later (d3, d31) and on d42 and were compared to values before the start of the study (d-5) by mixed models.
- Pearson's correlations were explored between the area under the curve (AUC) over 90 min of LCC ($AUC_{LCC}$,), LCC:NEU ($AUC_{LCC:NEU}$), CORT and NEU:LYM.

## Results

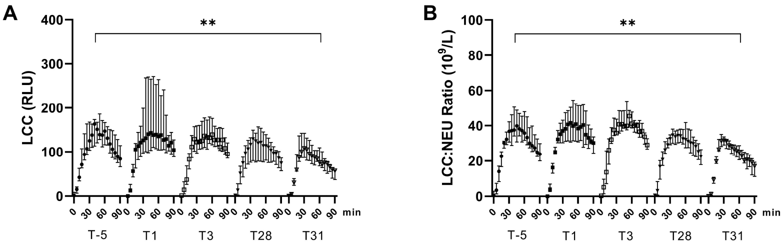


Fig. 2. The leukocyte coping capacity (LCC, A) expressed as relative light units (RLU) and LCC to neutrophilic granulocyte (NEU) count ratio (LCC:NEU ratio, B) is shown at each of the sampling timepoints. Symbols represent median and bars represent Q1-Q3, **, p<0.01.

**The $AUC_{LCC}$ (p=0.006) and $AUC_{LCC:NEU}$ (p=0.009) decreased significantly between T-5 and T31.**

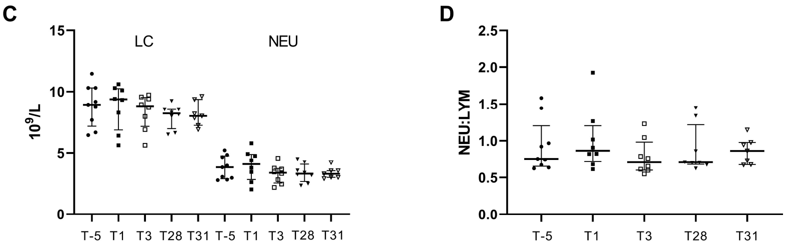


Fig. 3. The leukocyte (LC) count (C), the NEU count (C) and the NEU:LYM ratio (D) is shown for the sampling timepoints with symbols for each measurements, median (tick bars) and interquartile ranges (Q1-Q3, thin bars).

**The LC count (p=0.662), the NEU count (p=0.524), and the NEU:LYM ratio (p=0.701) were constant over the timepoints and were within the reference ranges.[4,5]**

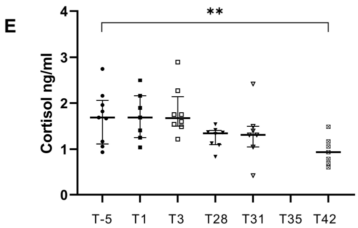


Fig. 4. Cortisol concentrations are shown for the sampling timepoints with symbols for each measurement, median (tick bars) and interquartile range (thin bars, Q1-Q3).

**The cortisol concentration decreased to levels even below the basal values of T-5. The difference over time obtained statistical significance between T-5 and T42** (**, p<0.01)**.**
**There was no correlations between $AUC_{LCC:NEU}$ and cortisol concentrations or NEU:LYM ratios.**

## Conclusion and Discussion

The progressive decrease of $AUC_{LCC}$, and $AUC_{LCC:NEU}$ may suggest increasing stress load over time. However, this interpretation is not supported by the time course of cortisol concentrations, the NEU:LYM ratios, or the correlations between these stress markers.

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References: 1) Palme et al., 1997, Int J Mamm Biol 62: 192-197. 2) Möstl et al., 1999, Zentralbl Veterinarmed A 46(10): 621-631. 3) Flauger et al., 2010, Vet Res Commun 34(2): 185-195. 4) Shawaf et al., 2018, Int J Vet Sci Med 6(1): 61-64. 5)Witkowska-Pilaszewicz et al., 2021, BMC Vet Res 17(1): 38.