**Lameness induced behavioural changes in dairy cows: independent of time of day and temperature effect**

**Application**: Behavioural changes can serve as indicators for detecting lameness in dairy cows. However, there is limited clarity regarding what behavioural changes are specifically attributed to lameness.

**Introduction:** Knowing and understanding the behavioural indicators could allow for development of an effective lameness detection systems. Particularly when other factors inducing natural behavioural alterations such as temperature and time of day are involved. Therefore, this study aimed to investigate behavioural changes attributed to lameness in dairy while accounting for temperature and time of day effects.

**Materials and Methods**: Data (total records: 14,587) were collected at 12 Dutch dairy farms in 2021 and 2022 from summer to early autumn. Visual behavioural observations (15 minutes per observation) were carried out per farm on healthy and lame cows on pasture for a week. For statistical analysis, duration (fraction of the portion of the 15-minutes) and frequency (counts per 15minutes) of different behaviours were computed from the processed data and used as response variables. The response variables were then tested against the predictors: health status (lame or healthy), time of day (time from start of observation), and temperature (maximum wet bulb). The behaviours included were: grazing, lying, standing, ruminating and walking. An array of quantile regression models to estimate the medians were then developed to examine how response variables relate to the predictors and their interactions. Various combinations of potential predictors which could explain a specific behaviour were tested. Models with the lowest AICs were selected regardless of the number of predictors involved and considered a result for a particular behaviour.

**Results:** Lame cows displayed increased walking and lying durations compared to healthy cows. Inversely, healthy cows increased rumination duration, while lame cows decreased when temperatures were average and hot. Interestingly, colder temperatures led to longer rumination in lame cows compared to the healthy. All cows decreased standing duration in average and hot temperatures, with lame cows consistently standing less. Grazing durations decreased for both groups in average temperatures in the early morning, with hotter days resulting in more grazing during midday. Even with these adjustment, lame cows consistently grazed lesser durations than healthy cows. Lame cows also showed increased lying frequency and decreased walking as well as standing compared to healthy cows. In contrast, all cows increased grazing and reduced rumination at midday when temperature were average and hot.

**Conclusion:** Despite temperature and time of day effects, differences in behaviour between lame and healthy cows persisted. Suggesting that some behaviours could be linked to lameness despite the influence of temperature and time.