



Rhythmicity as a welfare indicator – investigating the effect of extrinsic zeitgebers in equines as a model organism

Sonia Rey^a, Hassan Roland Nasser^b, Marianne Cockburn^{a,*} 

^a Equids, Swiss National Stud (SNS), Agroscope, Avenches, Switzerland

^b Digital Production, Agroscope, Tänikon, Switzerland

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ABSTRACT

To effectively assess and maintain animal well-being, reliable and accessible indicators are crucial. Researchers have proposed using rhythmicity as a measure of animal welfare. An animal in a good state of welfare would, ideally, follow the same daily rhythmic patterns while disruptions in these patterns may signal stress or illness. Although promising in certain cases, it remains unclear how rhythmicity reflects the well-being of animals in managed husbandry systems, where external (human) factors might impact their rhythmicity. This study investigated how extrinsic factors, such as feeding times, affect the rhythmicity of domesticated horses, aiming to assess its value as a welfare indicator. Twenty horses, of which 18 were included in the final analysis, were allocated to four groups and exposed to three feeding regimes according to a randomized crossover design: three 2-hour feeding periods during daylight hours, six 1-hour feeding periods distributed over 24 hours, and ad libitum access to hay through a slow-feeding net.

Continuous acceleration-derived activity data were recorded using accelerometers attached to the horses' left front legs. The resulting time-series data were used to determine lying duration based on the orientation of the accelerometer's y-axis. The data were also analysed using Fourier transformation to calculate the Degree of Functional Coupling (DFC), which quantifies the temporal organisation of the horses' daily locomotor activity patterns and the extent to which these patterns are coupled to periodic environmental cues; it does not represent the animals' overall biological rhythmicity. We established a linear mixed-effects model with DFC as the response variable. Bayesian Information Criterion-based model selection revealed that lying duration and feeding regime were the most effective predictors of DFC. Our analysis showed a significant effect of the experimentally imposed feeding schedules on the temporal organisation of the horses' daily locomotor activity patterns. In conclusion, activity-derived DFC should be interpreted cautiously in managed husbandry systems. Extrinsic factors that impose or reinforce rhythmic locomotor activity patterns should be considered when comparing DFC across systems.

1. Introduction

1.1. Animal welfare: a current topic

Animal welfare has shifted from a specialist concern to a major societal and political priority. Particularly in the equine sector, the social license to operate has recently been questioned (Fiedler & Slater, 2024). The equine sector encompasses a wide range of activities, including sport, recreation, breeding, work, and companionship, with horses also used for food production in some regions. The welfare risks and management priorities differ among these contexts (Holmes & Brown, 2022).

In equestrian sport, evidence-based management of training, competition, health, housing, and the horse–human relationship is considered essential for safeguarding both the physical and psychological welfare of horses (Williams et al., 2023). Maintaining high welfare standards is also increasingly important for preserving the social acceptability and social license of equestrian activities (Douglas et al., 2022). Irrespective of their use, horses require management that accommodates fundamental species-specific needs, particularly access to forage, freedom of movement, and social contact with conspecifics (Phelipon et al., 2024). Pets play an increasingly significant role within our society (Blouin, 2012), and the desire to meet their needs is becoming a central preoccupation.

* Corresponding author at: Agroscope Standort Posieux Avenches, Switzerland
E-mail address: marianne.cockburn@agroscope.admin.ch (M. Cockburn).

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In addition, consumers of animal products are concerned about what they eat, and thus animal welfare considerations are leading many consumers to change their dietary habits (Janssen et al., 2016). On the other hand, to meet this growing demand for higher animal welfare standards, political and legislative authorities have gradually adapted their strategies, resulting in stricter rules and regulations, on top of more demanding label requirements for animal welfare (Cardoso et al., 2017). Scientists address these concerns by working towards the development of objective and reproducible methods to measure animal welfare in livestock. For instance, in equines, scientists and practitioners typically assess equine well-being through behaviour (Hausberger et al., 2012; Rochais et al., 2016), body language (Heleski et al., 2002; Redbo et al., 1998), acoustic signals (Stomp et al., 2018) or physiological signals (Pawluski et al., 2017). However, these parameters are highly dependent on the context and on environmental factors (Lesimple, 2020) and their assessment is extremely labor intensive.

1.2. Rhythmicity as a welfare indicator

Another approach to assess an animal's welfare state is through the study of biological rhythms (Froy, 2011). In horses, biological processes are temporally organised across different timescales, particularly through circadian rhythms of approximately 24 hours and circannual rhythms occurring over the course of a year (Berger et al., 1999; Murphy, 2019; Aragona et al., 2024). The intensity, spectral composition, and timing of light exposure may also affect circadian organisation and should therefore be considered when studying biological rhythms and animal welfare (Lucas et al., 2024). These rhythms coordinate physiological and behavioural processes, including sleep and activity, body temperature, hormone secretion, feeding behaviour, metabolism, reproduction, and physical performance (Murphy, 2019; Aragona et al., 2024; Giannetto et al., 2022). In horses, the light–dark cycle is a major synchronising cue, while feeding schedules, exercise, social interactions, housing conditions, and other management practices may also influence the temporal expression of physiological and behavioural rhythms (Murphy, 2019; Aragona et al., 2024). This temporal organisation enables horses to anticipate predictable environmental changes and coordinate their internal functions accordingly. The maintenance of stable and appropriately synchronised biological rhythms contributes to physiological homeostasis. Conversely, alterations in their strength, timing, or synchronisation may indicate exposure to environmental or management-related challenges and may therefore provide information relevant to health and welfare assessment (Aragona et al., 2024). For example, accelerometer-derived 24-hour activity periodicity has recently been investigated as a potential welfare indicator in calves, although synchronous changes between animals also indicated an influence of shared environmental conditions (Rhodes et al., 2022). Housing conditions, feeding schedules, exercise, and seasonal changes may affect daily patterns of body temperature, locomotor activity, feeding behaviour, and other physiological variables in horses and other domestic animals (Murphy, 2019; Aragona et al., 2024). In horses, forage availability and feeder type have also been shown to affect behavioural time budgets and abnormal behaviours, while no corresponding treatment effect was detected in cortisol circadian rhythmicity (Carvalho Seabra et al., 2023). However, the interpretation of observed behavioural rhythms requires consideration of both endogenous regulation and the external conditions under which they are expressed.

1.3. Measuring animal rhythms

Circadian rhythms can be detected in various physiological markers such as heart rate variability or body temperature (Palacios et al., 2021; Piccione et al., 2003) as well as food intake (Parker et al., 2022) or locomotor activity (Piccione et al., 2005). Depending on the variable, time-series data may be obtained using bio-loggers for temperature and cardiac activity (Palacios et al., 2021), automated weighing feeders or

RFID-based systems for food intake (Parker et al., 2022), or wearable accelerometers for activity-derived 24-hour periodicity (Fuchs et al., 2022; Rhodes et al., 2022). All these parameters require time series data collection and processing to reveal rhythmic patterns in activity or processes. A large literature survey summarized the most common techniques for assessing periodicity in such data (Refinetti et al., 2007). Analysis of variance can identify time-dependent differences, whereas cosinor analysis characterises approximately sinusoidal rhythms using parameters such as the mesor, amplitude, and acrophase (Refinetti et al., 2007; Cornelissen, 2014). Fourier analysis decomposes a time series into its constituent frequency components (Refinetti et al., 2007). DFC is derived from Fourier analysis and quantifies the contribution of harmonic frequencies to the significant periodic components of the analysed time series (Scheibe et al., 1999; Nasser et al., 2025). DFC can therefore be calculated from different regularly sampled behavioural or physiological time series. In the present study, we selected DFC as the method for analysing rhythmicity because it provides a single index of harmonic organisation and has previously shown promising results in animal-welfare research (Fuchs et al., 2022).

1.4. The degree of functional coupling

Over the past years, the increasing desire to develop an objective and automatically accessible parameter to assess animal welfare has led to increased application of the Degree of Functional Coupling (DFC) in livestock research. Recent studies have applied DFC to investigate activity rhythms in dairy cows under automatic milking, cow–calf contact, and virtual-fencing systems (Fuchs et al., 2022; Schneider et al., 2024; Fuchs et al., 2025). The DFC is essentially computed by applying a Fourier Transformation to time-series data (Scheibe et al., 1999). When calculated from locomotor activity data, DFC quantifies the temporal organisation of recurring activity patterns and their coupling to periodic environmental cues; it does not measure the organism's overall circadian rhythmicity. DFC ranges from zero to one. Lower values indicate less harmonic organisation of the analysed time series, whereas values closer to one indicate stronger coupling of recurring activity patterns to periodic environmental cues (Sinz & Scheibe, 1976). The DFC has recently been made even more accessible to researchers, by being offered in an open-source CRAN package “DigiRhythm” version 1.1 (Nasser et al., 2025). The use of this parameter showed encouraging results in extensively managed sheep (Nunes Marsiglio Sarout et al., 2018) and housed dairy cows (Fuchs et al., 2022). Moreover, it has been successfully used to evaluate how wild horses engage in activity and feed (Berger et al., 1999). Thus, the relevance of the DFC is increasing in animal science.

The Degree of Functional Coupling (DFC) computed on time-series data of activity or feeding offers an estimate of the synchronization between an organism and the 24-hour environmental cycle (Scheibe et al., 1999). Most authors using the DFC computation (Fuchs et al., 2022; Nunes Marsiglio Sarout et al., 2018; Scheibe et al., 1999; Sinz & Scheibe, 1976) recommended using a 7-day rolling window of continuous data to compute one DFC value, thus one DFC value represents the synchronization with the environment for the 7 days covered in this specific rolling window. This further implies that any irregularity will cause a decrease of the DFC in any value including this event in the rolling window (Fig. 1).

1.5. Rhythmicity driven by extrinsic management cues

Despite the potential of rhythmicity as a welfare indicator for farmed livestock, currently, it remains uncertain how accurately the DFC mirrors the well-being of animals in extrinsically managed husbandry systems, where rhythmicity might be influenced by external factors such as feeding schedules, exercise routines, or other management-related activities.

Particularly in horses, humans influence daily activity patterns

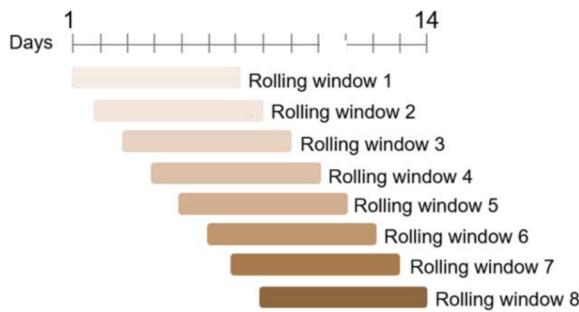


Fig. 1. DFC calculation over 7-day rolling windows.

through domestication. As highlighted by [Murphy \(2019\)](#), horses in their natural habitat tend to have a continuous and low level of exercise and constantly eat in small portions. However, in captivity, they are typically fed at specific times during the day and exercise with reduced frequency but higher intensity. In other words, wild horses are guided by their intrinsic motivation in the completion of their rhythmicity patterns whereas captive horses are subject to supplementary extrinsic factors such as feeding schedules determined by humans. To date, it is unclear how these extrinsic factors affect rhythmicity in farmed livestock, and as such it is unclear if this needs to be respected when using the DFC as a welfare indicator.

In the current study, we investigated the influence of extrinsic factors, such as feeding management, on the rhythmicity of managed horses, offering further insight into the quality of the DFC as an indicator for the well-being assessment of animals.

2. Materials and methods

2.1. Animal license

The current study was conducted in accordance with Swiss legislation on animal experimentation, and was approved by the Fribourg Cantonal authority under the authorization 2023-40-FR.

2.2. Animals, group allocation, housing, and management

The experiment took place in four paddock trail group housing systems (166 × 33 m each) at a specially designed research site. This trial included 20 research mares: 17 Swiss Warmbloods, one Holsteiner, one Oldenburg, and one Baden-Württemberg Warmblood. The horses were kept in four separate groups of five mares, with each group housed in a separate enclosure. The mares were not newly allocated to groups for the present study. They were maintained in four pre-existing, socially stable groups of five mares that had been established for the associated experiment and had remained unchanged for at least six months before the trial. The four groups were separated physically by an electric fence and chosen due to a convenience sample from an associated experiment (Roig-Pons et al., 2025). Visual and acoustic separation of the groups was not possible in this setup. In each of the enclosures, one shelter (14.5 × 3.70 m) was provided, with wood shavings, serving as bedding. Additionally, the enclosure included one drinking trough, situated on one side, while a hayrack and a sand rolling area were located on the opposite side (Fig. 2). The horses were fed hay exclusively. During the 3- and 6-feedings/day treatments, access to hay in the automatic hayrack was restricted to the programmed feeding periods. During the ad libitum treatment, hay was continuously available through a slow-feeding net. The hay net covered the forage with medium-sized mesh (approximately 4.5 cm). Straw was supplied inside the shelters, directly on the bedding material. The external surface available for the horses was stabilized with gravel and grids (Ecoraster floorgrids, Ecoraster, Arzberg, Germany). An aerial view of the housing system is provided (Fig. 2).

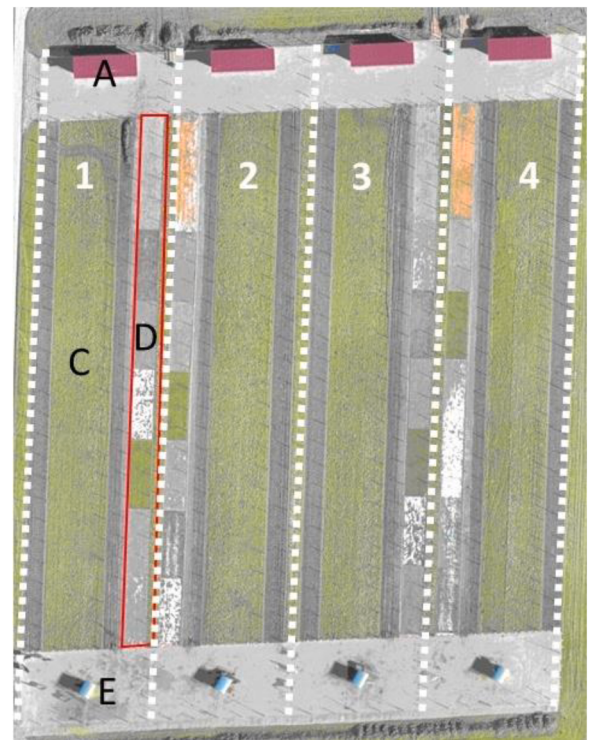


Fig. 2. Aerial view of the housing system. The numbers 1-4 and the dashed lines represent the enclosures separating the four groups of horses. The letters correspond to the different functional areas. A: shelter B: automatic drinking trough C: pasture (no access during the trial) D: paddock trail connecting functional areas E: automatic hayrack.

All mares were retired sport or breeding horses, had a mean age of 13 years (range: 6–18 years), and had been purchased specifically as research animals. The mares were specifically housed at this site for a 2-year research project investigating the interaction between soil and horses in paddock trail systems (Hiltebrand et al., 2026). This trial affected all groups in the same way, as they needed to cross the trails, voluntarily, across paddock grids arranged in a different order along the trail. Although the horses showed no acute clinical issues at the time of relocation to the paddock trail site, their medical histories reflected the reasons for their retirement from sport or breeding. Eighteen of the 20 mares had documented chronic or recurrent musculoskeletal conditions, including back pain or kissing spines, cervical osteoarthritis, navicular disease, club foot, tendon or suspensory-ligament disorders, and unilateral or bilateral chronic or recurrent lameness. Two mares had a history of colic, including one mare that had undergone colic surgery in 2012. One mare had no specific clinical diagnosis but had been retired because of unwillingness during work. Before relocation to the paddock-trail research site, the horses were housed at the Swiss National Stud in four separate group-housing systems, maintaining the same group compositions used during the present study. The groups had visual and acoustic contact with one another. Each housing system provided a straw-bedded shelter, drinking facilities, and a time-controlled hayrack that provided hay access four times per day for a total of 10 hours within each 24-hour period. The available enclosure area was approximately 210 m² per horse. The group compositions had remained stable for at least six months before relocation to the research site. Horses were transported for 6 km (about 10 minutes) in a horse lorry within their known groups.

At the research site, all horses were checked twice daily, at approximately 9:00 and 16:00, including weekends. Any minor injuries were recorded and treated daily on site. In the event of major injuries, horses were treated in a nearby horse clinic (6 km distance) and returned to the

group after their treatment was completed but excluded from the treatment of the trial. Thus, for medical reasons unrelated to the experiment, two mares (one from group 2, the other from group 3) were excluded from the experiment. The horses were not used for any activity and, hence, spent the entire day in the housing system, unless for medical reasons, the horse needed to be taken to the clinic. Cameras were installed inside the shelters to optimize animal observation but not analyzed as a part of this study. Animals were fed according to the feeding regimes presented in the following section of this paper. After the experiment was completed, the mares stayed on site and continued to be used in the underlying soil–horse interaction trial (Hiltebrand et al., 2026).

2.3. Treatments

The experimental design followed the approach described in Roig-Pons et al. (2025), a study conducted simultaneously. It is explained below: Three feeding regimes were considered and distributed across groups following a randomized crossover design (Table 1):

- **3 feedings per day:** Hay three times per day for two hours (7-9 a.m., 1-3 p.m. and 7-9 p.m.)
- **6 feedings per day:** Hay six times per day for one hour (3-4 a.m., 7-8 a.m., 11-12 a.m., 3-4 p.m., 7-8 p.m., 11-12 p.m.)
- **ad libitum:** 24h/24h with hay net

Each group of horses had their own automatic hay feeding station (B+M-Agrotech, Switzerland), which was programmed according to the time slots in their treatments.

2.4. Data collection

Data were collected with wireless accelerometers (MSR electronics GmbH, MSR145W2D, Winterthur, Switzerland) attached to the horses' left front leg in a neoprene pouch (crafted by the saddlery of the Swiss National Stud Farm, Agroscope, Avenches, Switzerland) according to the orientation presented in Fig. 3. This device has an IP67-rated housing, weighs 62 g, has a battery capacity of 900 mAh, and records triaxial acceleration over a measurement range of −15 to +15 g (Fig. 3). The device was suitable for determining the activity of horses and their recumbency time (Burla et al., 2014). Data from the three acceleration axes were collected at a sampling frequency of 1 Hz, corresponding to one measurement per second for each axis, and regularly transmitted to cloud storage by Wi-Fi. No additional sensor channels, such as temperature or humidity, were recorded with this device. The accelerometers were charged before each data collection and the battery charge usually lasted for the entire two-week data-collection period.

New treatments started with a three-week habituation period, followed by a two-week data collection phase.

During data collection, each sensor stored the measurements in an internal memory with a capacity of more than 1,000,000 measured values. The sensor automatically connected to the Wi-Fi network twice daily during predefined one-hour transmission windows and transferred the stored data to cloud storage. The exact duration of individual

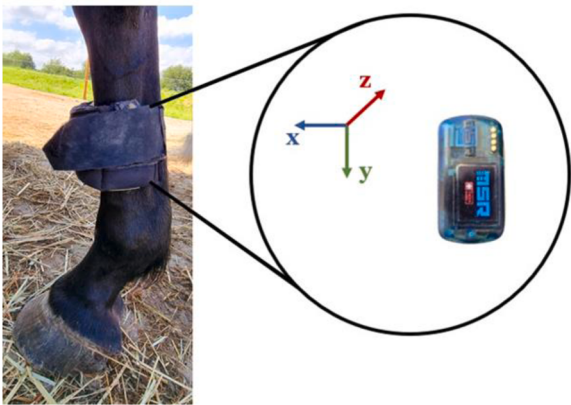


Fig. 3. The accelerometer was placed in a neoprene pouch on the front leg of the horse. The orientation of each acceleration axis is detailed on the right side of the figure.

transfers was not recorded. If transmission was unsuccessful, the data remained stored in the sensor's internal memory and were subsequently retrieved using a USB cable. Data recording continued during Wi-Fi transmission and was not interrupted.

2.5. Data processing

The data were downloaded from the cloud following each treatment. All computations were performed in R Version 4.3.3. First the data were separated by horse and group. The DigiRhythm R package, version 1.1 (Nasser et al., 2025), was used to assess periodicity using the `dgm_periodicity()` function. The rows in the data frames containing NAs either in the 'Timespan' column or in the accelerations were removed, likely due to a sensor synchronization error. Then, we applied `ggplot()` function (Wickham, 2022) to create a graphical representation of the raw data. The data were ultimately divided into sets containing a minimum of 7 consecutive days of time series acceleration data. Periods containing 24 hours or more of missing data were excluded from the analyses, and the corresponding data frames were segmented accordingly.. We then computed total acceleration of the three acceleration axes following Formula 1.

$$\text{Total Acceleration} = \sqrt{a_x^2 + a_y^2 + a_z^2}$$
 (1)

Formula 1 Total acceleration computation. a_x , a_y and a_z are the accelerations along the three cartesian axes.

Additionally, remaining outliers, identified by the 1.5 times inter-quartile range (IQR) rule in the Total Acceleration, were removed by replacing them with the signal's mean.

In the next step, the data were resampled from 1 Hz to 1/900 Hz, using the resampling function of the `digiRhythm` package (Nasser et al., 2023). This 15-minute interval is the most commonly used sampling frequency for time series DFC computation (Fuchs et al., 2022; Nunes Marsiglio Sarout et al., 2018; Scheibe et al., 1999; Sinz & Scheibe, 1976).

Once the pre-processing of raw data was completed, the `dfc` function was applied to compute the DFC as well as the harmonic parts, with a significance threshold of 0.05. Frequencies with power below this threshold were considered insignificant in the DFC computation. The index was then computed according to the computation presented in Formula 2. The DFC computed for each 7-day interval is presented in a figure and table. The values from the table were transferred to a final table containing the previously described variables and the additional computations presented below. If no significant frequencies were detected, the denominator equaled zero and the resulting DFC value was undefined. Such windows were excluded from subsequent analyses. Finally, as the DFC value is calculated using rolling windows for 7 days,

Table 1

Allocation of treatments according to the groups of horses. Each phase includes three weeks of habituation and two weeks of data collection. The dates indicate the complete experimental phases.

Group	Phase 1	Phase 2	Phase 3
Dates	February - March 2023	May - June 2023	June - July 2023
1	6 feedings per day	3 feedings per day	ad libitum
2	6 feedings per day	ad libitum	3 feedings per day
3	3 feedings per day	6 feedings per day	ad libitum
4	ad libitum	3 feedings per day	6 feedings per day

a DFC value was assigned for each day, representing the day at the beginning of the rolling window.

$$DFC = \frac{ssh}{sumsig} \quad (2)$$

Formula 2 DFC computation (Nasser et al., 2025). Ssh is the summed power of harmonic frequencies. Sumsig is the power of frequencies with significant peaks.

2.6. Additional computations

Further, lying behaviour was derived from the raw acceleration data using the triact R package (Simmler & Brouwers, 2024). The accelerometers did not classify behaviour during recording. Instead, the y-axis, which represented the vertical orientation of the instrumented leg, was analysed during post-processing. The triact algorithm filters the y-axis signal to isolate its gravitational component and applies an orientation

threshold to classify each observation as lying or standing. The graphical basis of this classification procedure, including the filtered acceleration signal and orientation threshold, is illustrated in Figs. 4 and 5 of Simmler and Brouwers (2024). Consecutive observations classified as lying were combined into lying bouts. Bouts lasting less than 30 seconds were reclassified as standing. Lying duration was subsequently calculated automatically from the classified time series. A rolling mean duration of total lying behaviour was computed and matched to each dfc 7-day DFC period. The total lying duration was summed for each day within the rolling window and then divided by the number of days considered. This duration is given in minutes.

Actograms and daily average activity plots were generated using the actogram() and daily_average_activity() functions from the DigiRhythm package (Nasser et al., 2023), respectively.

These activity patterns were computed using the squared x-axis acceleration values (horizontal axis) from raw data after outliers processing (see in the previous section). This axis was chosen as it

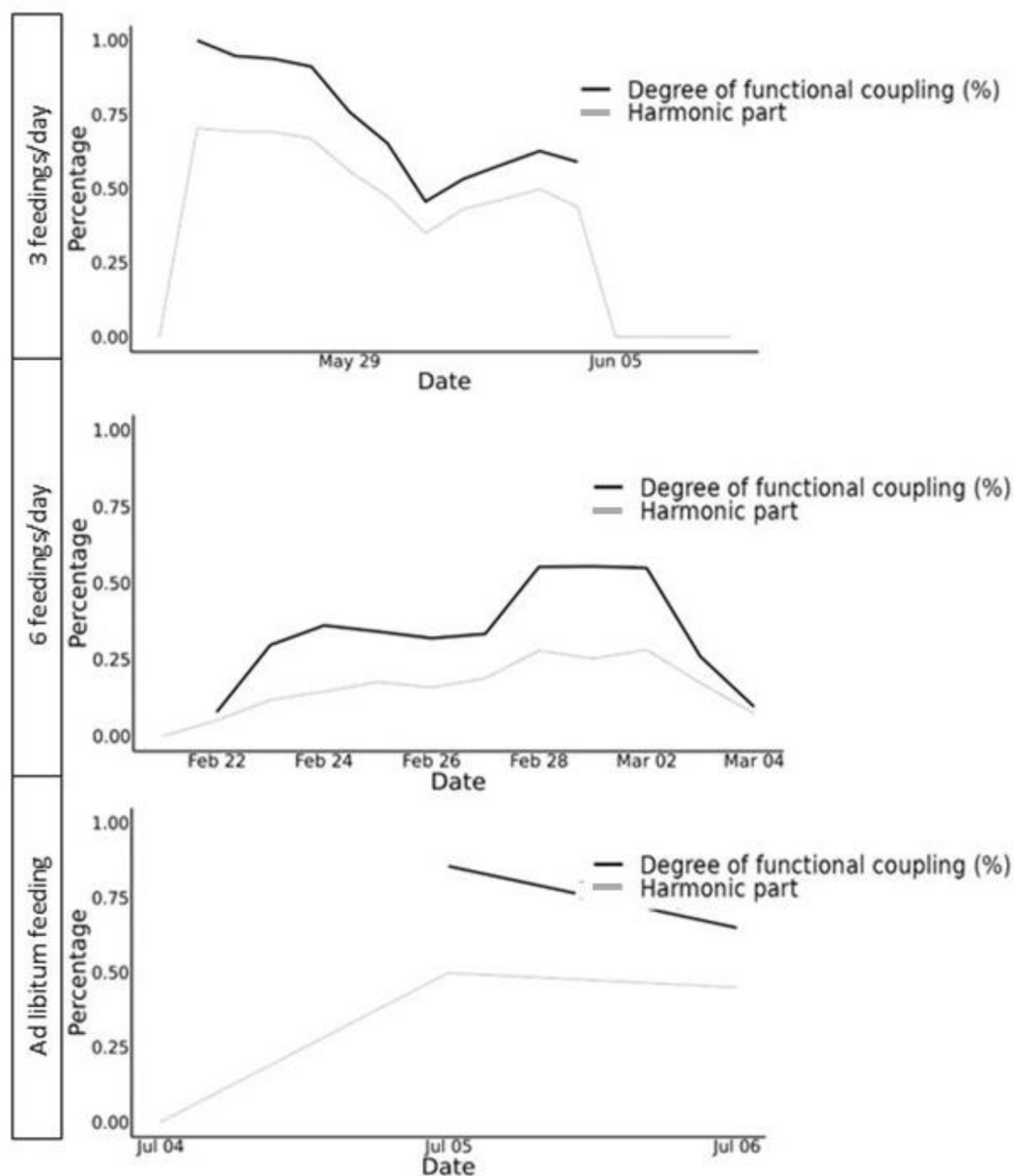


Fig. 4. Degree of functional coupling (DFC) of one example horse and its harmonic part (HP) in the three different treatments over time. The solid black line represents DFC, and the grey line represents the harmonic part. Each DFC value was calculated from one 7-day rolling window. The figure is presented descriptively to illustrate temporal variation within one horse; measurements were obtained during different experimental periods and at different times of year.

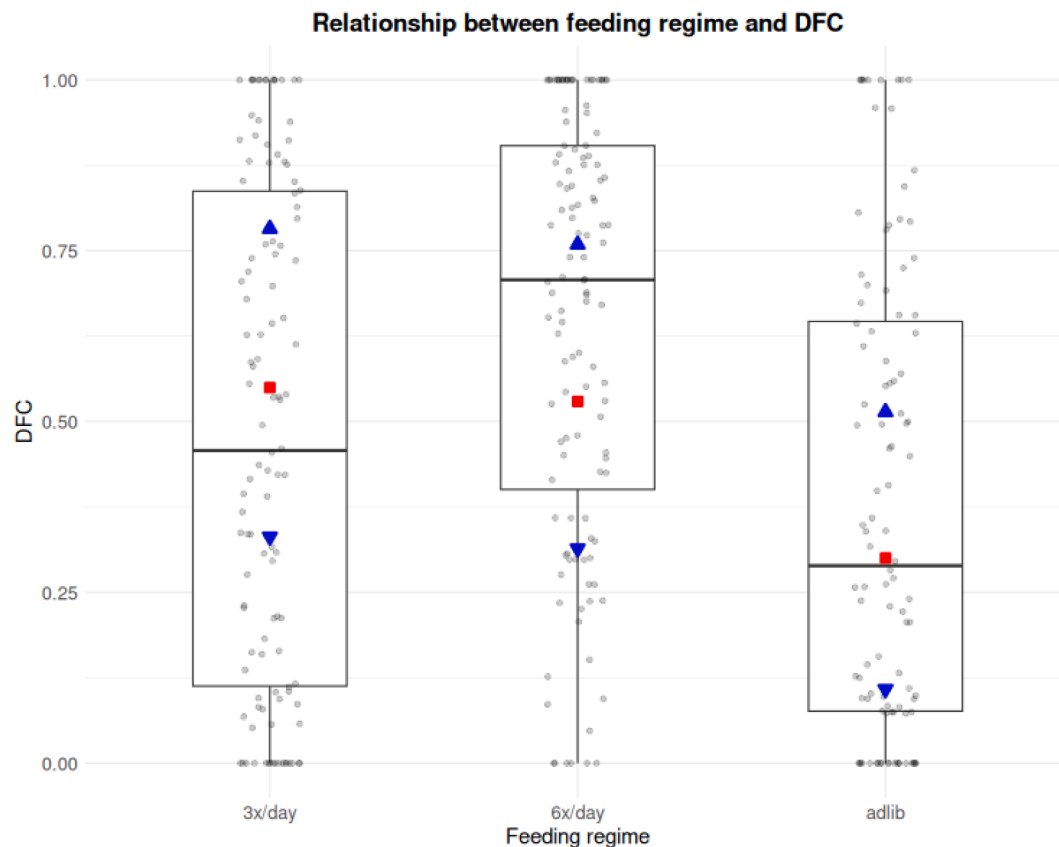


Fig. 5. Boxplots showing the distribution of the Degree of Functional Coupling (DFC) by feeding treatment. The centre line within each box represents the median, the lower and upper boundaries of the box represent the first and third quartiles, and the whiskers extend to observations within 1.5 times the interquartile range. Black dots represent raw data. Red squares indicate model-adjusted estimated marginal means derived from the BIC-selected model at mean lying duration. Downward- and upward-pointing blue triangles represent the lower and upper limits of the bootstrapped 95% confidence intervals, respectively.

represents the horizontal movement of horses when walking or displaying any other locomotor activity.

Representative actograms and daily average activity profiles derived from the accelerometer's x-axis are provided in Supplementary Figure S1. These illustrate the temporal distribution of locomotor activity under the three feeding regimes. Lying behaviour was classified separately from the y-axis orientation signal. The relationship between mean daily lying duration and DFC is presented in Supplementary Figure S3, while the descriptive distribution of lying duration across feeding regimes is shown in Supplementary Figure S4.

Further, we considered the Temperature Humidity Index (THI) in our analysis. These data extracted from a nearby weather station (MétéoSuisse (Swiss Federal Office of Meteorology)) were used to compute the Temperature Humidity Index (THI) (Formula 3). One THI value was calculated per day and added to the data table for each day.

$$THI = (0.8 * T) + \left[\frac{r^H}{100} * (T - 14.4) \right] + 46.4 \quad (3)$$

Formula 3 Temperature Humidity Index computation. r^H : relative humidity (%), T : temperature ($^{\circ}C$)

The day number variable varies from day one, which represents the first day of data available and ends day 137. Finally, repetition (Q1, Q2, Q3) was also considered in our experiment. Horse, date, DFC, treatment, mean lying duration, THI, day number, and repetition were compiled in the final dataset. Treatment, standardized mean lying duration, and repetition were considered as candidate fixed effects in the statistical analysis.

2.7. Unexpected event

A severe storm on 31 March 2023 caused a major disruption to the experiment. The shelters of the housing system were destroyed and needed to be rebuilt. All 20 mares were relocated during the reconstruction; 18 were subsequently included in the present analysis. After returning to the reconstructed experimental site on 15 May, the horses underwent a new three-week habituation period to the modified environment. The entire experiment was not repeated; only the second and third experimental phases were implemented thereafter.

During the six-week reconstruction, the horses were moved to the Swiss National Stud. Fifteen mares were housed in three group housing systems within their existing group, and one group of five horses was housed side by side in single stables with daily turnout in groups (2 and 3, mixing the pairs daily). Horses were fed hay four times per day with automatic feeders in the group housing systems and manually with hay on the ground in the single stables.

Treatment sequences were randomized across the established horse groups according to the predefined crossover design. No a priori power calculation was performed, as the sample size was determined by the number of horses available within the associated research project. Blinding of personnel implementing the feeding treatments was not possible because the feeding schedules required different programming of the automatic feeders. Data collection and behavioural classification were based on continuously recorded accelerometer data and predefined automated processing procedures. Data analysis was not blinded to treatment allocation.

2.8. Statistical analysis

We applied a linear mixed-effects model using the lmer function from the “lme4” package (Bates et al., 2015). The DFC was defined as the response variable. To ensure homogeneity of variance, a Tukey transformation was applied. The treatment (factor with three levels), the lying duration (continuous) and repetition (factor with three levels) were considered as fixed effects. Random intercepts were included for group, horse nested within group, and day number. Mean lying duration was standardized before analysis. Following model diagnostics, three observations with extreme residuals were excluded before fitting the final models. Statistical significance was assessed at an alpha level of 0.05.

Candidate models containing all possible subsets of the fixed effects were ranked using both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) with the dredge function from the MuMIn package. For each criterion, the difference between the two highest-ranked models and the corresponding evidence ratio were calculated. Because AIC provided only limited discrimination between its two highest-ranked models, whereas BIC showed substantially stronger support for the more parsimonious model, the BIC-selected model was used for the primary interpretation, model predictions, and graphical presentation. Results from the AIC-selected model were additionally reported as a sensitivity analysis.

Fixed-effect estimates were obtained from both selected models, whereas model predictions and graphical presentations were based on the BIC-selected model. Model predictions and their 95% confidence intervals were calculated using 1,000 parametric bootstrap simulations and back-transformed to the original DFC scale. Figures were generated using the ggplot2 package (Wickham, 2022) and combined raw observations with model-based predictions where applicable.

2.9. Ethical note

The animal experiment was rated with a severity degree of 0. Although long non-feeding periods could be perceived stressful for horses, they are common in practice. These feeding treatments were installed and authorized independently of the data evaluation for the current study. The data collection was carried out by trained personnel according to the Swiss animal experimentations laws. The current study caused minimal disturbance to the horses. During the current study horses were required to wear lightweight sensors. We checked the sensors regularly and never saw any signs of rubbing or discomfort caused by these pouches. However, “the average daily lying down time across all treatments was low [...in the horses on this research site...], ranging from 10 to 15 minutes, which is below reported averages in the literature (23.3 minutes to over 200 minutes in individual housing [...]). There was a considerable inter-individual variation in daily lying times, with individual values ranging from 0 to 191 minutes across treatments.” (Roig-Pons et al., 2025)

3. Results

3.1. DFC availability and variability

Of the 572 consecutive seven-day rolling windows, 231 windows (40.4%) yielded an undefined DFC value because no significant frequencies were detected in the Lomb–Scargle periodogram, resulting in a zero denominator in the DFC calculation. These windows were excluded as described in the Methods. DFC values could therefore be calculated for 341 rolling windows. Following the exclusion of three observations identified during model diagnostics, 338 observations were included in the final statistical analyses. Among the windows for which DFC could be calculated, values varied over time within individual horses and within feeding treatments (Fig. 4).

3.2. Selected fixed effects

Fixed effects, model estimates, and p-values are presented in Table 2. Representative actograms and daily average activity profiles are presented in Supplementary Figure S1. These plots were used descriptively and were not subjected to a separate inferential analysis.

3.3. DFC variation

The DFC values varied within and between treatments. Fig. 4 visualizes this variability for one of the 18 horses. The curves comprise 18, 5, and 14 data points for the three-feedings/day, six-feedings/day, and ad libitum treatments, respectively. Each data point of the DFC represents the computation of one 7-day rolling window. For the data presented in Fig. 4, the minimum DFC value is 0.07 and occurs during the ad libitum treatment. On the other hand, the DFC reaches its maximum value of 1 during the 3 feedings/day treatment but then decreases over time. Fig. 4 illustrates the temporal variation in DFC for one example horse and is not intended to represent the circadian or ultradian activity pattern of the entire study population. The observations were collected during different experimental periods and therefore at different times of year. Consequently, calendar time, seasonal conditions, and other period-specific environmental changes cannot be fully separated in this descriptive figure.

3.4. DFC and treatment

Model-adjusted DFC was lower under ad libitum feeding than under both scheduled feeding regimes (ad libitum vs. 6 feedings/day: $p < 0.001$; ad libitum vs. 3 feedings/day: $p < 0.001$). The 6 feedings/day and 3 feedings/day treatments did not differ ($p = 0.979$). Thus, scheduled feeding was associated with higher DFC than ad libitum feeding, but DFC did not increase with feeding frequency (Fig. 5).

Table 2

Fixed effect estimates from the models selected using AIC and BIC. Fixed-effect estimates, standard errors, and 95% confidence intervals are reported on the Tukey-transformed DFC scale. Mean lying duration was standardized before analysis. Model-adjusted marginal means and predictions presented in the figures were back-transformed to the original DFC scale. P-values are approximate two-sided Wald p-values.

Model selected by	Fixed effect	Estimate (ß)	SE	95% CI	p-value
AIC sensitivity model	Intercept	0.288	0.093	[0.106, 0.470]	0.002
	Repetition Q2 vs. Q1	0.051	0.058	[-0.063, 0.164]	0.382
	Repetition Q3 vs. Q1	0.144	0.065	[0.016, 0.272]	0.028
	Mean lying duration, standardized	-0.168	0.030	[-0.227, -0.110]	<0.001
	6 feedings/day vs. ad libitum	0.248	0.043	[0.164, 0.332]	<0.001
BIC primary model	3 feedings/day vs. ad libitum	0.257	0.045	[0.168, 0.346]	<0.001
	Intercept	0.360	0.077	[0.208, 0.511]	<0.001
	Mean lying duration, standardized	-0.152	0.028	[-0.207, -0.097]	<0.001
	6 feedings/day vs. ad libitum	0.223	0.041	[0.143, 0.303]	<0.001
	3 feedings/day vs. ad libitum	0.242	0.045	[0.153, 0.330]	<0.001

3.5. DFC and lying

In the BIC-selected model, greater standardized mean daily lying duration was associated with lower DFC ($\beta = -0.152$, $SE = 0.028$, 95% CI $[-0.207, -0.097]$, $p < 0.001$; Figure S3). However, we observed considerable variability and are thus cautious regarding its interpretation.

4. Discussion

The main findings were that DFC was associated with feeding regime and lying duration, whereas evidence for an additional effect of experimental repetition depended on the model-selection criterion. This suggests that DFC primarily reflected variation associated with feeding management and lying behaviour, although period-specific environmental conditions may also have contributed.

4.1. DFC as a rhythmicity indicator in horses: variability and data availability

The substantial proportion of rolling windows for which no DFC value could be calculated indicates that stable periodicity was not consistently detectable in the locomotor activity of the horses over seven-day periods. Together with the observed variation in DFC within individual horses and feeding treatments, this suggests that DFC may be sensitive to short-term changes in daily routines, environmental conditions, or both. Consequently, DFC should be interpreted as an indicator of detected rhythmic organisation rather than as a stable individual characteristic or a direct measure of welfare. The frequency of undefined values also represents a practical limitation when applying DFC to equine activity data and when comparing rhythmicity between treatments.

4.2. DFC affected by extrinsic feeding schedule

As shown in the results, the different feeding regimes influenced activity-derived DFC and therefore the temporal organisation of the recorded locomotor activity patterns. DFC was similarly higher under both scheduled feeding regimes than under ad libitum feeding, whereas the two scheduled regimes did not differ. These findings indicate that activity-derived DFC, as calculated in the present study, is strongly influenced by externally imposed feeding schedules and should therefore not be interpreted as a standalone welfare indicator in managed husbandry systems. Roig-Pons et al. (2025), who studied the same horses at the same experimental site, reported more agonistic behaviour and related injuries under the 6 feedings/day treatment than under ad libitum slow feeding. In contrast, both scheduled feeding regimes were associated with similarly higher DFC values than ad libitum feeding in the present study. This discrepancy indicates that a higher DFC does not necessarily correspond to better welfare. Hence, activity-derived DFC should not be used as a standalone welfare indicator in managed husbandry systems.

Thus, predictable externally imposed routines may strengthen detectable rhythmic activity patterns, irrespective of their welfare consequences. Therefore, the DFC computations differ according to the management of the animals considered. Our data show that the rhythmicity patterns are strongly related to feeding management, and thus may reflect feeding management more strongly than the animals' underlying welfare state.

4.3. DFC and lying behaviour

Greater mean daily lying duration was associated with lower DFC, as presented in Figure S3.

Initially, this association appears unexpected because DFC has been proposed as a welfare-related measure in animals, while recumbency

has been proposed as a welfare indicator in horses (Berger et al., 2003; Kelemen et al., 2021). However, lying duration cannot be equated directly with total sleep because horses can rest while standing and sleep polyphasically, whereas recumbency is required for REM sleep (Aleman et al., 2008; Greening & McBride, 2022). Lying behaviour is also influenced by housing conditions, social rank, and the availability of suitable lying areas (Chaplin & Gretgrix, 2010; Kjellberg et al., 2021).

In contrast, mid-lactation dairy cows spend more than 12 hours a day lying and ruminating (Cook et al., 2007), potentially resulting in less variable locomotor patterns than those observed in horses (Fuchs et al., 2022).

The timing, frequency, and duration of lying bouts may therefore influence activity-derived DFC in horses (Helmerich et al., 2025). However, the considerable variability in lying behaviour limits interpretation, and further studies are required to determine how fragmented recumbency affects DFC calculations.

Most horses spent less time in recumbency than previously reported (Greening et al., 2021), and mean daily lying duration showed considerable variation. Because sleep stages were not recorded, lying duration could not be equated directly with sleep duration. Fourteen of the 18 horses showed signs of pseudo-narcolepsy, although evidence regarding the optimal duration of recumbency in horses remains limited.

Descriptively, mean daily lying duration appeared lower during the 6 feedings/day treatment than during the other feeding regimes (Figure S4). This result seems plausible, as two of the six feeding periods occurred during the night (03:00-04:00 and 23:00-24:00), when much of horses' REM sleep and recumbency occurs (Greening & McBride, 2022). Horses may adjust their lying behaviour to temporal variation in food availability under both natural and managed conditions. In the present study, however, food availability was externally imposed at fixed times, including during typical nocturnal resting periods, which may have influenced recumbency. This descriptive pattern raises the possibility that feeding during typical nocturnal resting periods may interfere with recumbency and warrants further targeted investigation.

4.4. Limitations

Despite the randomized design of our trial, we would like to address some limitations. All animals in our trial were female and could therefore constitute a possible bias. As highlighted by Górecka-Bruzda et al. (2022), mares have been identified as being more socially dependent than geldings. As a result, our mares may have synchronized their activities with the group, dominant individuals, or simply a "good friend". This synchronization could have occurred when they engaged in activities such as eating, resting, playing, or moving from one side to the other side of the housing system. Further, the experimental mares' behaviour may have been affected through synchronization between groups, as groups had visual, vocal and olfactory contact over the fence.

Additionally, the interruption of the trial due to the destruction of the housing system on the 31st of March 2023 after a storm impacted our data collection. The trial was interrupted for 2 months, and the orientation of the shelters was changed, to prevent future storm damage. Thus, the mares experienced the destruction of the housing system and were also accommodated in emergency housing during reconstruction of the site. Because repetition was retained in the AIC-selected model but not in the BIC-selected model, a period-specific effect cannot be excluded. As experimental period coincided with storm-related relocation, reconstruction, and changes in shelter orientation, the effects of experimental period and environmental modification could not be separated.

Finally, the animals were on average 13 years old ($SD = 2.44$) and had a different background before taking part in the experiment. Most of them were retired sport horses due to injury or underperformance. Although none of the mares showed acute clinical signs during enrolment, most had a history of chronic or recurrent musculoskeletal conditions. Such conditions may affect locomotor activity or lying

behaviour and could therefore have contributed to inter-individual variation in DFC and lying duration. Therefore, this population may not be representative. The horses had been housed in their groups for over six months before onset of the trial. During this time, they had become well integrated into their herds. However, when we moved them to the paddock trail system, the lying behaviour of all horses reduced. Despite efforts to ensure their comfort during the building of the housing system (National animal welfare regulations regarding lying area were exceeded), they displayed reluctance to lie down. As a result, some of the animals showed signs of atonic collapses. The reduced recumbency and observed signs of atonic collapses may have affected the horses behaviour.

5. Conclusion

Our findings show that activity-derived DFC was strongly associated with externally imposed feeding schedules and lying duration. Period-specific environmental conditions may also have contributed, although their effects could not be separated from experimental repetition. Consequently, activity-derived DFC, as calculated in the present study, should be interpreted cautiously and not as a standalone welfare indicator in managed husbandry systems. Further studies are also needed to evaluate the impact of fragmented lying behaviour on DFC. Differences in species-specific lying patterns and management practices may limit the comparability of activity-derived DFC across studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.crbeha.2026.100220](https://doi.org/10.1016/j.crbeha.2026.100220).

Data availability

The data and analysis script supporting the findings of this study are available in Zenodo under DOI: [10.5281/zenodo.17628705](https://doi.org/10.5281/zenodo.17628705).

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