Measuring Behavior 2024

13th International Conference on Methods and Techniques in Behavioral Research, Aberdeen, Scotland

15 – 17 May 2024

Proceedings

Proceedings of Measuring Behavior 2024

13th International Conference on Methods and Techniques in Behavioral Research in Aberdeen, Scotland, May 15 – 17

DOI: 10.6084/m9.figshare.25897855

ISBN: 9789074821957

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Method comparison to analyse the activity rhythm of dairy cows during early lactation

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Abstract

Several methods for analysing activity rhythms have been developed and compared in the past. However, the Degree of Functional Coupling (DFC) was never included in comparative studies. Therefore, we set out to compare the DFC with two other methods of analysing the circadian activity rhythm, Interdaily Stability (IS) and Spectral Entropy (SE), regarding the impact of oestrus and regrouping. Both oestrus and regrouping are known to affect the circadian activity rhythm of dairy cows. The study used data from two herds of German Holstein cows housed in a mirror imaged free stall barn. The days on which oestrus and regrouping occurred were documented. Accelerometers recorded cow activity from two weeks before expected calving until 82 days after calving. The DFC, IS and SE were calculated from 15-minute intervals of the activity using a sliding 7-day window. A linear mixed effect model was used to calculate the correlation between each pair of rhythmicity measures. Furthermore, the influence of oestrus and regrouping on each rhythmicity measure was analysed visually using boxplots. The correlation between the measures ranged from low to moderate, with the highest correlation found between DFC and IS, which were similarly interpreted as the alignment of the activity rhythm with the natural 24-hour cycle. The visual analysis indicated that all rhythmicity measures decreased during oestrus. Additionally, the DFC and the IS decreased during regrouping, while SE remained unaffected. In conclusion, DFC seems to be a reliable and comparable but also sensitive method for analysing the influence of oestrus and regrouping on the activity rhythms of dairy cows.

Introduction

In recent years, various measures of rhythmicity have been developed and used in different areas of biological rhythm research. Refinetti et al. [1] provided a guide for selecting a method for circadian rhythms analysis. They explained, when to use a Fourier Analysis, a Lomb-Scargle Periodogram, the Cosinor method or other methods [1]. However, they did not include non-parametric measures (other than the Kruskal-Wallis-Test) such as the Interdaily Stability (IS) or other measures, that include Fourier Analysis or the Lomb-Scargle Periodogram, but go further to classify the circadian rhythm, such as the Degree of Functional Coupling (DFC) or the Spectral Entropy (SE).

The DFC, which was developed by Sinz and Scheibe [2], analyses the alignment of animal activity rhythms with the natural 24-hour cycle by calculating the proportion of harmonic periods in all expressed periods. To date, the DFC has been used to analyse the circadian rhythms of various animals, including alpacas, Prezwalski horses, red deer and moufflons under free-ranging conditions [3] [4] as well as ewes under extensive conditions [5]. A pilot study also analysed the circadian activity rhythm of dairy cows milked by automatic milking systems using the DFC [6]. The SE is another rhythmicity measure, calculating the irregularity and complexity of activity rhythms [7]. It can be used as a frequency domain feature to improve the automatic classification of locomotor-associated sickness behaviour [8] or automatically classify cows' behaviour by comparing leg- and neck-mounted accelerometers [9]. A pilot study by McPherson et al. [10] used the SE to analyse the circadian rhythms of cows and how they are affected by cow-calf contact. Finally, the IS [11] which is a non-parametric indicator that measures the stability of activity rhythms between different days is a well-known measure of rhythmicity in human science, but has had less impact in animal sciences.

Various factors can affect the circadian activity rhythm of animals. For instance, changes in dairy cows' circadian activity rhythm were reported during oestrus, calving, or several diseases such as lameness, mastitis, or ruminal acidosis [12] [13]. Additionally, Wagner et al. [13] reported an effect of regrouping on cows' circadian activity rhythm. However, the effect of these influencing factors on the DFC, IS or SE, measured in dairy cows has not been previously studied.

The aim of this study was to compare three different rhythmicity measures (DFC, IS, SE) with respect the potential effects of oestrus and regrouping in dairy cows.

Animals, Housing and methods

The study was conducted on the research farm of the Thünen Institute of Organic Farming in Germany. Two dynamic herds were kept in the mirror imaged free stall barn. One herd consisted of an average of 40 polled German Holstein cows (28-48), while the other consisted of an average of 43 horned German Holstein cows (37-47). The cows were milked twice daily and fed with a total mixed ration, which was provided at the feeding table during the milking times. Cows were kept on pasture both day and night during the vegetative period. Around calving, they were held in single maternity pens until 4 ± 1 d after calving. As the cows were kept under their normal living conditions, without performing procedures deviating from standard husbandry and commercially available sensors were used, no ethical approval of the study was required.

Data collection and editing

The experiment was conducted from August 2020 to April 2021 and from August 2021 to June 2022. The study included all cows that calved between August and January or August and March. Since oestrus and regrouping can potentially affect the activity rhythms of cows they were recorded during the experimental period. The farm staff noted the date the cow was regrouped with the main herd, and the days of oestrus were recorded by either farm staff or the management system. The cows' activity data was collected at a frequency of 16 Hz using IceTags (Peacock Technology, Stirling, UK). The data was recorded from two weeks before expected calving until day 82 after calving to analyse the effect of regrouping in the herd after calving and the occurrence of oestrus events. As being on pasture affects the cows' activity, these data were excluded from the dataset.

The IceManager Software (Peacock Technology, Stirling, UK) was used to calculate the Motion Index and Steps from the activity data. These variables were used to identify incorrect data caused by technical issues. A technical issue was identified if either Steps or Motion Index were zero for more than 12 hours, as the cows had to move within that time when walking to the milking parlour twice a day. If a technical issue was identified, the entire day was excluded from the dataset.

Calculation of Rhythmicity

The Motion Index was used to analyse the activity rhythms, using the DFC, IS and SE. As the data were collected during European summer and winter time, they were converted from CEST and CET to GMT. Afterwards, the Motion Index was summed up to 15-minute intervals, following the method of [6] . To analyse a circadian activity rhythm, multiple consecutive days are necessary [4]. In a similar way to previous studies using the DFC, IS and SE, a sliding window of 7 days (today and the following 6 days) was used to calculate each of the rhythmicity measures [11][7][6].

The DFC analyses the alignment of the cows' activity rhythms with the natural 24-hour cycle by calculating the proportion of harmonic periods in all expressed periods. Harmonic periods are defined as those that can be obtained by dividing 24 h by an integer, resulting in 24 h, 12 h, 8 h, 6 h, 4.8 h etc. (all periods, that fit in with 24 h). The DFC can take on values between 0 and 1 and was calculated using the digiRhythm package [14]. First, the activity rhythm frequencies were extracted using a Lomb-Scargle-Periodogram [15][16], which is more suitable for uneven data than Fourier Transformation [17] as used by Sinz and Scheibe [2]. Subsequently, the Baluev method [18] was used to identify significant frequencies, as it is one of the most effective methods for calculating the false alarm probability [19]. Finally, the sum of significant harmonic periods was divided by the sum of all significant periods (harmonic and non-harmonic).

The IS is a non-parametric indicator that measures the stability of activity rhythms between different days [11][20]. It compares the activity pattern of each day with the average pattern across days. The higher the IS (ranging between 0 and 1), the more aligned the expressed rhythms are with the natural 24-hour cycle [20] . In this study, the IS was calculated using the nparACT package [21].

The SE measures the irregularity and the complexity of the activity rhythms [7]. A higher SE value (theoretical max. is infinite) indicates a more random and less predictable rhythm due to increased irregularity and complexity. In this study, the spectrum was extracted using an autocorrelation function. Additionally, the SE was calculated based on a Fourier Transformation. However, as the autocorrelation and Fourier Transformation-based SE did not differ, the autocorrelation-based SE was used. It was calculated using the R package tsfeatures [22] .

Data analysis

After calculating the rhythmicity measures, we excluded cows with less than 15 data points per lactation. The dataset included 68 cows, with 13 of which had two lactations, resulting in a dataset of 4208 datapoints spread over 81 cow datasets.

A linear mixed effects model (R package lme4, [23]) was used to analyse the linear correlation between each pair of rhythmicity measures. This method accounts for the data structure of repeated measurements. Following Christensen [24], one rhythmicity measure was set as the target and the other one as the fixed variable. The lactation number nested in the cow was used as the random effect. Afterwards, the coefficient of determination was calculated using the R package MuMIn [25]. The square root of this coefficient was used to analyse the correlation and was interpreted using the definition by Hinkle et al. [26]. Additionally, the effects of oestrus and regrouping on each rhythmicity measure were analysed visually using boxplots (using the R package ggplot, [27].

Results

The pairwise comparison of the rhythmicity measures showed correlation coefficients ranging from low to moderate. The correlation between DFC and IS was the highest at 0.60, followed by the correlation between IS and SE at 0.53. The correlation between DFC and SE was classified as low at 0.43.

The visual analysis indicated that oestrus and regrouping affected DFC and IS, whereas only oestrus had a visible effect on SE. The median DFC value for the entire dataset was 1.00 (IQR = 0.00, 1.00). During oestrus, it decreased to 0.18 (IQR = 0.00, 0.52), and during regrouping, it decreased to 0.58 (IQR = 0.00, 1.00). Similarly, the IS showed a median of 0.37 (IQR = 0.27, 0.44), which decreased to 0.24 (IQR = 0.18, 0.29) during oestrus and to 0.33 (IQR = 0.27, 0.39) during regrouping. In contrast, the SE decreased from a median of 0.98 (IQR = 0.96, 0.99) to 0.94 (IQR = 0.90, 0.97) during oestrus but was not affected by regrouping.

Discussion and conclusion

Among the three pairs of rhythmicity measures compared, DFC and IS had the highest correlation. They additionally showed a greater response to oestrus and regrouping compared to SE. However, the interpretation of DFC and IS, both of which show an alignment with the natural 24-hour cycle, is very similar. SE, on the other hand, deviates from this interpretation, as it refers to the irregularity and the complexity of a circadian rhythm. DFC showed the lowest median during oestrus, the second lowest median during regrouping. The DFC additionally had the highest median in the entire dataset, which indicates the highest alignment with the natural 24- hour cycle. However, the DFC is the only rhythmicity measure used in this study that includes ultradian rhythms and thus uses more information in its calculation process, which might explain the higher sensitivity to changes in the rhythm. Nonetheless, IS as a non-parametric rhythmicity measure, also seems to be a suitable method for detecting changes in the circadian activity rhythm of dairy cows caused by oestrus and regrouping. However, the SE measure demonstrated a weaker correlation with both methods. It is important to note that the SE measure has a different value range and interpretation compared to the other measures. An increase in DFC and IS indicates a higher alignment with the natural 24-hour cycle, while an increase in SE indicates greater irregularity and complexity of the rhythm.

The lower values in DFC and IS during oestrus and regrouping suggests a lower alignment with the natural 24-hour cycle on these days. However, the lower value in SE indicates a lower variability of the rhythm. This reduction could be explained by a lower behavioural variability, which animals are known to display under several conditions, such as during sickness or when exhibiting stereotypic behaviour (Miller et al., 2020). Given that during oestrus, certain behaviours such as mounting or chin resting on other cows and being mounted are frequently repeated [28], a decrease in behavioural diversity is reasonable. In contrast, in our visual analysis, SE was not affected by regrouping. This indicates that the variability of the cows' behaviour was not affected by regrouping, although their activity increases [29]. However, as DFC and IS had lower values during regrouping than in the entire dataset, the cows' circadian activity rhythm was influenced by this effect, even though the impact was lower than during oestrus.

In conclusion, oestrus altered all tested rhythmicity measures. Furthermore, DFC and IS were also affected by regrouping in our visual analysis. However, DFC appears to be more sensitive in detecting changes in the circadian activity rhythm of dairy cows, caused by oestrus and regrouping.

Acknowledgements

A great thank you to the farm and technical staff for their help in collecting the data.

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