Behavior Characteristics of Holstein Friesian Cows During Estrus Using Sensor and Meteorological Measurements

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SUMMARY

This study evaluated oestrus detection, one of the major challenges in herd management for dairy farms worldwide, in Holstein Friesian cows using the Cow-Manager chip sensor system. A total of 105 estrus events from 15 cows were recorded over an 18-month research period using the ear-tag system. Throughout the study, behavior data were collected for a total of 3081 days. Of these, 84 were confirmed as estrus, and 21 were classified as suspicious estrus. The average duration of estrus was calculated to be 9.8 hours. These values were found as the highest values <70 groups in the THI group, in multiparous cows in the parity group, and 151+ group in the DIM group. Upon general examination, it was observed that estrus values in cows increased at night (32.4%) and decreased in the afternoon (19.3%). These values in the THI>70 group and primiparous cows were found at their highest levels in the morning. During estrus, cows exhibited respective increases of 124%, 4.8%, and 7.6% in high-active, active, and eating behaviors compared to non-estrus days, while not-active and rumination values decreased by 15.3% and 23.1%, respectively. According to non-estrus days, during estrus, high-active, active, and eating values increased by 124%, 4.8%, and 7.6%, respectively, while not-active and rumination values decreased by 15.3% and 23.1%. Findings from this study suggest that high-active and active values tend to increase towards estrus, reaching their peak at the onset of estrus, and gradually decrease towards the end of estrus. In addition, rumination values are lower than normal during the estrus period, beginning at their lowest level at the start of the period and decreasing further as estrus approaches. The research indicates that elevated temperatures can reduce the length of estrus and negatively affect reproductive performance. This study provides valuable insights into behavioral changes and estrus duration in Holstein Friesian cows, highlights the importance of monitoring physical activity and rumination patterns for accurate estrus detection.

KEY WORDS

Holstein; behaviour; estrus; sensor; THI.

INTRODUCTION

Detecting a high percentage of cows in estrus is essential to maintain reproductive performance in dairy herds using AI (1). Estrus detection remains a major challenge for dairy farms worldwide despite reproductive management advancements (2-3-4). Increasing age, milk production, and environmental factors (greater ambient temperature, uncomfortable housing, and so on) can also negatively affect the length and intensity of estrus expression (5,6). Climate change-induced global warming further stresses dairy cows, disrupting their reproductive efficiency, hormonal activities, and estrus behaviors, thereby reducing their fertility and causing significant financial losses for breeders (7-8-9). Direct observation by farm personnel is the primary method for detecting cows in heat[6] with efficiency ranging from less than 50% to 90% (2). Undetected estrus leads to longer calving intervals and reduced milk production, causing substantial economic losses (4).

The behavior of dairy cows during the estrus period is characterized by certain behaviors or signs. Dairy cows exhibit specific behaviors during estrus, such as mounting, increased mobility, and reduced inactivity (1,6,10-11-12). Some other behaviors such as licking, sniffing, and restlessness are also observed (1). Changes in feeding and rumination patterns accompany estrus during the estrus period (1,13). Automated estrus detection (AED) technologies are an available alternative to supplement or replace visual estrus detection (1). Technological advancements, particularly in Precision Livestock Farming, offer automated estrus monitoring solutions to alleviate breeder workload (13). To determine the accuracy of a specific AED technology, estrus events identified by the technology algorithm (a set of criteria used to determine "estrus") are compared with a gold standard such as visual observation, ultrasonography, blood or milk P4 levels, or a combination of these factors (1). Remote sensing devices, like the CowManager SensOor system, enable non-disruptive monitoring of animal behavior via computer or mobile phone (14). This electronic sensor detects ear and head

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movements, classifying behaviors like rumination and activity levels in real-time through algorithms (15). Animals in estrus are not ruminating and usually not eating; their frequent head movements mimic feeding behavior and can be mistaken for eating activity and the algorithm characterizes this movement as eating. While it may not be eating, the combined high activity with low rumination coupled to this continual head movement almost makes 100% accuracy in confirming heat and estrus (16). Another study reported that the CowManager system can quickly identify animals in estrus by seeing changes in a cow's behavior and mate her in a timely manner (17).

The purpose of this study using chip ear tags was to investigate the duration and circadian distribution of estrus in cows and analyze its relationship with various factors such as air temperature, parity, and days in milk (DIM). Additionally, the study aimed to assess behavioral changes associated with the estrus period, particularly focusing on physical activity, and feeding behaviors, to provide insights into estrus detection and reproductive management strategies in dairy farming.

MATERIAL AND METHODS

Animal and Management

The research was conducted at the International Center for Livestock Research and Training (39°97 N, 33°10 E; elevation 826m) located in Ankara Province of Turkey. The study was carried out between October 01, 2020, and March 31, 2022 (18 months). The animal material consisted of 15 Holstein Friesian (HF) cows. The ages of the cows ranged between 3 and 7 years old. The cows were on average 1.8 (SD±0.775) lactations. Six of these 15 cows were in their first lactation, while the others were in their 2nd (n=6) or 3rd lactation (n=3). The average weight of the cows was around 650 kg. All of the animals were dairy cows, and they were all housed in the same shelter compartment. Only healthy cows were included in the study.

Cows were housed in a 50x8m semi-open barn with a total of 20-25 milking cows. Animals were separated individually for 10 days of transition dry period. Dry cows were housed with other cows in a 20x10 m-sized compartment with a total of 6-8 cows. The cows gave birth in a single calving pen, and their calves were taken away from them immediately after birth. After being kept in the individual pen for 4 days, they were sent to the dairy cow pen.

Total mixed ration (TMR) and water were given ad libitum to the cows, taking into account their dry matter intake requirements. Components of TMR included corn silage, meadow grass, dry alfalfa, concentrate, straw, and additives. The composition of TMR was calculated to meet the requirements of cows with a daily milk yield of about 25-30 kg. Fresh TMR was supplied twice per day at 9:00 am and 9:00 pm. No additional concentrate was fed. Cows were milked between 8:00 am and 8:00 pm.

Meteorological Measurements

Meteorological records were taken from the meteorology station established in the barn. These records were collected at daily intervals and included temperature (T) and humidity (H). Then, using these records, the daily Temperature-Humidity Index (THI) was calculated. The formulas used in the calculation of index values are presented below:

$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times H) (1.8 \times T - 26.8)]$ (NRC,1971). THI values were also divided into 2 groups and threshold stress is accepted as 70 (18).

Sensor Data

CowManager Sensoor (Agis, Harmelen, Netherlands) was used for behavior data recording. The system was previously used in another project on the same farm, and there were no issues with functionality and data transfer. The system consists of three parts: a sensor with a chip, transmitting antennas, and computer software. Data were recorded using computer software by attaching an electronic ear tag. The observed behaviors were high-active, active, not-active, eating, and rumination on a minute-by-minute basis. Then, they were transferred to the 'heat index' by the software. The behavior data were recorded in the system on a minute-by-minute basis. Subsequently, all data were transferred to the computer environment (Excel format) on an hourly basis. Finally, the hourly and daily collected data were converted to percentages (%) in Excel and made ready for analysis.

In this system, estrus is directly determined by high-active behavior, while rumination serves as a secondary marker (Figure 1). The 'heat index' was calculated using these two parameters. Figure 1 depicts two instances of estrus detection, showing high-active (red line), rumination (turquoise line), and estrus (heat index with blue line). When the blue line enters the 'light green' zone, estrus is considered suspicious, and when it enters the 'bold green' zone, estrus is assumed to be certain. A 'heat index' between 2.8-3.5 (light green) indicates suspicious estrus, while above 3.5 indicates certain estrus.

Cows were not inseminated or impregnated during this study for 1.5 years, and artificial insemination was applied in the last two estrus. A total of 105 estrus events from cows, identified using the ear-tag system, were recorded during the research. In addition to the CowManager system cows estrus were determined by herd staff observation of behavioral indicators of cows in estrus as standing while mounted by other cows. Among these, 84 events were classified as certain estrus, while 21 were classified as suspicious estrus. Only data from cows determined to be in certain estrus (n=84) according to the system were included in the analysis. The cows were classified into lactation groups as primiparous (n=36) and multiparous (n=48), and further grouped based on lactation stage: 1. DIM≤150 (n=16) and 2. DIM≥151 (n=68). The distribution of certain estrus occurrences by groups was as follows: 1. THI:<70 (n=78), 2. THI>70 (n=6).

Statistical Analysis

In the study, firstly the behavioral times of the cows according to the system were calculated. Secondly, the distribution of estrus durations of cows according to the periods of the day was calculated. The day was divided into 4 categories to observe the distribution of estrus values throughout the day. These; night (00:00-05:59 h), morning (06:00-11:59 h), noon (12:00-17:59 h), and evening (18:00-23:59 h). At whatever hour/hours of the day estrus values were observed, hour totals were added to the category corresponding to that hour. Then, these totals were divided into percentages (%), and the distribution of heat during the day was calculated.

Figure 1. A Cow's Estrus for 1 Month

Thirdly, changes in the cows' behavior during estrus compared to non-estrus periods were calculated. Here, it assessed the estrus periods and differences between estrus and non-estrus days using 3081 days of behavioral records. The categorical effects of heat status and behavior values were analyzed separately. Daily averages were considered for non-estrus time, while only values during estrus were analyzed. Fourthly, behavioral values at estrus were compared with pre- and post-estrus values. Here, behaviors are divided into five periods: 12-24 hours before estrus, 0-12 hours before estrus, estrus moment, 0-12 hours after estrus, and 12-24 hours after estrus. Behavior values were included as categorical effects and analyzed separately.

Minitab 16 statistic program was used to evaluate the data obtained in the study (19). Mixed model analysis was applied by the General Linear Model to infer associations between estrus values. The differences between group means were determined by the Tukey Multiple Comparison Test at the 5 percent significance level. The model used for analysis; Model: $Y_{ijkl}=\mu+a_i+b_j+c_k+e_{ijkl.}$ The symbols in the formula: μ: overall mean; a_i: i. the effect of THI (<70, >70); b_j: j. the effect of parity group (primipar, multipar); c_k: k. the effect of DIM (0-150, 150+); e_{ijkl}: error.

RESULTS

In the study, the duration (hours) of estrus of the cows created by the Cowmanager system is presented in Figure 2. The average estrus duration was calculated as 9.8±1.434 hours. These values were found as the highest values below 70 in the THI group, in multiparous cows in the parity group, and the 151+ group in the DIM group.

The daily changes in the estrus of the cows according to the

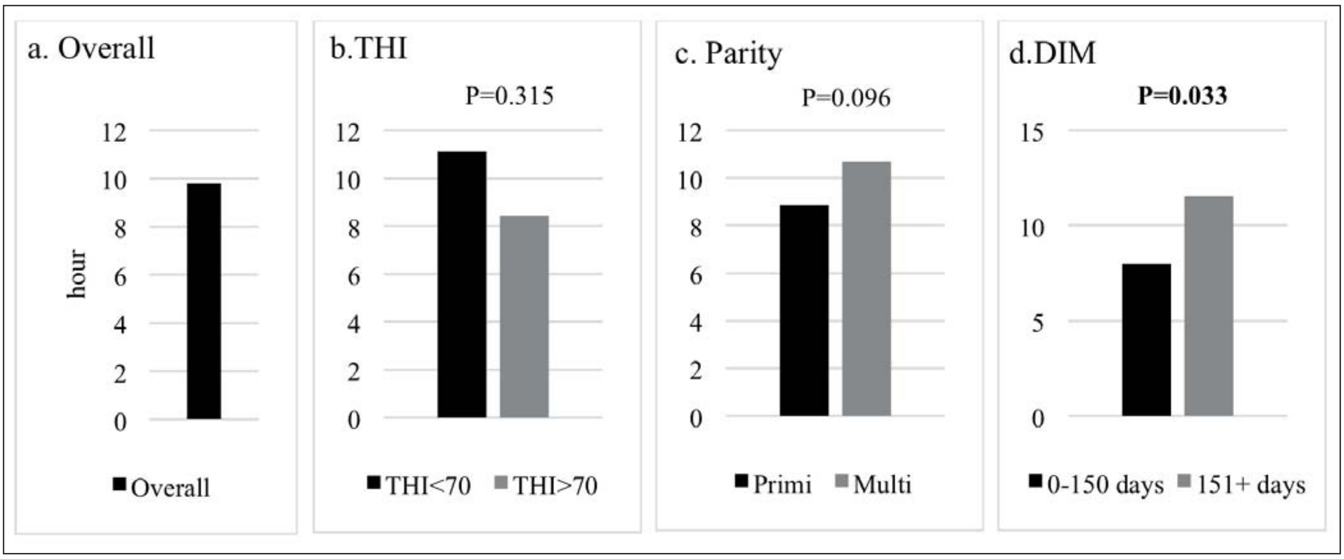

Figure 2. Duration of Estrus of the Cows (Hours) According to General (a), THI (b), Parity (c), and DIM (d)

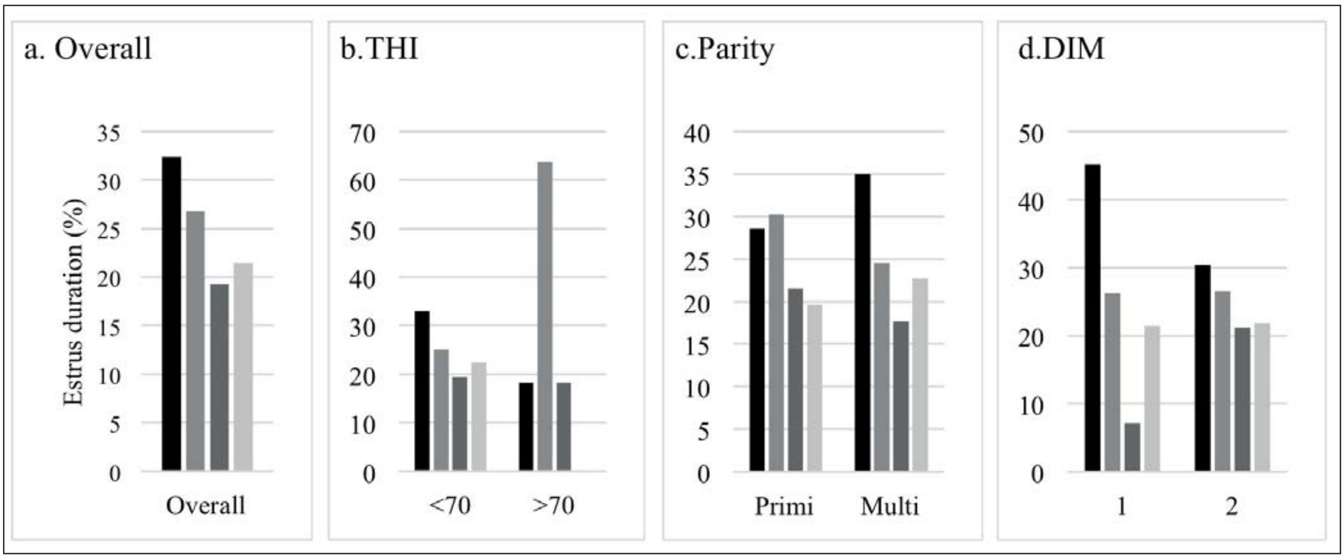

Figure 3. The Variation (%) of Estrus Duration During Estrus Day (Black: Night, Grey: Morning, Dark Grey: Noon, Light Grey: Evening) According to General (a), THI (b), Parity (c), and DIM (d).

system are presented in Figure 3. When examined in general, it is seen that the estrus values of cows increase at night and decrease during the afternoon. These values in the THI>70 group and primiparous cows were found at their highest levels in the morning.

Changes in cow behavior between estrus and non-estrus days are summarized in Table 1. During estrus, high-active, active, and eating values increased by 124%, 4.8%, and 7.6%, respectively, while not-active and rumination values decreased by 15.3% and 23.1%.

Changes in cow behavior within 24 hours before and after estrus were analyzed in 12-hour intervals (Figure 4). High-active and active values peaked at the onset of estrus and gradually declined thereafter, while not-active values exhibited an opposite trend. Eating behavior peaked 12 hours before estrus and gradually decreased afterward, whereas rumination values declined towards estrus, reaching their lowest point at its onset, and remained below normal levels throughout the estrus period.

DISCUSSION

Estrus Duration

Accurate estrus detection is of paramount importance for optimizing the reproductive efficiency of livestock. Cattle do not exhibit equal heat intensity and duration (10). Traditional methods are often labor-intensive. The cow estrus period, which only lasts 12-24 hours in a cycle that repeats every 18-24 days, causes the opportunity to mate or perform artificial insemination to be missed (20). In this study, the mean estrus duration was recorded as 9.8±1.434 hours overall. Similar results were reported by Løvendahl and Chagunda (21) using AI technology in Holstein, Jersey, and Red Dane breeds, with heifers showing a mean duration of 9.24 hours and cows 8.12 hours. Another study using automatic measurement monitors reported that cows in the open air (12.4h) had a longer duration of estrus compared to cows in indoors (9.9h) (22). However, comparing estrus periods with Schweinzer et al. (23), also utilizing AI technology, revealed a

Table 1 - Differences in the Behavior of Cows between Non-Estrus and Estrus (%) According to THI, Parity, and DIM by Least Square Mean.

Feature	High Active			Active			Not Active			Eating			Rumination		
	NE	E	%	NE	E	%	NE	E	%	NE	E	%	NE	E	%
Overall	9.2	20.6	+124	6.2	6.5	+4.8	26.1	22.1	-15.3	28.8	31.0	+7.6	29.6	19.8	-23.1
THI	***	*		***	**		***	NS		NS	NS		***	*	
< 70	6.4	16.6	+159	3.7	5.1	+37.8	28.2	23.0	-18.4	28.9	29.6	+2.4	32.7	25.8	-21.1
> 70	12.0	25.0	+208	8.7	8.1	-6.9	24.0	21.2	-11.7	28.6	32.7	+14.3	26.6	13.1	-50.8
Parity	***	NS		***	NS		NS	NS		***	NS		***	NS	
Primipar	9.7	21.2	+119	5.7	6.5	+14.0	26.3	21.7	-17.5	28.4	29.8	+4.9	30.0	20.8	-30.7
Multipar	8.8	19.1	+217	6.7	6.3	-6.0	26.0	21.7	-16.5	29.2	31.0	+6.2	29.3	21.8	-25.6
DIM	***	***		***	NS		***	*		***	NS		***	NS	
0-150	9.5	22.1	+133	6.5	6.2	-4.6	25.0	23.9	-4.4	28.9	28.4	-1.7	30.1	19.4	-35.5
151+	9.0	18.8	+109	6.0	6.9	+15.0	27.2	20.1	-26.1	28.6	34.0	+18.9	29.2	20.2	-30.8

The means within columns with different superscripts are significantly different at P<0.05. ***(P<0.001), **(P<0.01), *(P<0.05), NS: Non-significant. THI: Temperature-Humidity Index, DIM: Daily in milk, NE: Non-estrus period, Estrus period.

lower duration than the reported 16.9 hours in HF cows.
Our study found a significant decrease in cow estrus duration with rising air temperature (Figure 2), indicating a negative impact on reproduction. Similarly, Borchardt et al. (24) noted shorter estrus durations in Holstein cows during summer (12.7 hours) compared to other seasons. Peralta et al. (25) reported decreasing estrus durations in June, July, and August, correlating with higher THI values. Bülbül and Ataman (26) highlighted reduced estrous rates and reproductive performance in cows when THI exceeded 72 during July and August. Our findings regarding THI variations in estrus duration, with shorter durations observed during summer months, corroborate previous research.
Our study observed longer estrus durations in multiparous cows (Figure 2), possibly due to optimized management practices. Schweinzer et al. (23) also found higher estrus durations in second-parity cows. However, contrasting studies suggest increased estrus activity in primiparous cows, declining with parity (12,21). The longer estrus duration in multiparous cows could indeed be influenced by factors such as improved environmental conditions and feeding programs.
Additionally, our study noted longer estrus durations with advancing days in milk (DIM) (Figure 2), likely linked to milk yield. This aligns with previous reports of shorter estrus periods in early lactation compared to later stages (8,23). The observed increase in estrus duration as the DIM period progresses is consistent with the notion that milk yield may influence reproductive processes in cows.

Circadian Distribution of Estrus

Our study analyzed hourly behavioral and estrus data at 6-h intervals to elucidate daily circadian rhythms. Notably, 32.4% of estrus periods occurred at night (Figure 3a), consistent with findings by Peralta et al. (25) who reported a higher estrus frequency (42.5%) between 00:01 and 06:00 hours. Also, Schweinzer et al. (4) and Brehme et al. (11) ob-

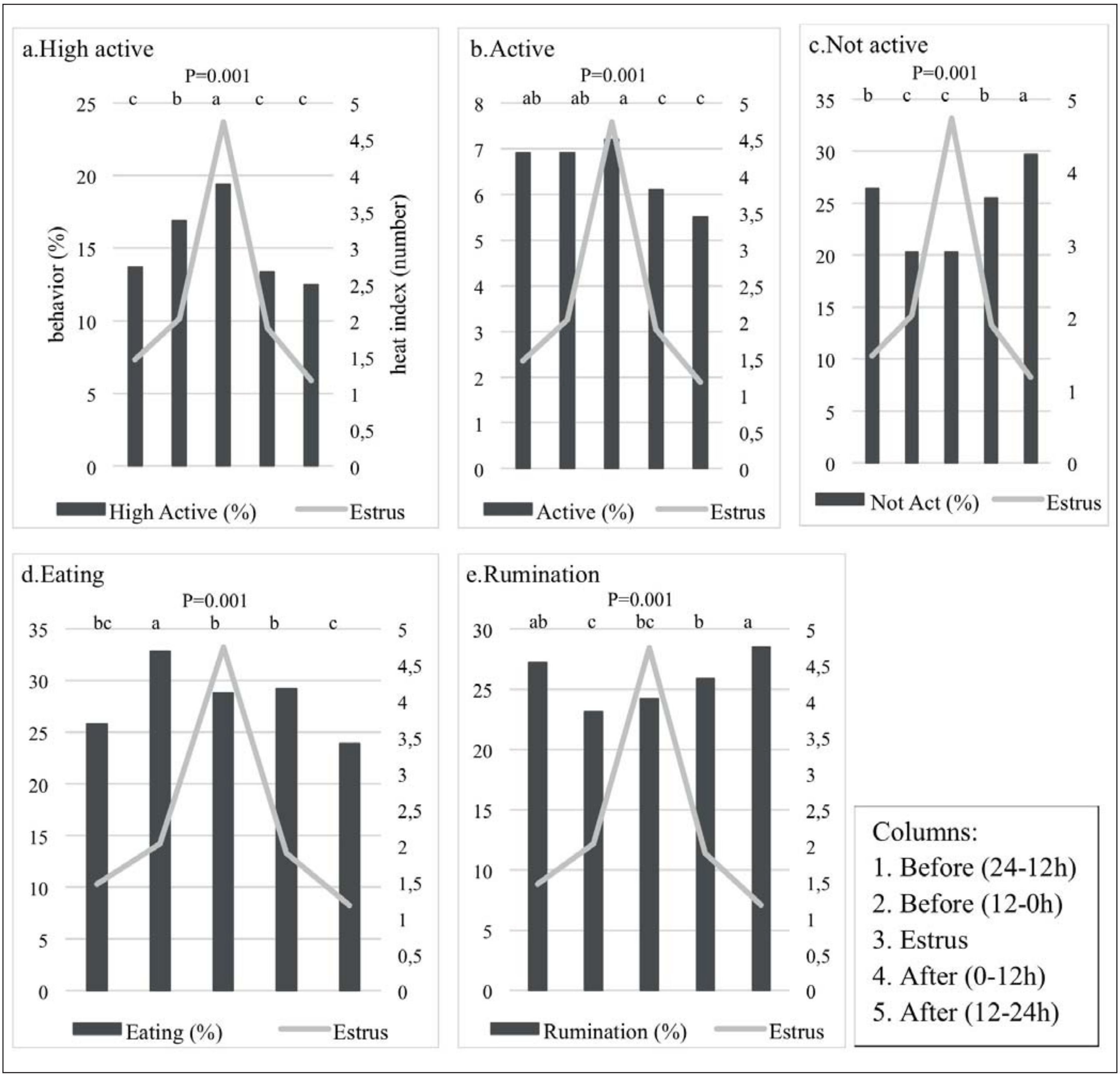

Figure 4. Before and After Display of Behaviors During Estrus.

served increased estrous activity at night. These results suggest cows are more likely to exhibit standing activity when less distracted by feeding, milking, and barn cleaning. This assumption is reasonable and supported by the literature. This highlights the potential influence of management practices on estrus behavior and the importance of considering environmental factors in reproductive management strategies. In addition, Minegishi et al. (27) reported that ovulation times, when cows tend to ruminate, are interrupted by daytime grazing behavior.

Additionally, our study suggests that estrus prevalence at night may be due to reduced disturbing activities like insemination and treatment. Pahl et al. (13) noted nighttime as the most promising time for detecting behavioral changes in cows. Moreover, higher estrus rates in the morning, when SNI values were above 70, may result from fewer samples in periods >70. Increased animal mobility during daylight, especially in high temperatures, may also contribute to more estrus values displayed by sensor systems. This is important to account for such factors when interpreting estrus data.

Differences between Estrus and Non-Estrus Periods

Since only values above 3.5, which are defined as definitive estrus, are taken into account in the calculation of estrus values in the system, it is expected that these values will increase compared to non-estrus days. Mayo et al. (28) observed significant increases in activity during estrus compared to non-estrus days, ranging from 69% to 170% across different monitoring systems (Table 1). High-active and rumination behaviors showed the most notable differences, underscoring their importance in identifying cow estrus.

In the study, high-active behavior increased by 124% during estrus compared to non-heat days. Various monitoring systems reported mobility increases ranging from 118.5% to 309.4% on estrus days (1). Another study noted a 38.7% rise in activity during estrus (29). It has been reported in various studies that these increases in activity during estrus in cows may be related to the release of estrogens (estradiol-17) that begins in the pre-estrus period (2,5,29). Although parity groups showed similar high-active changes, primiparous cows exhibited a 14% increase compared to multiparous cows. This aligns with previous studies indicating higher activity in first-lactation cows during estrus (1,5). In another study, it was reported that when the estrus day (day 0) was compared with the reference period (-3, -2, -1, +1, +2, +3 days compared to the reference period), an increase in daily activity was observed in 96% of estrus events and a decrease in daily rumination was observed in 82% of estrus events (27).

During estrus, there's a decrease (-15.3%) in not-active values compared to non-estrus days. This was reported by Dolecheck et al. (1) and found to be compatible with the data. Increased activity during estrus naturally reduces inactivity time. Restlessness during estrus shortens lying time (10), with cows reported to not lie down for 6 to 17 hours (11). This behavior, especially in cows with low observed inactivity, can aid in estrus detection. Here, a noteworthy finding, the disparity between estrus and non-estrus values increases with advancing days in milk (DIM), likely due to decreased milk yield and increased inactivity.

Estrogen hormones, known for increasing activity in cows during estrus, typically reduce rumination and feeding times (12). Pahl et al. (13) observed a significant decrease (58 min) in feeding time on cows' estrus days. However, our study, corroborated by Contrary to these findings, in our study, eating time increased (+7.6%) during estrus compared to the non-estrus period. Similarly, Dolecheck et al. (1), using the Cow-Manager system, noted increase (8.0 min/h) in eating time during estrus. Lukas et al. (30) found DMI increased 0.61 kg/d during estrus. This discrepancy may be attributed to the system's reliance on accelerometer-based feeding-related ear movements, possibly influenced by increased restlessness and head movements in cows during estrus.

The most significant behavioral change during cows' estrus is a notable decrease in rumination values (-23.1%) following increased activity levels (Table 1). The increase in the activity of cows during estrus can be the main reason for the decrease in rumination times. Dolecheck et al. (1) reported similar declines of -43.8% and -37.9% using Cow-Manager and HR Tag systems, respectively. Reith and Hoy (12) and Reith et al. (29) observed reductions of 17.0% and 19.6% in rumination during estrus in studies evaluating 265 and 453 estrus events. Schweinzer et al. (4) reported a 19.6% decrease in rumination time, with 86.0% of cows spending less time ruminating on estrus days. A significant decrease (-50.8%) in rumination values during high heat stress conditions (THI>70) suggests increased mobility may contribute to reduced rumination time. Primiparous cows showed a higher decrease (-30.7%) in rumination times, likely linked to their activity behavior and these values were found to be compatible with the values reported by Schweinzer et al. (4).

Behaviors Before and After Estrus

Considering that estrus in cows begins at any time of the day, it is difficult to classify and evaluate its distribution during the day. Our study recorded behavior and estrus data hourly, focusing on individual cows during estrus for comparison over the preceding and subsequent 24 hours. This approach allows for detailed examination without daily classification constraints.

Estrus behavior in cows is characterized by increased physical activity (2,4). Restlessness and increased activity are also signs of estrus (21). According to the change in these behavioral values, it was determined that the most affected behavior in cows during estrus, both before and after estrus, was high-active (Table 1). Numerous pedometer studies have noted a significant increase in the number of steps during estrus (21,25,28), suggesting high-active as a crucial factor in determining estrus time. High-active values typically begin to rise the day before estrus, peak on the estrus day, and decrease thereafter, aligning with findings from accelerometer studies (4,8,29). Similarly, not-active values, indicative of resting time, decrease before estrus, reach their lowest point on the estrus day, and gradually increase thereafter (4,11).

The study revealed a significant impact of estrus onset on rumination in cows. Reith and Hoy (12) observed a 17% (74 min) decrease in rumination time during estrus compared to surrounding days. Similarly, Pahl et al. (13) noted a decrease of 19.3% on the first day and 19.8% on the previous day of inseminations leading to pregnancy. This decrease in rumination time, starting even a day before insemination, may be closely linked to increased physical activity, a key indicator of estrous behavior initiation. Thus, the gradual decline in rumination values preceding estrus onset presents a contrasting dynamic with active values.

The study observed that eating times were affected by estrus, albeit irregularly. This fluctuation could be attributed to the Cow-Manager system's percentage-based calculation, where a decrease in one behavior may lead to an increase in another. Consequently, there wasn't a significant change in eating time during estrus compared to non-estrus days (Figure 4d). Pahl et al. (13) reported lower feed intake and higher feeding rates the day before insemination, with significant reductions observed only on the insemination day. Changes in feeding behavior during estrus are linked to increased physical activity and restlessness, as noted by (10). Thus, the decrease in feeding and rumination activities before and during insemination may be a result of reduced time available for feed intake. In a study conducted using the CowManager system, it was reported that high active increased and rumination decreased to a great extent, while the high level of «eating» value may actually be due to continuous head movements that mimic the feeding behavior and movement of animals in estrus (16).

CONCLUSION

In conclusion, this study provides valuable insights into behavioral changes and estrus duration in dairy cows and sheds light on factors that influence reproductive patterns. Since an increase in physical activity and a decrease in rumination times were observed during the estrus period in cows, the use of behavioral monitoring systems such as pedometers or smart sensors can improve estrus detection and timing. In particular, the increased activity and decreased rumination times before estrus can serve as an early warning system. By monitoring these changes, farmers can detect the estrus period earlier and perform insemination at the appropriate time. The study shows that high temperatures can shorten the duration of estrus and have a negative impact on reproductive performance. Therefore, it is important to provide appropriate cooling systems such as fans, sprinkler systems, and shaded areas to cope with heat stress, especially during the summer months. These results, behavioral changes during estrus, including increased physical activity and altered rumination and eating patterns highlight the importance of monitoring these parameters for estrus detection.

Ethical Approval

This study's Ethics Committee Certificate with the date 29.03.2022-199 was obtained from the International Center for Livestock Research and Training.

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Author Contributions

Conceptualization: Ç.M.S., İ.A.; Methodology: Ç.M.S., Y.E., B.A., M.Y., İ.A.; Data collections: Ç.M.S., Y.E., B.A.; Statistical analysis: Ç.M.S., M.Y., İ.A.; Article writing: Ç.M.S., M.Y., İ.A.

Conflict of Interest Statement

The authors of the study kindly declare no competing interest.

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