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Characterization of the physical and ruminal activities related to estrus in dairy cows raised on Chinese commercial farms

Xiaojing Zhou[1, 2], Chuang Xu[2*], Matheus Gomes Lopes[3], Juan J Loor[3], Yunhong Shao[1, 2], Bin Jia[4], Guang Shao[4], and BaoYin Huang[4]

[1]*Department of Information and Computing Science, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province, China*
[2]*Heilongjiang Provincial Key Laboratory of Prevention and Control of Bovine Diseases, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Heilongjiang Province, China*
[3]*Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, USA*
[4]*Animal Husbandry and Veterinary Branch of Heilongjiang Academy of Agricultural Science, Qiqihaer, Heilongjiang, China*

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ABSTRACT

This study aimed to describe the estrus-related changes in dairy cattle in parameters automatically recorded through an HR-Tag (SCR Engineers Ltd., Netanya, Israel) or a neck collar (Nedap Livestock Management, Groenlo, Netherlands). On two commercial dairy farms, the baseline period was defined as the mean of 3 d before and 3 d after estrus day. In the HR-Tag monitored herd, changes in physical activity and behavioral parameters (lying bouts, lying duration, total lying time, lying ratio) were studied in 78 estrous cycles. The cows were classified in groups according to parity (primiparous, n = 34; and multiparous, n = 44), milk production (MK1, n = 7, > 47 kg/d; MK2, n = 12, 42-47 kg/d; MK3, n = 43, 31-42 kg/d; and MK4, n = 16, < 31 kg/d) and lactation stage (DIM1, n = 34, ≤ 60 d; DIM2, n = 22, 61-120 d; and DIM3, n = 22, ≥ 121 d). In the neck-collar monitored herd, we investigated physical activity on estrus day and total daily feeding time, rumination time and inactivity time in 35 dairy cows during the peri-estrus period. On estrus day, the effects of parity and milk production on physical activity, and the differences in total daily feeding time, rumination time and inactivity time between estrus day and the baseline period were evaluated. The cows were classified in groups according to parity (primiparous, n = 9; and multiparous, n = 26), and milk production (MK1, n = 5, > 41 kg/d; MK2, n = 17, 32-41 kg/d; and MK3, n = 13, < 32 kg/d). ANOVA was performed to determine differences in the continuous data, and comparing means was performed to estimate differences within and between groups, using SPSS 23.0. Ordinal logistic regression was adopted to analyze the factors affecting physical activity on estrus day. Results from the HR-Tag monitored herd demonstrated that on estrus day, physical activity increased compared with the baseline period, and average number lying bouts on estrus day was markedly lower than at baseline, as well as total lying time and lying ratio, with values of -3.53 ± 0.55, -188.02 ± 21.46 and -14.05 ± 1.37, respectively. For the neck

*Corresponding author:
Chuang Xu, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Xinyang Rd. 5, 163319 Daqing, China. E-mail: xuchuang7175@163.com

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collar-monitored herd, when the TFT (total feeding time) of MK2 cows increased by one unit, physical activity was 3.19 times (P=0.03) more likely to increase by one unit compared to those without increased TFT. Overall, our data underscored the important physical, behavioral and ruminal changes related to estrus, which were accurately detected through the automated monitoring systems. Thus, it is suggested that HR-Tags and neck collars are suitable tools for estrus detection in commercial dairy herds.

Key words: automated monitoring system; estrus; lying behavior; reproductive management; rumination

Introduction

Today, the use of technologies plays a fundamental role in dairy herds, and process automation allows for greater precision, coupled with a reduction in physical labor (RUTTEN et al., 2013). Automated systems for monitoring physical activity and rumination time can provide the farm manager with important information about nutrition, reproduction and health status. Application of data from automated monitoring systems (AMS) has been investigated in key fields of study, such as: periparturient diseases (CALAMARI et. al., 2014; LIBOREIRO et al., 2015), estrus identification (AUNGIER et al., 2012; KAMPHUIS et al., 2012), and reproductive performance (GALVÃO et al., 2013).

In the field of reproduction, estrus detection is a critical point to guarantee artificial insemination (AI) efficiency and, although there are tools for estrus identification, the most-common mode is still visual observation (DOLECHECK et al., 2015). However, environmental and behavioral factors can directly influence the intensity of estrus expression, leading to a significant decrease in estrus detection rate (LOPEZ et al., 2004). In the last few decades, a marked decline from ~18 to ~8 h in estrus duration has been reported in Holstein cows (REAMES et al., 2011), which renders visual observation even more challenging.

The behavior of dairy cows during the estrus period is often characterized by standing to be mounted, restlessness, sniffing, licking, or resting with the chin on the back of another cow (LøVENDAHL and CHAGUNDA, 2010; ZEBARI et al., 2018). In addition, estrus is also accompanied by decreased feeding and rumination time (DOLECHECK et al., 2015; PAHL et al., 2014; MINEGISHI et al., 2019), with rumination widely proven as the most sensitive indicator of a cow's well-being, and one that deserves individual daily monitoring to establish reference values for each cow (REITER et al., 2018). With observation of changes in rumination time, farmers can quickly identify individual cows that need attention and make timely decisions that optimize individual and herd production. In that context, integration of information collected by AMS is supplemented with other behavioral parameters, to allow for more precise advice, including whether to inseminate a cow or not (MAYO et al., 2019).

Private dairy farms in several countries have implemented AMS in recent years, and commercial tags are routinely the chosen method (SCHIRMANN et al., 2012; PAHL et al., 2014; LIBOREIRO et al., 2015). For instance, the HR-Tag system (SCR Engineers Ltd., Netanya, Israel) was previously validated with proven applicability in monitoring physical and ruminal behavior in dairy cows (SCHIRMANN et al., 2009; REITH and HOY, 2012; STANGAFERRO et al., 2016). In addition, the use of a triaxial accelerometer, coupled to an adjustable neck collar (Nedap Livestock Management, Groenlo, Netherlands), has previously been validated and utilized as a useful system for analysis of eating behavior (VAN ERP-VAN DER KOOIJ et al., 2016; ECKELKAMP and BEWLEY, 2020). Although extensively investigated in recent years, there is still a lack of knowledge about the behavior characteristics and changes in rumination time of dairy cows on days around estrus, monitored by AMS in the northeast of China.

The objectives of this study were to evaluate (1) estrus-related changes to individual cow activity recorded by HR-Tags; and (2) the effect of feeding time, rumination time and inactivity time on physical activity on estrus day in cows monitored by a neck collar. A specific focus was to characterize the feeding and rumination time variations on estrus day and in the baseline period.

Materials and methods

Animals, Housing, and Feeding. The study was conducted on two commercial farms located in the northeast of China, with 800 and 450 lactating Holstein cows. One farm was located between longitude 126.956 and 126.972, latitude 46.627 and 46.633, with a cold and temperate continental monsoon climate. The annual average temperature is 2°C, with average temperature 22.4°C in July. For the herd studied on this farm, data on physical activity around estrus events were recorded with the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) from April 2019 to January 2020. In total, 166 verified estrous cycles were observed in 94 cows with AI, and led to conception, and 78 cows were confirmed pregnant by double confirmation. The other commercial farm was located between longitude 126.738 and 126.746, latitude 45.603 and 45.606, with a cold and temperate continental monsoon climate. The annual average temperature is 0.4°C, with average temperature 21.3°C in July. For the herd studied on this farm, data on physical activity on estrus day and rumination characteristics around estrus events were recorded with neck collars (Nedap Livestock Management, Groenlo, Netherlands) from November 2019 to May 2020. In total, 66 verified estrous cycles in 48 cows were observed with AI and led to conception, and 35 cows were confirmed pregnant by double confirmation.

The baseline period was defined as the mean of 3 d before and 3 d after estrus day in both herds. Records collected on the cows for both herds included calving date, lactation number (1-6 range), AI services, AI date, days in milk (DIM) at the beginning of the estrus synchronization protocol, and pregnancy confirmation. The cows were checked daily for signs of diseases or injuries by the farm staff. Each evaluated group was housed in uniform pens with identical characteristics: the feeding and water areas in each pen, feed bunk, and access to an exercise yard for 1 h/d. Cows were fed a total mixed ration (TMR) twice daily (0500 h and 1300 h) with ad libitum access to fresh water. To fulfill energy and protein requirements, the delivered TMR was formulated with corn silage, alfalfa silage, *leymus chinensis*, and a grain mix (Supplemental Table S1). The cows were milked three times a day (0300 h, 1100 h and 1900 h).

Supplementary material

Supplemental Table S1.	Value (kg)	Percentage (%)
Ingredient		
Corn silage	0.30	0.69
Alfalfa silage	22.00	50.47
Leymus chinensis	2.20	5.05
Ground corn grain	0.07	0.16
Soybean hulls	1.60	3.67
Staple fiber feed	1.70	3.90
Ground barley grain	0.32	0.73
Water	6.00	13.76
Lactating material	9.40	21.56
Nutrient		
NDF		35.45
ADF		16.90
ADL		0.85

Supplementary material (continued)

Supplemental Table S1.	Value (kg)	Percentage (%)
Starch-AG		22.78
Ca		0.79
P-total		0.41
Mg		0.34
K		1.04
Na		0.24
Trace mineral-vitamin mixture		0.30
S		0.25
CP		16.43
CFAT		4.22

Study Design. On both farms, the cows enrolled in the study were artificially inseminated according to the estrus synchronization protocol. In brief, the first phase of the estrus synchronization protocol started at 38 d after calving as follows: day 0 (GnRH1), day 7 (PG1) and day 10 (GnRH2). Seven days later, the second phase of the estrus synchronization protocol started and included day 0 (GnRH3), day 7 (PG2), and day 9 (PGF2α) to allow for expression of estrus. Estrus day was defined as the day when estrus was detected both by visual observation and by AMS (i.e. the amount of physical activity monitored by the HR-Tag for one herd, and physical activity monitored with a neck collar for the other herd).

In the HR-Tag-monitored herd, 78 cows (average milk production 11,268.17 kg/year, DIM 101.18 ± 54.75 d) were found to be successfully pregnant after double confirmation (28-35 days and 60-70 days after AI). Physical activity (no.), bouts of lying down (no.), total lying time (min/d) and lying ratio (%) for each cow were measured each day. Estrus day identification was identical to the AI day.

In the neck collar-monitored herd, 35 cows (average milk production 9,870.92 kg/year, DIM 155.54 ± 37.01) were found to be successfully pregnant after double confirmation (28-35 days and 60-70 days after AI). Physical activity on estrus day, denoted as multiple ordinal variables by a number from 1 to 9, as well as total rumination time (TRT) (h/d), total feeding time (TFT) (h/d) and total inactivity time (TIAT) (h/d) around the estrus period (3 d before and 3 d after estrus) were downloaded each day and used for statistical analysis. In the data editing process, some records were deleted according to the following criteria: records of the parameters monitored by the AMS without data; cows missing data 7 d before and 7 d after estrus day in a group; a cow that was moved between herds more than twice within a lactation; cows without AI due to some special reason, e.g. acute health disorders during the experimental period.

Group Division. In the HR-Tag-monitored herd, the cows were classified in stratified groups according to parity, milk production and lactation stage. Two groups were defined according to parity (2.03 ± 1.25): primiparous (n = 34; average milk production 10,060.73 kg/year; 122 ± 59 DIM) and multiparous (n = 44; average milk production 12,201.19 kg/year; 74 ± 33 DIM; 2.82 ± 1.15 parity). Four groups were defined according to milk production: MK1 (n = 7; > 47 kg/d), MK2 (n = 12; 42-47 kg/d), MK3 (n = 43; 31-42 kg/d) and MK4 (n = 16; < 31 kg/d), and three groups were defined according to lactation stage: DIM1 (n = 34; ≤ 60 d), DIM2 (n = 22; 61-120 d) and DIM3 (n = 22; ≥ 121 d).

In the neck collar-monitored herd, cows were classified in stratified groups according to parity and milk production (for all cows enrolled, DIM ranged from 101 to 200 days; groups according to lactation stage were not considered). Two groups were defined according to parity (2.60 ± 1.17): primiparous (n = 9; average milk production 9,870.92 kg/year) and multiparous (n = 26; average milk production 10,616.73kg/year, 3.15 ± 0.78 parity). Three groups were defined according to milk production: MK1 (n = 5; > 41 kg/d), MK2 (n = 17; 32-41 kg/d) and MK3 (n = 13; < 32 kg/d).

Statistical Analysis. For the data on the two herds downloaded from the AMS applied on both farms, ANOVA was performed in SPSS 23.0 (SPSS Inc., Chicago, IL) to determine differences in the continuous data. Comparing means was performed to estimate differences within and between the groups according to parity, milk production and lactation stage, and interactions among them. Pearson correlation coefficients and significance levels were calculated for the relationships between the variables. Ordinal logistic regression was adopted to analyze the factors affecting physical activity on estrus day. Statistical significance was considered at $P \leq 0.05$.

Results

HR-tag Monitored Herd

Peak estrus activity. We calculated the average physical activity on estrus day (APAE) and the average percentage of relative increase in physical activity (ratio of physical activity on estrus day to the average physical activity 3 d before and 3 d after estrus day) according to parity, milk production, and lactation stage, which had a marked association with APAE. Peak estrus activity (physical activity on estrus day) showed a marked increase in all evaluated cows (304.43 %; Table 1). In addition, the largest relative increase in physical activity was 317.81%, 350.79% and 320.26% for primiparous, MK4, and DIM1 cows, respectively.

Table 1. Means ± SD for peak estrus activity and percentage of relative increase in activity according to parity, milk production, and lactation stage.

Item	No.	Peak activity	Relative increase[1] (%)
Total	78	498.47 (159.53)	304.43 (105.00)
Parity			
Primiparous	34	549.44^{a} (173.78)	317.81^{a} (105.14)
Multiparous	44	459.09^{b} (136.94)	294.10^{b} (104.92)
Milk production (kg/d)			
MK1(>47)	7	354.14^{a} (101.03)	228.71^{a} (51.65)
MK2(42-45)	12	492.42^{ab} (173.95)	281.74^{ab} (89.06)
MK3(31-41)	43	497.09^{bc} (147.40)	305.84^{bc} (116.55)
MK4(<31)	16	569.88^{bd} (168.34)	350.79^{bd} (79.31)
Lactation stage			
DIM≤60	34	529.82^{a} (161.49)	320.26^{a} (109.18)
DIM61-120	22	435.86^{b} (151.62)	264.52^{b} (92.73)
DIM≥121	22	512.64^{ab} (158.09)	319.87^{ab} (103.79)

[1]Relative increase is the ratio of peak estrus activity (on estrus day) to the average physical activity at baseline time. [ac]Different superscript lowercase letters in each group by parity and milk production indicate significant differences (P <0.05).

According to parity, the APAE in primiparous was greater than multiparous cows [mean difference 90.35, P=0.012, 95% CI (20.30, 160.39)]. According to milk production, the APAE in MK1 was significantly lower than MK3 [mean difference -142.95, P=0.025, 95% CI (-267.18, -18.72)], and MK4 cows [mean difference -215.73, P = 0.003, 95% CI (-353.86, -77.61)]. According to lactation stage, the APAE in DIM≤60 was greater than DIM61-120 [mean difference 94.00, P=0.031, 95% CI (8.69, 179.23)].

There were significant interactions between parity and lactation stage (F = 2.511, P=0.038). Primiparous cows in the DIM3 group (DIM ≥ 121 d) had the highest average physical activity on estrus day, with the value of 656.33 ± 83.58, and multiparous cows in the DIM2 group had the lowest average physical activity on estrus day, with a value of 393.93 ± 94.63. Weak interactions between parity and milk production groups were detected (F = 2.265, P=0.047). Primiparous cows in the MK2 group expressed the highest average physical activity on estrus day, with a value of 667.00, and multiparous cows in MK1, expressed the lowest average physical activity on estrus day, with a value of 354.14 ± 101.03. Average physical activity on estrus day was not significantly influenced by the interactions of milk production and lactation stage.

Correlations between physical activity on estrus day and behavioral parameters affecting estrus. APAE was negatively correlated with total lying time (min/d) (-0.247; P=0.029) (Table 2). According to parity, APAE in multiparous cows was negatively correlated with lying bouts (-0.468; P=0.001), total lying time (-0.463; P=0.002), and lying ratio (-0.555; P≤0.001) (Table 2). However, APAE in primiparous cows was positively correlated with total lying time (0.416; P=0.015), and lying ratio 0.477 (P=0.004) (Table 2). According to milk production, correlations between APAE and lying ratio were observed in MK1 (-0.807; P=0.028) and MK2 (-0.620; P=0.031) cows. According to lactation stage, correlations between APAE and lying bouts (-0.586; P=0.005), total lying time (-0.594; P=0.005), and lying ratio (-0.592; P= 0.005) were observed in DIM≥121 cows.

Table 2. Coefficients and P-value between peak estrus activity and lying bouts, lying duration, total lying time, lying ratio according to parity, milk production, and lactation stage.

Item		Lying bouts	Lying duration	Total lying time	Lying ratio
Peak estrus activity	Coefficient	-0.149	-0.039	-0.247*	-0.187
	P-value	0.192	0.737	0.029	0.101
Parity					
Primiparous	Coefficient	0.227	0.180	0.416*	0.477**
	P-value	0.197	0.309	0.015	0.004
Multiparous	Coefficient	-0.468**	-0.027	-0.463**	-0.555**
	P-value	0.001	0.862	0.002	P<0.001
Milk production (kg/d)					
MK1(>47)	Coefficient	-0.545	-0.021	-0.716	-0.807*
	P-value	0.206	0.965	0.070	0.028
MK2(42-45)	Coefficient	-0.525	-0.126	-0.573	-0.620*
	P-value	0.080	0.697	0.051	0.031
MK3(31-42)	Coefficient	-0.189	0.090	-0.167	-0.109
	P-value	0.224	0.564	0.283	0.488
MK4(<31)	Coefficient	0.029	-0.046	0.125	0.418
	P-value	0.916	0.866	0.646	0.107

Table 2. Coefficients and P-value between peak estrus activity and lying bouts, lying duration, total lying time, lying ratio according to parity, milk production, and lactation stage. (continued)

Item		Lying bouts	Lying duration	Total lying time	Lying ratio
Lactation stage					
DIM≤60	Coefficient	-0.039	-0.140	-0.189	-0.099
	P-value	0.826	0.431	0.285	0.579
DIM61-120	Coefficient	-0.281	0.303	0.087	0.190
	P-value	0.195	0.159	0.693	0.385
DIM≥121	Coefficient	-0.586**	-0.053	-0.594**	-0.592**
	P-value	0.005	0.821	0.005	0.005

Comparison of differences between activity and behavioral parameters and the baseline on estrus day. The mean differences ± SD, 95% CI or 99% CI and *P*-value derived among groups are reported in Table 3. With the exception of lying duration, there was a significant difference between APAE and the baseline in all other behavioral parameters. The mean difference between APAE and baseline was 330.39 ± 18.43 [P≤0.001, 95% CI (293.64, 367.12)], with the largest difference 374.52 ± 30.04 in primiparous cows, 407.71 ± 42.59 in MK4 cows, and 359.79 ± 28.38 in DIM1. Lying bouts on estrus day were -3.53 ± 0.55 lower than the baseline for the 78 cows analyzed, especially primiparous, with a value of -4.55 ± 0.87.

Table 3. Differences between physical activity and behavioral parameters on estrus day and baseline, in groups defined according to parity, milk production and lactation stage.

Item	Physical activity	Lying bouts	Lying duration	Total lying time	Lying ratio
MD_{total}[1]	330.39 (18.43)	-3.53 (0.55)	2.87 (2.82)	-188.02 (21.46)	-14.05 (1.37)
CI_{total}[2]	[293.64, 367.12]*	[-4.97, -2.09]**	[-2.76, 8.49]	[-244.05, -131.99]*	[-17.63, -10.46]*
P-value	<0.001	<0.001	>0.05	<0.001	<0.001
MD_{Pri}[1]	374.52 (30.04)	-4.55 (0.87)	-2.49 (3.59)	-189.44 (29.64)*	-14.02 (1.91)*
CI_{Pri}[2]	[313.48, 435.55]*	[-6.87, -2.24]**	[-9.62, 4.65]	[-248.80, -130.08]	[-17.85, -10.20]
P-value	<0.001	<0.001	>0.05	<0.001	<0.001
MD_{Mul}[1]	296.30 (21.57)	-2.74 (0.49)	0.38 (4.33)	-186.92 (30.46)*	-14.06 (1.94)*
CI_{Mul}[2]	[253.00, 339.60]*	[-4.04, -1.45]**	[-8.26, 9.02]	[-247.47, -126.37]	[-19.16, -8.97]
P-value	<0.001	<0.001	>0.05	<0.001	<0.001
MD_{MK1}[1]	197.5 (41.31)	-1.43 (0.68)*	-5.33 (8.00)	-101.71 (106.97)	-9.69 (7.26)
CI_{MK1}[2]	[102.21, 292.79]*	[-2.06, -0.80]	[-12.73, 2.06]	[-200.64, -2.79]	[-16.40, -2.98]
P-value	0.001	0.039	>0.05	>0.05	>0.05
MD_{MK2}[1]	290.36 (76.98)	-2.40 (2.46)*	4.40 (11.85)	-190.04 (127.59)	-14.58 (9.17)
CI_{MK2}[2]	[122.64, 458.08]*	[-3.96, -0.84]	[-3.13, 11.93]	[-271.11, -108.98]	[-20.41, -8.76]
P-value	0.003	<0.001	>0.05	>0.05	>0.05
MD_{MK3}[1]	326.89 (23.23)	-3.84 (2.92)*	-1.57 (13.71)	-189.30 (138.60)	-13.55 (8.36)

Table 3. Differences between physical activity and behavioral parameters on estrus day and baseline, in groups defined according to parity, milk production and lactation stage. (continued)

Item	Physical activity	Lying bouts	Lying duration	Total lying time	Lying ratio
CI_{MK3}[2]	[280.70, 373.08]*	[-4.74, -2.94]	[-5.79, 2.64]	[-231.96, -146.65]	[-16.13, -10.98]
P-value	<0.001	<0.001	>0.05	>0.05	>0.05
MD_{MK4}[1]	407.71 (42.59)	-4.48 (2.79)*	-2.76 (9.73)	-220.81 (129.52)	-16.88 (7.11)
CI_{MK4}[2]	[317.30, 498.12]*	[-5.96, -2.99]	[-7.94, 2.42]	[-289.83, -151.80]	[-20.67, -13.08]
P-value	<0.001	<0.001	>0.05	>0.05	>0.05
MD_{DIM1}[1]	359.79 (28.38)	-4.28 (3.37)*	-1.49 (12.07)	-186.09 (148.24)	-13.93 (8.19)
CI_{DIM1}[2]	[302.25, 417.33]*	[-5.46, -3.10]	[-5.70, -2.73]	[-237.81, -134.37]	[-16.79, -11.07]
P-value	<0.001	<0.001	>0.05	>0.05	>0.05
MD_{DIM2}[1]	266.95 (33.24)	-2.79 (1.64)*	-2.82 (11.12)	-165.80 (105.41)	-11.28 (7.64)
CI_{DIM2}[2]	[198.26, 335.64]*	[-3.52, -2.06]	[-7.75, 2.11]	[-212.53, -119.06]	[-14.67, -7.89]
P-value	<0.001	<0.001	>0.05	>0.05	>0.05
MD_{DIM3}[1]	345.89 (34.79)	-3.12 (2.60)	0.73 (14.20)	-213.23 (136.65)	-17.00 (8.20)
CI_{DIM3}[2]	[273.68, 418.10]*	[-4.27, -1.97]	[-5.56, 7.03]	[-273.81, -152.64]	[-20.63, -13.37]
P-value	<0.001	>0.05	>0.05	>0.05	>0.05

[1]MD_{total}, MD_{Pri}, MD_{Mul}, MD_{MK1}, MD_{MK2}, MD_{MK3}, MD_{MK4}, MD_{DIM1}, MD_{DIM2}, MD_{DIM3} represent mean differences (standard deviation) between peak estrus activity and behavioral parameters on estrus day and baseline for the total of 78 cows. Cows were in groups defined according to parity, milk production and lactation stage.
[2]CI_{total}, CI_{Pri}, CI_{Mul}, CI_{MK1}, CI_{MK2}, CI_{MK3}, CI_{MK4}, CI_{DIM1}, CI_{DIM2}, CI_{DIM3} represent the confidence intervals of mean differences for the 78 cows. Cows were divided according to parity, milk production and lactation stage.

Differences between lying bouts on estrus day and baseline for groups by milk production are reported in Figure 1. In MK1 cows, the difference was greater than MK3 with a mean difference of 2.41 [P=0.032, 95% CI (-0.21, 4.61)], and MK4 cows with a mean difference of 3.05 [P=0.015, 95% CI (0.60, 5.50)]. A similar result was observed between MK2 and MK4 cows, with mean difference of 2.08 [P=0.049, CI 95% (0.01, 4.14)].

In DIM2, the difference between lying ratio on estrus day and baseline was greater than DIM3, with a mean difference of 5.72 [P=0.021, 95% CI (0.88, 10.55)]. There was no significant difference between different groups for behavioral parameters of lying duration or total lying time. Milk production on estrus day was not correlated with activity or behavioral parameters.

Neck Collar-Monitored Herd

Correlations between physical activity on estrus day and TFT, TRT and TIAT. On estrus day, TFT was negatively correlated with TIAT (-0.599; P<0.001), and was greater in primiparous than multiparous cows, with a mean difference of 1.37 ± 0.55 [P= 0.018, 95% CI (0.25, 2.48)]. This response was coupled with lower total inactivity time, with a mean difference of -3.26 ± 1.01 [P=0.003, 95% CI (-5.31, -1.20)]. There were no significant differences between the three groups when divided according to milk production.

Means ± SD for activity counts on estrus day, TFT, TRT and TIAT on estrus day according to parity and milk production are reported in Table 4. We analyzed the effects of parity and milk production on the amount of inactivity on estrus day detected by AMS, as well as differences in TFT, TRT and TIAT on estrus day and the baseline. The significance of the Log-Likelihood test and the paralleled lines test indicated that using multiple ordinal Logistic regression to analyze the data was of statistical significance. The TFT in multiparous cows increased by one unit, physical activity on estrus day increased one unit 0.60 times (P=0.028), while no similar results with statistical significance were obtained either for the total 35 cows or the primiparous cows (Table 5). For the neck collar-monitored herd, when the TRT on estrus day of multiparous cows decreased by one unit, physical activity was 0.60 times (P=0.028) more likely to increase by one unit compared to those without decreasing TRT. When the TFT of MK2 cows increased by one unit, physical activity was 3.19 times (P=0.03) more likely to increase one unit compared to those without increased TFT.

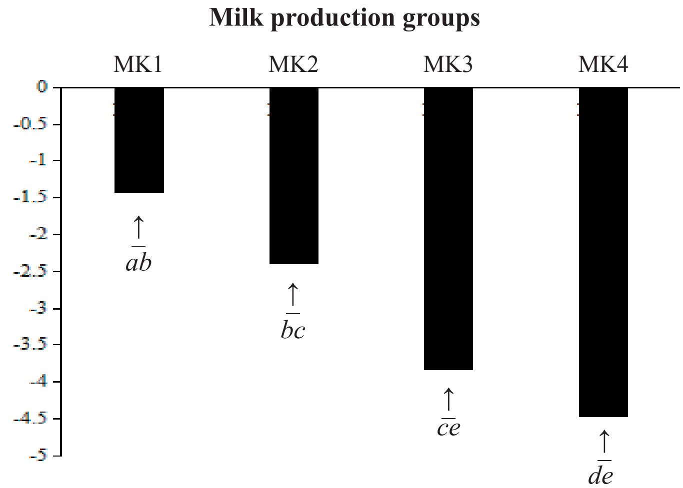

Figure 1. Differences between lying bouts on estrus day and baseline for cows grouped according to milk production.
Differences between lying bouts on estrus day and baseline where MK1 (n = 7; > 47 kg/d) was lower than MK3 (n = 43; 31-42 kg/d) [mean difference 2.41, P = 0.032, 95% CI (-0.21, 4.61)] and MK4 (n = 16; <31 kg/d) [mean difference 3.05, P = 0.015, 95% CI (0.60, 5.50)]. Different superscript lowercase letters in the bar charts indicate significant differences (P < 0.05).

Table 4. Means ± SD for physical activity on estrus day, TFT, TRT and TIAT on estrus day according to parity and milk production.

Item	No.	Physical activity on estrus day (h/d)	TFT (h/d)	TRT (h/d)	TIAT (h/d)
Total	35	4.68 (1.56)	5.28 (1.53)	6.71 (1.73)	8.47 (2.97)
Parity					
Primiparous	9	4.22^{a} (1.20)	6.32^{a}(1.13)	6.83^{a} (1.82)	6.01^{a} (1.89)
Multiparous	26	4.82^{ab} (1.66)	4.95^{b} (1.51)	6.68^{ab} (1.73)	9.27^{b} (2.83)
Milk production (kg/d)					
MK1(>41)	5	3.60^{ab} (0.89)	4.68^{ab} (2.18)	7.34^{ab} (1.49)	10.53^{a} (2.22)
MK2(32-41)	17	4.95^{ac} (1.43)	5.46^{ac} (1.28)	6.38^{ac} (1.83)	8.62^{ab} (3.44)
MK3(<32)	13	4.69^{bc} (1.84)	5.59^{bc} (1.44)	6.68^{bc} (1.72)	7.35^{b} (2.39)

[ac]Different superscript lowercase letters in each group by parity and milk production indicate significant differences (P <0.05).

Table 5. Analysis of physical activity on estrus day using multiple ordinal Logistic.

Variables	Sig. 1[1]	Parameter Estimates			Sig. 2
		Estimate(SE)	Significance	95% CI	
TFT of multiparous cows	0.018	0.60 (1.26)	0.028	[0.38, 0.94]	0.978
TFT of MK2	0.02	3.19 (1.71)	0.03	[1.12, 9.10]	0.990
TRT of MK2	0.02	0.57 (1.37)	0.08	[0.31, 0.78]	0.990

[1]Sig. 1 denotes the significance of the model log-likelihood test in fitting information. This was performed using the multiple ordinal Logistic analysis of regression in SPSS software. Sig. 2 denotes significance of the test of parallel lines.

Differences in TFT, TRT and TIAT and the baseline on estrus day. For all enrolled cows analyzed in this trial and in the multiparous group, TFT on estrus day was significantly greater than 3 d before estrus and 3 d after estrus. The TIAT on estrus day was significantly lower than 3 d before estrus and 3 d after estrus. For TFT, the average relative percentage increase was 33.8% (estrus day vs. 3 d before estrus) and 28.7% (estrus day vs. 3 d after estrus) for all cows. The average relative percentage increase for TFT at 3 d after estrus reached the highest for multiparous cows with a value of 39.6%. For TIAT, the average relative percentage decrease was 25.1% (estrus day vs. 3 d before estrus) and 43.8% (estrus day vs. 3 d after estrus) for all cows, 21.8% (estrus day vs. 3 d before estrus) and 38.6% (estrus day vs. 3 d after estrus) for multiparous cows. The TRT on estrus day was markedly lower than 3 d before and 3 d after for all cows, and for primiparous cows. In addition, the average relative percentage decrease was 12.8% (estrus day vs. 3 d before estrus) and 13.6% (estrus day vs. 3 d after estrus) for all cows. Differences between TFT, TRT, and TIAT and the baseline on estrus day are reported in Table 6 and Figure 2. There was no significant difference in the TFT or TIAT between 3 d before and 3 d after estrus day.

Table 6. Means ± SD for differences between TFT, TRT, ITAT on estrus day and 3 d before and 3 d after estrus. P-value for all cows and groups according to parity.

Variables	Estrus day vs. 3d before estrus				Estrus day vs. 3d after estrus			
	Mean difference	P-value	95% CI	Average Relative percentage (%)	Mean difference	P-value	95% CI	Average Relative percentage (%)
All cows								
TFT	1.03 (0.35)	0.005	[0.32, 1.75]	33.8	0.90 (0.36)	0.014	[0.18, 1.61]	28.7
TRT	-0.96 (0.35)	0.007	[-1.65, -0.26]	12.8	-1.02 (0.35)	0.004	[-0.63, 0.76]	13.6
TIAT	-2.80 (0.64)	<0.001	[-4.07, -1.53]	25.1	-2.79 (0.64)	<0.001	[-4.06, -1.52]	43.8
Multiparous								
TFT	1.18 (0.38)	0.002	[0.42, 1.92]	39.6	1.08 (0.38)	0.005	[0.34, 1.83]	36.4

Table 6. Means ± SD for differences between TFT, TRT, ITAT on estrus day and 3 d before and 3 d after estrus. P-value for all cows and groups according to parity. (continued)

Variables	Estrus day vs. 3d before estrus				Estrus day vs. 3d after estrus			
	Mean difference	P-value	95% CI	Average Relative percentage (%)	Mean difference	P-value	95% CI	Average Relative percentage (%)
TRT	-0.75 (1.28)	0.56	[-3.30, 1.80]	10.4	-0.78 (1.28)	0.55	[-3.33, 1.77]	11.2
TIAT	-2.70 (0.69)	<0.001	[-4.07, -1.33]	21.8	-2.78 (0.69)	<0.001	[-4.15, -1.41]	38.6
Primiparous								
TFT	0.63 (0.58)	0.29	[-0.56, 1.82]	17.1	0.36 (0.58)	0.54	[-0.83, 1.55]	6.4
TRT	-1.66 (0.67)	0.021	[-3.05, -0.28]	19.5	-1.72 (0.67)	0.017	[-3.11, -0.34]	20.1
TIAT	-3.09 (0.83)	0.001	[-4.81, -1.38]	34.5	-2.82 (0.83)	0.002	[-4.54, -1.10]	58.9

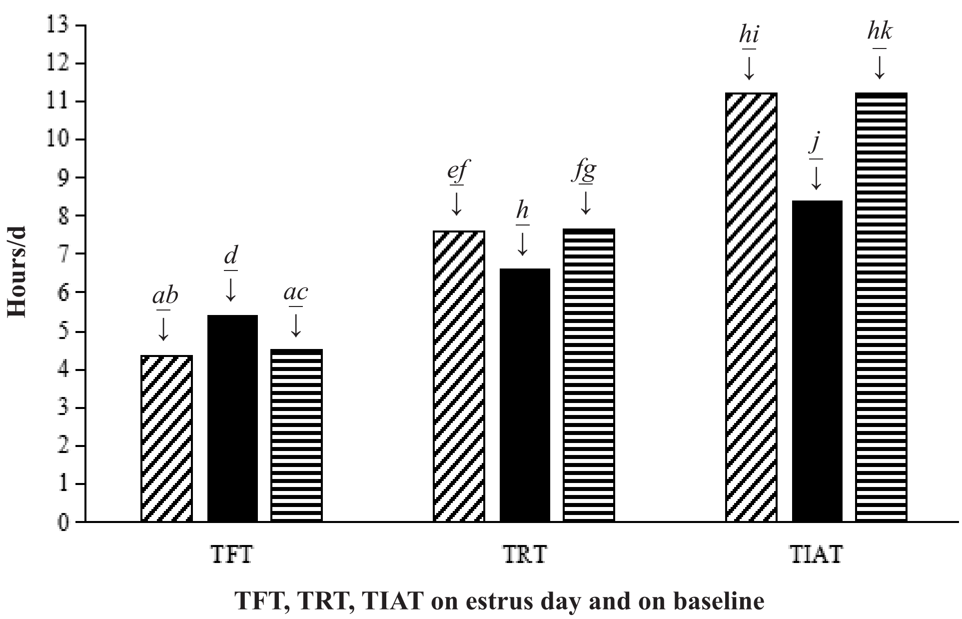

Figure 2. Difference of total feeding time (TFT) (h/d), total rumination time (TRT) (h/d) and total inactivity time (TIAT) (h/d) on estrus day and 3 d before and 3 d after estrus day for all 35 animals.
Slash lines denote the variable on 3 d before estrus, black denotes the variable on estrus day, and horizontal lines denote the variable on 3 d after estrus day. Compared to 3 d before estrus and 3 d after estrus day, TRT and TIAT both decreased significantly in all cows, while TFT markedly increased on estrus day compared to the baseline. Different superscript lowercase letters in the bar charts indicate significant differences ($P < 0.05$), while the same letters denote lack of significance ($P > 0.05$).

For groups defined by milk production, there was no significant difference between TFT and TRT on estrus day and the baseline. The TIAT on estrus day for MK1 (high-yielding) was greater than MK3 (lower-yielding) cows [mean difference 3.18 ± 1.55, P=0.048, 95% CI (0.02, 6.33)].

Discussion

The marked increase in physical activity on estrus day (304.43 %) observed in the present study for all cows in the HR-Tag-monitored herd was in accordance with previous reports (~300 %) (ARNEY et al., 1994; SCHOFIELD et al., 1991). Compared with multiparous cows and lower-yielding cows (<40 kg/d) on estrus day (REITH et al., 2014) using the same HR-Tag as an AMS, the present study revealed that primiparous cows and high-yielding cows (>40 kg/d) had greater activity behavior. Thus, the greater activity in primiparous cows when starting their first lactation, along with the greater activity of high-yielding cows to support a possible increase in feed intake due to the greater demands for nutrients in the mammary gland, might have led to the behaviors observed in the present study.

The decreased bouts of lying down observed in all cows in the HR-Tag monitored herd and in multiparous vs. primiparous cows on estrus day were similar to previous reports with AMS assessment (MAYO et al., 2019). The decreased total lying time for all cows observed on estrus day and 3 d before or 3 d after estrus was also reported by ZEBARI et al. (2018). In fact, the restless behavior on the days around estrus led to a marked decrease in total lying time (DOLECHECK et al., 2015; SAINT-DIZIER and CHASTANT-MAILLARD, 2018). Previous studies demonstrated this marked difference in the lying time between multiparous and primiparous cows under different systems (SEPúLVEDA -VARAS et al., 2014; MASELYNE et al., 2017). A recent study evaluating lying and eating behavior through a longitudinal analysis revealed that multiparous cows have longer lying time compared with primiparous cows during the first DIM. However, multiparous cows reduced their lying time over the lactation period, while primiparous cows remained stable during the first 6 wk of lactation (MUNKSGAARD et al., 2020). Clearly, primiparous and multiparous cows have different responses in lying behavior, which might be directly related to the greater impact suffered by primiparous cows when starting their first lactation in a new group. Similarly, these different responses were also reported previously in the days around estrus, with more lying time in multiparous cows at least until the first half of DIM (SILPER et al., 2017).

The influence of lactation stage on lying time has been well demonstrated, with the lying time increasing overall as lactation progresses (BEWLEY et al., 2010; VASSEUR et al., 2012; SOLANO et al., 2016; WESTIN et al., 2016). However, according to the longitudinal analysis by MASELYNE et al. (2017), the first month of lactation is characterized by a decrease in total lying time, especially in cows in mid-lactation, where this behavior stabilized, with a plateau up until the end of lactation. These changes can be partly explained by the effects of lactation stage on feeding behavior as lactation progresses. A previous study reported an increase in feeding time during the first week of lactation, which reached a plateau during lactation, and supports the results showing a stabilization in total lying time (HUZZEY et al., 2006).

As expected, the negative correlation between TFT and TIAT in the neck collar-monitored herd corroborated a previous study showing that cows with lower TIAT had greater TFT on estrus day (SCHWEINZER et al., 2020). This correlation is supported by observing that the primiparous cows with lower TIAT had greater TFT. A previous study showed that primiparous cows had greater activity during estrus day, which led to a consequent greater TFT (REITH et al., 2014). This behavior was also supported when comparing the differences between TFT, TRT, and TIAT on estrus day and the baseline. Clearly, cows that had lower TIAT, increased their feeding frequency, resulting in an increased TFT. In this way, the TRT was also affected, culminating in a lower time for rumination. A previous study using AMS reported an average 17% decrease in rumination time on estrus day (REITH and HOY, 2012). In the present study, the average relative decrease in TRT of ~13% confirmed this behavior.

However, it is important to note that there was an individual variation between cows previously reported by REITH and HOY (2012), where the rumination time could vary between a decrease of ~70% and an increase of ~16% (Mayo et al., 2019).

A previous study reported a decrease in feeding and rumination time on estrus day and 1 d before estrus (PAHL, et al., 2015); however, no differences were observed in rumination time between multiparous and primiparous cows. In the present study, the decreased rumination time observed in all cows on estrus day vs. baseline, and in multiparous vs. primiparous cows was similar to previous studies (REITH and HOY, 2012; MAYO et al., 2019). In fact, rumination time has proven to be one of the most reliable variables for identifying changes through AMS, and constant rumination monitoring can provide information for making early decisions (REITER et al., 2018). This variable can signal problems before clinical signs appear and before milk yield is affected (SORIANI et al., 2013; STANGAFERRO et al., 2016). Similarly, decreased rumination can be an early indicator of the effectiveness of nutritional changes, group adjustments, or veterinary treatments.

Conclusions

Overall, our study underscored the physical, behavioral, and ruminal changes related to estrus day through AMS monitoring, which provided important information for the management of the herd. In the HR-Tag monitored herd, these changes were supported by a marked increase in physical activity on estrus day compared to the baseline period. In the neck collar-monitored herd, changes were supported by a marked decrease in total rumination time and total inactivity time, and a marked increase in total feeding time compared to the baseline period. In addition, we observed marked changes according to parity, milk production, and lactation stage, which underscored the importance of more detailed management planning according to cow grouping. The use of both forms of AMS proved them to be useful tools for identifying changes associated with estrus, and they can help farmers make important management decisions.

Conflict of interest

Authors declare that no conflicts of interest exist.

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Authors' Contributions

XZ and CX conceived the study; YS, BJ and CX carried out experiments and data analysis; XZ, JJL, GS, BYH and CX interpreted the data. XZ, JJL, MGL, YS and CX wrote the manuscript. All authors approved the final version.

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SAŽETAK

Cilj je ovog istraživanja bio opisati promjene parametara povezanih s estrusom koje se automatski bilježe pomoću HR-Tag-a (SCR Engineers Ltd., Netanya, Izrael) ili ovratnika (Nedap Livestock Management, Groenlo, Nizozemska). Početna faza istraživanja na dvjema komercijalnim farmama definirana je kao prosječna vrijednost koja obuhvaća 3 dana prije i 3 dana poslije estrusa. U stadu praćenom HR-Tag-om u kod 78 krava tijekom estrusnog ciklusa bilježene su promjene u tjelesnoj aktivnosti i ponašanju (ležanje, trajanje ležanja, ukupno vrijeme ležanja, omjer ležanja). Krave su razvrstane u skupine prema paritetu (primipare, n = 34; multipare, n = 44), proizvodnji mlijeka (MK1, n = 7, > 47 kg/d; MK2, n = 12, 42 – 47 kg/d; MK3, n = 43, 31 – 42 kg/d; MK4, n = 16, < 31 kg/d) i stadiju laktacije (DIM1, n = 34, ≤ 60 d; DIM2, n = 22, 61-120 d; DIM3, n = 22, ≥ 121 d). U stadu praćenom pomoću ovratnika zabilježena je tjelesna aktivnost na dan estrusa i ukupno vrijeme hranjenja u danu (TFT), vrijeme preživanja i vrijeme neaktivnosti u 35 mliječnih krava za vrijeme periestrusa. Na dan estrusa procijenjeni su učinci pariteta i proizvodnje mlijeka na tjelesnu aktivnost te razlika u ukupnom dnevnom vremenu hranjenja, vremenu preživanja i vremenu neaktivnosti između dana estrusa i početne faze istraživanja. Krave su razvrstane u skupine prema paritetu (primipare, n = 9; multipare, n = 26) i proizvodnji mlijeka (MK1, n = 5, > 41 kg/d; MK2, n = 17, 32 – 41 kg/d; and MK3, n = 13, < 32 kg/d). Proveden je test ANOVA kako bi se ustanovile razlike u podacima i usporedile metode kojima su se procjenjivale razlike unutar skupine i među skupinama putem SPSS 23.0. Za prilagodbu podataka korištena je ordinalna logistička regresija kako bi se analizirali čimbenici koji utječu na tjelesnu aktivnost na dan estrusa. Rezultati u stadu praćenom HR-Tag-om pokazali su da se na dan estrusa tjelesna aktivnost povećala u odnosu na početno razdoblje, a prosječne su vrijednosti koje se odnose na ležanje na dan estrusa bile znatno niže nego u početnoj fazi, kao i ukupno vrijeme ležanja i omjer ležanja, s vrijednostima od -3,53 ± 0,55, -188,02 ± 21,46 i -14,05 ± 1,37. U stadu praćenom ovratnikom, kada se TFT u skupini MK2 povećao za jednu jedinicu, tjelesna aktivnost povećala se 3,19 puta (P = 0,03) u usporedbi sa skupinama u kojima se TFT nije povećao. Rezultati ovog istraživanja općenito upućuju na tjelesne i ponašajne promjene te promjene u preživanju koje su vezane za estrus, a otkrivene su automatiziranim sustavom praćenja. Pretpostavlja se stoga da sustav HR-Tag i ovratnici mogu biti prikladan način detekcije estrusa u komercijalnim stadima mliječnih krava.

Ključne riječi: automatizirani nadzorni sustav; estrus; ležanje; upravljanje reprodukcijom; ruminacija