Rumination Time and Physical Activity Monitoring, Milk Yield Changes Around Estrus and First Service Pregnancy Rate in Dairy Cows Assigned to Voluntary Waiting Period

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ABSTRACT
In high milk-yielding dairy cows, the energy balance challenges in early lactation and impact reproductive performance often affects estrus intensity, duration and detection. This study determined the role of a voluntary waiting period on rumination time, physical activity and milk yield changes during the peri-estrus period in the first service and further fertility in high-yielding dairy cows. To the best of our knowledge, this is the first study to demonstrate a relationship between voluntary waiting period and estrus-related parameters. This study was conducted on 145 Holstein-Friesian dairy cows aged 2 to 6 years of age. Cows were assigned a short voluntary waiting period, 45 to 70 day in milk (SVWP; n=73), and the long voluntary waiting period, 71 to 95 day in milk (LVWP; n=72). Rumination time, physical activity and milk yield changes around estrus in the first service were detected by an automated monitoring system. Artificial insemination procedures were performed between 45 to 70 days in milk for SVWP and 71 to 95 days in milk for LVWP. The barn's daily temperature and humidity index (THI) was recorded throughout the study period and was <72. Basal rumination time were significantly lower in SVWP (376.82±74.58 min/day) than LVWP group at the day of estrus (352,07±68.90 min/day) (P<0.01). First service pregnancy rate was 23.72% and 38.46% in short and long voluntary waiting period, respectively (p<0.05). Physical activity intensity and milk yield changes were similar among groups during the peri-estrus period and on the day of estrus. The results indicated that the short voluntary waiting period was associated with the lower basal rumination time around estrus and lower first service pregnancy rate.

Keywords: Cattle; Estrus; Lactation; Rumination.

INTRODUCTION
For years, the global dairy farm industry has been changing because of economic pressure, technological innovations and consumer expectations. In developed countries, dairy herd size has increased, while herd numbers have decreased over the last four decades (1). In contrast to herd size, increased milk production is associated with decreased dairy cow health. Adverse effects from high production manifest as disrupted metabolic adaptations during the postpartum period. Thus, increase severity of negative energy balance,
mobilized body fat reserves, and altered fat-protein ratios in milk remain for 6 to 8 weeks after parturition. However, this situation negatively affects the uterus and ovaries for an even longer period, lasting between 80 and 100 days postpartum. This carryover effect triggers fertility-related problems, such as anestrus and sub-estrus (2). In addition to these problems, poor visual estrus signs and detection problems appear (3). Furthermore, housing, genetics, milking frequency and yield also negatively affect estrus signs (intensity and duration) in modern dairy herds (4).

The gold standard for detecting estrus is the visual observation of dairy cows (5); however, global dairy farming systems make visual detection of estrus difficult (6). Thus, automated monitoring systems are often used to detect estrus in dairy farm industries (7). Automated monitoring systems evaluate the daily yield, temperature, electrical conductivity, and progesterone levels in the milk, as well as rumination time and activity on dairy farms (8). Automated systems also help diagnose uterine and metabolic diseases (9). These devices detect primary estrus-related behaviors, such as walking, standing and mounting, as well as help to monitor secondary estrus-related behaviors, such as rumination time and decreased milk yield near the peri-estrus period (10, 11, 12).

Rumination time, physical activity and milk yield changes reflect estrus related behaviors such as standing for mounting, decreased appetite, and restlessness during peri-estrus, and can detect metabolic adaptation to lactation in the early lactation period.

The rumination, physical activity and daily milk yield monitoring may be used as indicators of the welfare in individual animals. Also, the slower increase of rumination time, physical activity and daily milk yield after calving might lead to a decrease in the first service pregnancy rate in dairy cows due to influences by several factors.

This study aimed to determine the role of a voluntary waiting period on physical activity, rumination and milk yield changes during the peri-estrus period and further fertility in high-yielding dairy cows, exhibiting estrus behavior.

**MATERIALS AND METHODS**

**Animal Management and Conditions**

This study was conducted on a commercial dairy farm housing 700 dairy cows, in Karacabey-Bursa, Turkey (40°18’50.0” N; 28°26’28.9” E) between March and November 2015. Mean temperature over the last five years was 14°C (38.5°C max; 9.7°C min), and mean relative humidity over the last four years was between 14% and 78% (mean 69%). One hundred forty-five Holstein-Friesian dairy cows aged between 2 and 6 years were enrolled in the study. Cows were housed in free-stall barns consisting of a resting area and fresh potable water. During the transition period, all cows were inspected daily, and all health-related problems and treatments administered through the transition period were recorded. The cows were milked three times daily (06:00 h, 14:00 h, 22:00 h), and the milk yield per cow was recorded on each occasion (Data Flow II, SCR Engineers, Netanya, Israel). Cows were fed the same total mixed ration twice daily, at approximately 08:00 h and 20:00 h during the study period.

Pens were equipped with 2 axial flow fans (0.75 kW; 90 cm in diameter; 22,500 m³/h maximum airflow rate). Fans were built-in at a height of 2.5 m and angled downward vertically 10°. The fan speed was thermostatically controlled and activated at 25°C. Sprinklers spaced at 150-cm intervals were placed along the feed alley, which were thermostatically controlled and switched on at 27°C. Five minutes of ventilation alone was followed by 50 seconds of showering and ventilation.

**Study Design**

Two voluntary waiting periods were available to the herd veterinarians between calving and initiation of breeding. Each cow was assigned randomly to either the “short voluntary waiting period” (SVWP) or “long voluntary waiting period” (LVWP) group after parturition. Cows were randomized using the random number function in Microsoft Excel (Redmond, WA, USA) and imported into the farm’s Data Flow II software (SCR Engineers, Netanya, Israel Ltd.).

Only cows showing visible signs of estrus 45 days after parturition were enrolled in the study (n=145). Each group consisted of a similar percentage of primiparous and pluriparous cows. The SVWP group (n=73) was inseminated between 45 and 70 day in milk (DIM) whereas the LVWP group (n=72) was inseminated between 71 and 95 DIM. Each cow was only inseminated once during the study period. Each cows’ estrus was detected by visual observation, and all rumination times, physical activities and milk yield data were recorded for the peri-estrus period (3 days before and 3 days after estrus).
Artificial Insemination

Cows were selected for insemination by visual observation, conducted by 2 farm technicians. The same farm veterinarian inseminated the cows after detecting estrus. Rumination time, physical activity and milk yield changes were not used as selection criteria. Forty days after insemination, the cows were evaluated for pregnancy using ultrasound.

Monitoring Herd Health

Prior to beginning the study, rectal palpation and vaginal examinations were performed to confirm ovulation, uterine involution and absence of uterine infection on postpartum day 35. An individual herd health profile based on serology confirmed that all cows were negative for infectious bovine rhinotracheitis, bovine viral diarrhea, tuberculosis and brucellosis.

MEASUREMENTS AND ANALYSES

Microclimatic conditions

Temperature and relative humidity inside the barn were recorded daily during the study period. The daily maximum and minimum temperature and humidity index (THI) was calculated by the THI (t.rh)= (1.8xt+32)- (0.55-0.0055xhrh) x (1.8xt-26) formula (13), where t is temperature in degrees Celsius and rh is relative humidity as a percentage. THI < 72 indicates no heat stress.

Rumination time, physical activity and milk yield changes during peri-estrus period

Rumination time, physical activity and milk yield changes in the peri-estrus period were detected by a system using data loggers (placed on the cows’ necks), stationary readers (placed at the milking parlor entrance) and computer software (Data Flow II, SCR Engineers, Netanya, Israel) for analysis (14). The cows’ daily physical activity during peri-estrus was measured by an acceleration sensor-based method using the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) attached to each cow’s neck collar (14). Acceleration sensors recorded each cow’s activity related to estrus, every 2 hours, 12 times daily and calculated the general activity, “index in activity units (u)”. The measured data were downloaded and stored to the herd computer for analysis by Data Flow II software (SCR Engineers Ltd., Netanya, Israel) at each milking or anytime the cows passed under a tag reader. Rumination time monitoring during the peri-estrus period was recorded by the same system (14). Tags were attached to the left side of the neck and consisted of a microphone enclosed in a plastic cover to record the distinctive sounds of rumination and regurgitation at 2-hour intervals, twelve times daily. Data were then sent to the farm computer for further analysis, as described previously (14). Milk yield data per cow were recorded at each milking by Data Flow II software.

Statistical Analysis

Before performing the statistical analysis, all data were examined using the Kolmogorov-Smirnov test for normality and Levene’s test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as the mean ± standard deviation. T-test was used to evaluate the difference between the groups for milk yield and temperature-humidity index. General linear models with a repeated measures design were used to test the differences in physical activity, rumination time and milk yield between time sampling in groups. Multiple tests with Bonferroni’s adjustment were used as post hoc tests for variables that were statistically significant. For all comparisons, differences with a minimum P-value of 0.05 were considered significant. All statistical analyses were performed by using SPSS® 14.1 for Windows (License no: 9869264).

RESULTS

Rumination time on the day of estrus was clearly affected by the voluntary waiting period. It was determined between 352.07±68.90 to 417±75.65 min/day and 376.82±74.58 to 443.11±75.81 min/day for the short voluntary waiting period (SWVP) and long voluntary waiting period (LVWP) respectively. Long voluntary waiting period group showed higher basal rumination time (376.82±74.58 min/day) than SVWP group on the day of estrus (352.07±68.90 min/day; p<0.05), but the arithmetic decreases in rumination times were similar as 15.7% and 14.19% in the SVWP and LVWP, respectively. Rumination time changes over time within each group were also statistically significant (p<0.001; Figure 1).

Although all cows were cyclic before artificial inseminations, first service pregnancy rates differed significantly (p<0.05) between the groups, at 23.72% and 38.46% for the SVWP and LVWP groups, respectively.
Mean physical activity changes during the peri-estrus period were statistically significant within groups \( (p<0.001) \) (Figure 2). Activity measurements ranged from 543.09±128.52 to 792.54±6.58 u/d, and from 563.60±138.26 to 886.29±224.17 u/d for the SVWP and LVWP groups, respectively. The arithmetic increases in physical activity were 27.8% and 33.37% in the SVWP and LVWP, respectively. No statistical difference was observed between physical activity increases on the day of estrus between the groups \( (p>0.05) \).

Mean milk yield changes around estrus within the SVWP and LVWP differed significantly \( (p<0.05) \) (Figure 3); however, changes in milk yield among the groups did not. The average 305-day milk yields were 37±8.81 and 36±8.73 for the SVWP and LVWP groups, respectively, with no statistical significance between the groups. The average minimum and maximum THI inside the barn through the study period (from March to November) were 60.92±8.23 and 62.72±7.44 respectively. No cows experienced a max THI > 72.

**DISCUSSION**

Rumination time, physical activity and changes in milk yield were investigated as estrus-related behavior. A limited numbers of studies presenting data on physical activity and rumination time measurements by sensor-based technologies have been published previously (10, 11, 12, 15).

Pregnancy rate, estrus detection rate, and pregnancy losses are important parameters that endanger dairy herd performance (16). First service pregnancy rate can be influenced by many factors, including poor estrus detection, energy balance, high milk production, heat stress, diet and disease (17, 18). During the study period, no cows had postpartum disorder or were exposed to heat stress (THI<72). Although milk production was similar in both groups, first service pregnancy rate differed significantly between them, being higher at late voluntary waiting period. One interpretation of this result is that for the cow to establish a successful pregnancy, the animal’s physiology must progress to a state of positive energy balance, whereby dry matter intake matches metabolic demands.

Rumination behavior is characterized by regurgitation, re-mastication and re-swallowing, which can be measured directly by visual observation; however, this is difficult in large herds, thus automated monitoring systems are used. Rumination time is influenced by estrus, milk yield, physiological status, health status, heat stress and nutritional factors such as feed digestibility, NDF intake, dietary composition and forage quality (19, 20). Decreases in rumination time before parturition can be used to detect the beginning of parturition, as these times increase rapidly and usually reach maximal daily rumination time after parturition (21). The present study showed that basal rumination times differed in both groups, and rumination time changed during early lactation. Previous studies stated different rumination times between 340 and 540 min/d; however, these focused solely on the calving period and different dietary formulations (22, 23, 24, 25). Milk yield is another factor that affects rumination time, and some studies reported that high milk yield was associated with a high rumination time (26, 27). In the present study, milk yields were similar, but rumination times differed when the animals were fed the same ration. It is proposed...
that differences in basal rumination time may result from the physiological energy status in early lactating cows. After the voluntary waiting period, energy status returns to become positive, thereby improving dry matter intake and increasing basal rumination time. Such changes can be explained by the positive relationship between dry matter intake and rumination time (28, 29). Rumination times change during the peri-estrus period, indicating the influence of estrus, as changes in rumination are closely related to physical activity during peri-estrus (10). On the day of estrus, blood estrogen levels are higher than those of the pre-post estrus period (30, 31). Estrogens reduce appetite and feed consumption (32), which is also closely related to the decreased rumination time (33).

Physical activity is markedly influenced by estrus. Arney et al. (34) indicated that activity on the day of estrus was clearly influenced by the lactation stage. Contrary to our results, their study found high activity in the short voluntary waiting period, while in contrast to Arney et al. (34), López-Gatius et al. (35) and Yániz et al. (36) stated that the lactation stage had no effect on physical activity in estrus. Similar findings were seen in the present study. A plausible reason for these different results may be the sample size used in each study. Arney et al. (34) used a small sample size, which may have masked the results.

Rajamahendran et al. (37) found no relationship between milk yield changes and signs of estrus during peri-estrus. However, Schofield et al. (38) and Malz et al. (39) stated that milk yield was significantly reduced on the day of estrus in a proportion of cows. Similar findings were encountered in the present study, in which estrus was characterized by increased physical activity and decreased dry matter intake, which directly affect milk production on the day of estrus.

In conclusion, there appears to be a relationship between rumination time during peri-estrus and pregnancy rates in dairy cows. The present study showed that sensor-based collar technology measurements could be used to identify estrus. Physical activities increased in both groups on the day of estrus, whereas rumination time and milk yield decreased. The voluntary waiting period had no effect on physical activity and milk yield changes during the peri-estrus period or on the day of estrus. However, it is clear that rumination time and first service pregnancy rate were influenced by the voluntary waiting period. The differences in endocrine adaptation to negative energy balance depending on lactation stage are likely critical factors influencing rumination behavior. Rumination time may be affected by the cattle’s energy status and could be used to indicate and determine energy status before artificial insemination on dairy farms.

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REFERENCES