

Article

Identification of Changes In Rumination Behavior Registered With An Online Sensor System In Cows With Subclinical Mastitis

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Simple summary. Objectives was to determine the relation between subclinical mastitis and rumination behavior registered with an online sensor system. According results of our study we found that rumination time and rumination chews can act as biomarkers of subclinical mastitis. Changes in rumination behavior could be possibly used to identify subclinical mastitis in dairy cows. It can be a great advantage for the farmers to recognize the affected cows and treat the udder inflammation at an early stage. Veterinarians and farmers can consider online sensor system which registers rumination behavior biomarkers for identification of subclinical mastitis. This would allow early identification of sick cows and reduce the economic losses to the farm due to this disease.

Abstract: The aim of the current study was to determine the relation between subclinical mastitis and rumination behavior registered with an online sensor system. According to the results of the general clinical examination of 650 milking cows, 10 cows with subclinical mastitis (SCM) and 10 clinical healthy cows (HG) were selected (without clinical signs of any diseases). Rumination behavior biomarkers were registered with RumiWatch noseband sensor (RWS; ITIN + HOCH GmbH, Fütterungstechnik, Liestal, Switzerland). Sensors were implanted on the first day after calving. Data from the RWS from 13th days before the diagnosis and 13 days after diagnosis of SCM were used to compare with HG data during the same period. The healthy cows were monitored during the same days as the SCM cows.

We found that healthy cows spent more time for rumination and drinking ($p < 0.05$) and had a higher number of boluses per rumination. Rumination time in the subclinical mastitis cows decreased from day -4 to day 0 by 60.91%, drinking time by 48.47%, and number of boluses per rumination by 8.67% ($p < 0.05$). Subclinical mastitis effects rumination time and rumination chews registered with sensor systems. Furthermore, impact of heat stress, oestrus and others effects on rumination behavior biomarkers should be evaluated. Veterinarians and farmers can consider online sensor system which registers rumination behavior biomarkers for identification of subclinical mastitis. This would allow early identification of sick cows and reduce the economic losses to the farm due to this disease. Further studies should focus more on the factors such as heat stress, oestrus and other effects which have impact on rumination behavior biomarkers with more animals. Also, in the following studies the values of RWS and locomotion should be analyzed together.

Keywords: subclinical mastitis; rumination; activity; early diagnosis; precision agriculture

1. Introduction.

Mastitis is defined as an inflammatory disorder of the mammary gland, the primary cause of which is bacterial in nature [1]. Mastitis is common around the world and is linked to decreased milk supply, discharged milk, reduced milk quality, increased activities of veterinarians, medicine, labor expenditures, shortened longevity and increased culling rate [2]. Furthermore, mastitis disrupts energy balance and immune potency, making cows more susceptible to metabolic diseases [3]. Biomarkers for subclinical mastitis detection are: somatic cell count (SCC), l-lactate dehydrogenase, milk lactose, protein, fat, minerals (Cl, Ca, and Na), and lactate; milk pH and temperature; acute phase proteins such as milk haptoglobin and serum amyloid A and C-reactive protein; and others such as plasma-carotene and ceruloplasmin [4].

Electrical milk conductivity (EC) alone cannot provide the sensitivity needed to detect infected portions [5]. Incorporating additional information (e.g., milk yield, milk flow, number of incomplete milkings) may improve detection accuracy and capacity to detect early beginning of mastitis. Using EC data alone cannot give the needed precision to discover contaminated quarters. For the early detection of mastitis in AMS herds, various information and alternative methodologies must be combined [6]. Sensor-generated data can be used alone or in conjunction with established health-monitoring techniques to detect cows suffering from health problems [7,8]. Furthermore, continuous monitoring of behavior and physiological markers may allow the detection of subtle changes prior to the appearance of obvious clinical indications. Early disease detection may help cows by avoiding disease development and boosting treatment response [9]. Health problems in the early postpartum period affect a large number of lactating dairy cows, having a severe impact on their health, welfare, and performance [10]. Future study is needed to develop criteria for differentiating and treating specific health conditions based on data from the automatic health monitoring system (AHMS) [9]. Retained placenta, mastitis, displaced abomasum, metritis, or lameness, for example, could have altered the animals' walking activity behavior [11]. Early detection is critical in decreasing the deleterious effects of mastitis [12]. Among the methods used to diagnose this condition, rumination sensors have proven to be efficient in detecting mastitis and other diseases in their early stages [12]. In previous investigations, we attempted to identify the relationship between subclinical ketosis (SCK), locomotor behavior (feeding time with head up, feeding time with head down, frequency of switching between activities, and walking activity), and ambient temperature (average, minimal and maximal temperature). Cows with subclinical ketosis were shown to be more prone to changing their behavior (between ruminating, feeding, and drinking) more frequently. Lower minimum and maximum ambient temperatures are associated with the beginning of SCK [13].

According to the literature we hypothesized that subclinical mastitis affects rumination behavior in cows (such as rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus) registered with sensor systems. To achieve this, we set objectives to determine changes in rumination behavior (such as rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus) registered with an online sensor system in cows with subclinical mastitis.

2. Materials and Methods

2.1. Location and animals.

This research was carried out with the provisions of the Republic of Lithuania's Law on Animal Welfare and Protection, as well as the European Directive 2010/63/EU for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Official Journal 2007, No. 49) (1883, No. 49-1884). The study approval number was PK016965. This investigation was conducted between July 1 and December 15, 2021 at the

Lithuanian University of Health Sciences and a commercial dairy farm (54.9754° N, 23.7684° E). The average milk production per cow was 10,500 kg per year.

Since mastitis is a disease that is influenced by many factors, therefore, when selecting cows for the study, we paid special attention to the homogeneity of the samples so that all factors and external processes that can affect the data remained constant throughout the study period. The current examination comprised 20 cows out of a herd of 650 (on average 2.58 ± 0.34 lactation and 187.65 ± 2.6 days in milk). We have found that in this farm the highest probability of mastitis in cows is 187.65 ± 2.6 days in milk.

The average milk yield (MY) of selected cows was 37.6 ± 0.3 kg, with milk fat (MF) of 4.31 ± 0.04 percent, milk protein (MP) of 3.51 ± 0.02 percent, milk fat-to-protein ratio (F/P) of 1.23 ± 0.01 , milk lactose (ML) of 4.60 ± 0.01 percent, milk urea (MU) of 27.02 ± 0.15 mg/dL, and milk somatic cell count (SCC) of 286.65 ± 35 thousand cells/mL. The cows were kept in a free housing system and fed a total mixed ration (TMR) two times per day at a specified time throughout the year, balanced to meet the physiological needs of a 550 kg Holstein cow producing 38 kg milk per day. TMR was created in such a way that it met or exceeded the requirements. The ration's dry matter (DM) % value was 48.00, acid detergent fibre (% of DM) was 18.00, neutral detergent fibre (% of DM) was 27.00, non-fibre carbohydrates (% of DM) was 38.00, crude protein (% of DM) was 17.00, and net energy for lactation was 1.7 (Mcal/kg). On a five-point scale, cows had an average body condition score of 3.65 ± 0.56 . According to the results of the general clinical examination 10 cows with subclinical mastitis and 10 clinically healthy cows (without clinical signs of any diseases) out of 650 milking cows were selected.

Subclinical mastitis group (SCM) (n=13). On the basis of SCC subclinical mastitis cases were found. Cows with an SCC more than 200,000 cells/mL (average – 540 (± 45 cells/mL)) were determined to have SCM [14]. SCC was evaluated one time per week, during all experiment. According to a general clinical assessment, none of these cows displayed clinical signs consistent with any disease and other factors such as heat stress, oestrus and ect. Cows with these factors were excluded from the study (n=3). In this group total number of cows was – 10.

Subclinical mastitis treatment protocol –_SCC bolus ([Groothandel Carton B.V. Julekesweg 7, 7451 PB Holten](#)) and nonsteroidal anti-inflammatory drugs RIMADYL Cattle 50 mg/ml Solution for Injection (Zoetis Belgium S.A. Cherrywood Business Park, Loughlinstown Co Dublin Ireland). Protocol Composition of a 90g SCC-Bolus : Garlic extract, microcrystalline cellulose, lactose, ethylcellulose, maltodextrin, magnesium stearate. Cows were treated with one orally-administered bolus once. Anti-inflammatory drugs. Cows were treated with single subcutaneous injection at a dosage of 2.5 ml/100 kg body weight.

Clinically healthy group (HG) (n=10). According Nielen et al. [14] cows were defined as healthy if the SCC was <200,000 cells/mL (average – 158 (± 25 cells/mL)). Additionally, these cows did not exhibit any clinical signs consistent with any disease, according to a general clinical assessment.

2.2. Measurements

Rumination behavior biomarkers were registered with RumiWatch noseband sensor (RWS; ITIN + HOCH GmbH, Fütterungstechnik, Liestal, Switzerland). Sensors were implanted on the first day after calving. Zehner et al. [15] and Alsaad et al. [16] developed and confirmed these sensors.

Measurements were started on the first day after calving and continued for 13 days after SCM diagnosis. Table 1 summarizes the registered biomarkers.

The RWS is comprised of a noseband halter with an integrated pressure detector and a liquid-filled pressure tube. The pressure sensor generates a pressure signal, which is transmitted to a data logger, which is housed in a secure plastic box on the same halter. There is also an acceleration sensor for detecting triaxial head movements and a durable

memory cardholder. At a frequency of 10 Hz, the accelerations and pressure readings are stored as binary files. A wireless data transmitter connects the halter to the RumiWatch Manager software, allowing for live collecting data. Simple algorithms employed in the RWC software process the customized classification of behavioral features from 10 Hz pressure data in various time summaries that can be selected. Because the consolidation of 10 Hz pressure data in 1-min time summaries only allows for a binary output of the behavioral characteristics of drinking, rumination, eating, and other activity, whether performed within the respective minute or not, additional behavioral parameters are convertible from raw pressure data in 1-h time summaries. Then, a wireless data transmitter is connected to the RumiWatch Manager software, which collects real-time data. The algorithms classify objects based on the recognition of distinct pressure peak clusters produced by jaw movements that are classified according to their behavioral characteristics.

Periods of measurements. Data from the RWS from in a period of 13 days before the diagnosis and 13 days after diagnosis of SCM was used to compare with HG data during the same period. The healthy cows were monitored during the same days as the SCM cows.

Table 1. Biomarkers of rumination behavior identified using real-time sensors and quantified using the RumiWatch noseband sensor and their description.

| Parameter | Description |
|-----------------------|--|
| Rumination time (RT) | Time spent for rumination chews including chewing interruptions up to 5 s |
| Eating time (ET) | Time spent for eating chew, including interruptions between eating chews up to 5 s |
| Drinking time (DT) | Time spent for drinking, including interruptions between drinking gulps up to 5 s |
| Rumination chews (RC) | Chews during rumination for mechanical breakdown of the regurgitated materials into finer particles using the molars |
| Eating chews (EC) | Total number of prehension bites and mastication chews while eating |
| Drinking gulps (DG) | Total number of drinking gulps while drinking |
| Bolus (B) | Number of boluses per rumination |

s - seconds.

2.3. Data analysis and statistics

Hypothesis testing was performed using descriptive statistics to summarize RumiWatch noseband data (mean and standard deviation). In addition to descriptive statistics, in hypothesis testing studies, we used inferential statistics to test the hypothesis of possible differences between groups of HG and SCM cows according to measures of ruminant behavior (rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus).

All statistical analyses were performed using SPSS 25.0 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) with indicators whose normal distribution was confirmed using the Shapiro-Wilk normality test.

The study lasted for a total of 27 days. Days “-13” to “0” denote the experimental period 1 (before the onset of diagnosis of subclinical mastitis - day “0”), and days “1” to “13” indicate the experimental period 2 (after the start of treatment).

The design of repeated measurements was used, including measurements (time periods) of the same RumiWatch indicator according to the days of the experiment. The mean and standard error of the mean were calculated for the estimated variables. The LSD criterion was used to compare the difference in mean between groups values.

To achieve the goal of this work and to determine the relationship between subclinical mastitis and rumination behavior registered with an online sensor system, we performed a correlation and logistic regression analysis.

The Pearson correlation coefficient was calculated by evaluating the relationships of the studied parameters by groups.

To analyze the factors influencing the likelihood of mastitis, multivariable logistic regression models were used, in which a backward stepwise logistic model was used to exclude all non-essential explanatory variables (according to the significance of the Wald test). The estimates and 95% Wald limits were used to calculate odds ratio OR (as the ratio of the probability of success and the probability of failure) and 95% confidence interval CI. The entire final statistical model included only three significant explanatory variables.

All RumiWatch variables (explanatory variables) have been grouped into two classes based on the arithmetic mean: class G0 \leq arithmetic mean, G1 $>$ arithmetic mean of all tested cows in the herd four days before mastitis detection in the SCM group of cows (when a significant change in the observed parameters was identified).

A probability of less than 0.05 was considered as being significant ($p < 0.05$) for all tests.

3. Results

According to the results of our study, we found that HG cows spent more time for rumination and drinking ($p < 0.05$) and had a higher number of boluses per rumination than SCM cows (Table 2). In the period from "-13" to "0" days, rumination time in healthy cows was 10.62% longer ($p < 0.05$), these cows needed 11.90% more rumination chews ($p < 0.05$), their drinking time was 4.83% longer ($p < 0.05$), gulps 1.91% less, and number of boluses per rumination 2.12 % more compared to cows of the SCM. Similar trends in the difference in indicators persisted in the second period of the study (from days 1 to 13) but the differences between groups of cows were statistically not significant (Table 2).

The results showed that on the day of diagnosis the rumination time of SCM cows was 39.79% and the drinking time was 16.95% longer, the number of boluses per rumination was 7.35% higher, but the eating time by 21.84% shorter, eating chews by 14.00% and drinking gulps by 4.34% less compared to the group of healthy cows ($p < 0.05$).

Table 2. Rumination behavior biomarkers registered with real-time sensors measured by the RumiWatch noseband sensor by group cows and experimental periods.

| RumiWatch noseband indicator | HG | SCM |
|------------------------------|-----------------------------------|-----------------------------------|
| | <u>Period 1 (- 13 - 0 days)</u> | |
| | Mean \pm standard deviation | |
| Rumination time (min/h) | 25.70 \pm 2.02 ^a | 22.97 \pm 1.99 ^b |
| Eating time (min/h) | 8.87 \pm 1.94 ^a | 8.91 \pm 2.07 ^a |
| Drinking time (min/h) | 1.24 \pm 0.20 ^a | 1.18 \pm 0.18 ^b |
| Rumination chews (n/h) | 1704.54 \pm 235.67 ^a | 1501.58 \pm 276.92 ^b |
| Eating chews (n/h) | 504.89 \pm 88.02 ^a | 522.19 \pm 127.073 ^a |
| Drinking gulps (n/h) | 164.87 \pm 40.03 ^a | 168.03 \pm 48.927 ^a |
| Bolus (n/rumination) | 26.46 \pm 1.97 ^a | 25.90 \pm 2.30 ^a |
| | <u>Period 2 (1 - 13 days)</u> | |

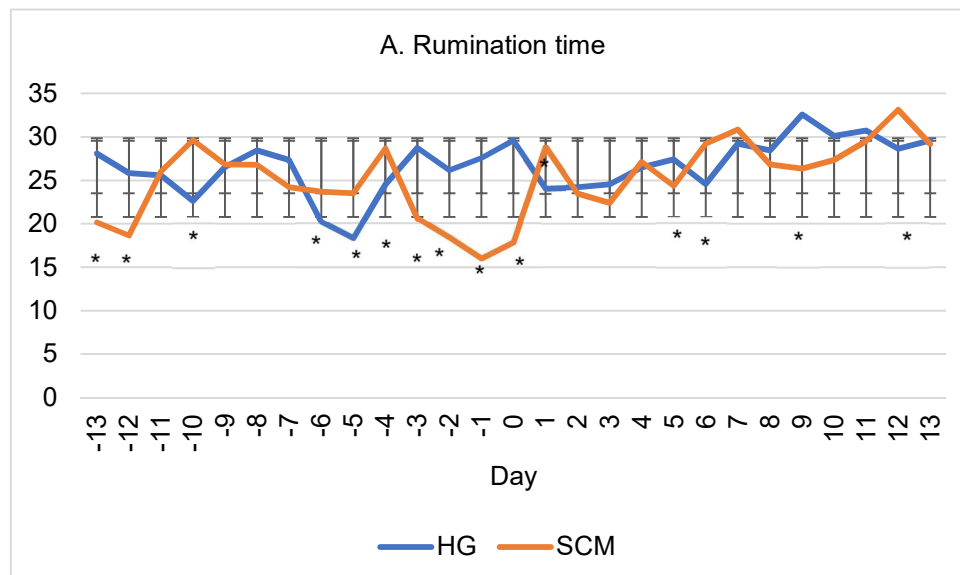
| | Mean \pm standard deviation | Mean \pm standard deviation |
|-------------------------|-----------------------------------|-----------------------------------|
| Rumination time (min/h) | 27.74 \pm 2.03 ^a | 27.59 \pm 0.79 ^a |
| Eating time (min/h) | 9.62 \pm 1.91 ^a | 10.33 \pm 1.79 ^a |
| Drinking time (min/h) | 1.17 \pm 0.23 ^a | 1.07 \pm 0.06 ^b |
| Rumination chews (n/h) | 1798.68 \pm 184.91 ^a | 1776.95 \pm 168.97 ^a |
| Eating chews (n/h) | 621.20 \pm 151.34 ^a | 643.63 \pm 113.18 ^a |
| Drinking gulps (n/h) | 226.05 \pm 64.61 ^a | 237.37 \pm 67.44 ^a |
| Bolus (n/rumination) | 27.97 \pm 3.114 ^a | 28.94 \pm 2.70 ^a |

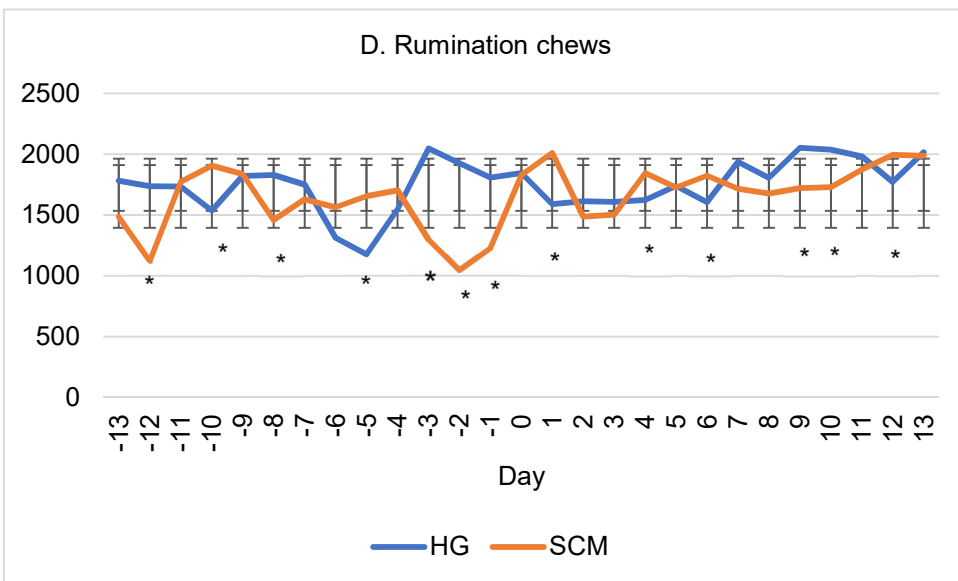
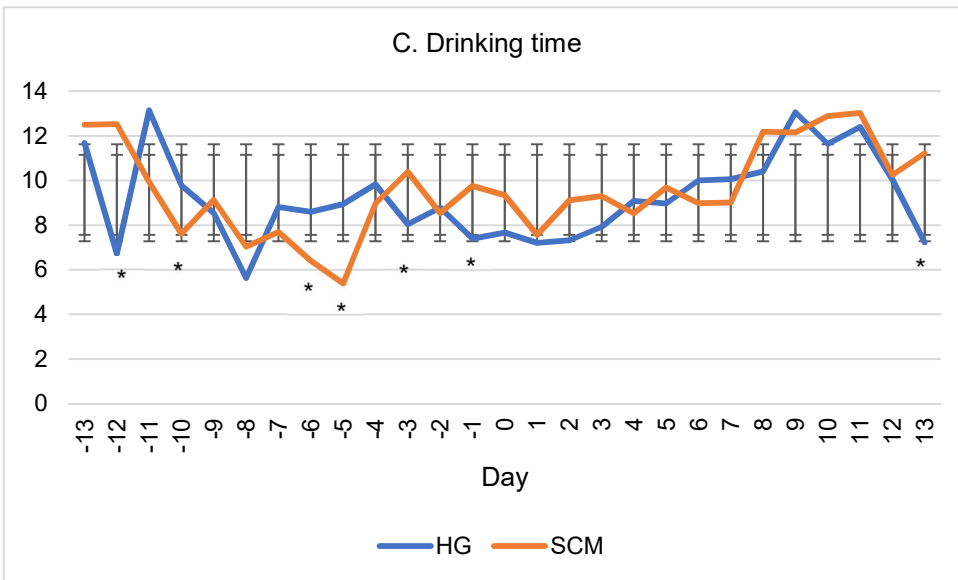
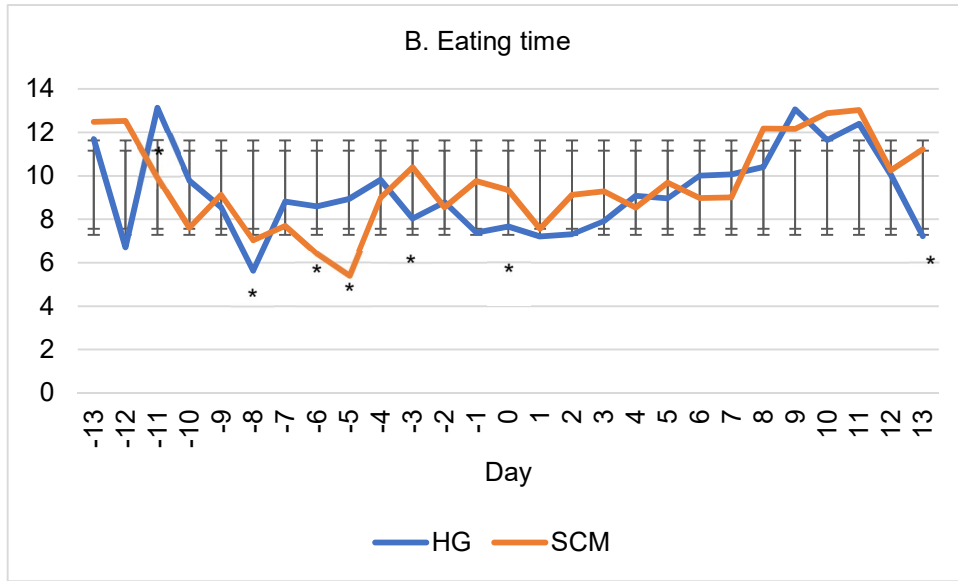
HG - Clinically healthy group; SCM - Subclinical mastitis group. ^{ab} - The difference between the mean values of the groups HG and SCM marked with different letters is statistically significant: $p < 0.05$.

Rumination time in the SCM group from day -4 to day 0 decreased by 60.91%, drinking time by 48.47%, and number of boluses per rumination by 8.67% ($p < 0.05$). On the other hand, during this five-day period, the eating time of SCM cows increased by 4.00%, the number of rumination chews increased by 6.83%, and drinking gulps by 12.66% ($p < 0.05$). In the second period experiment, a few significant differences were observed between the SCM group and the indicators of healthy cows (Figure 1).

There were no significant differences between the time of eating and drinking in the groups from day 0 to day 12 of the experiment.

Analyzing the change in the studied parameters during the entire period of the experiment, uneven changes were observed in cows of both groups, but in the experimental group, more pronounced changes were found from about the fourth day before the diagnosis of the disease was established. The difference between the mean values of the groups is statistically significant: * = $p < 0.05$ (figure 1).





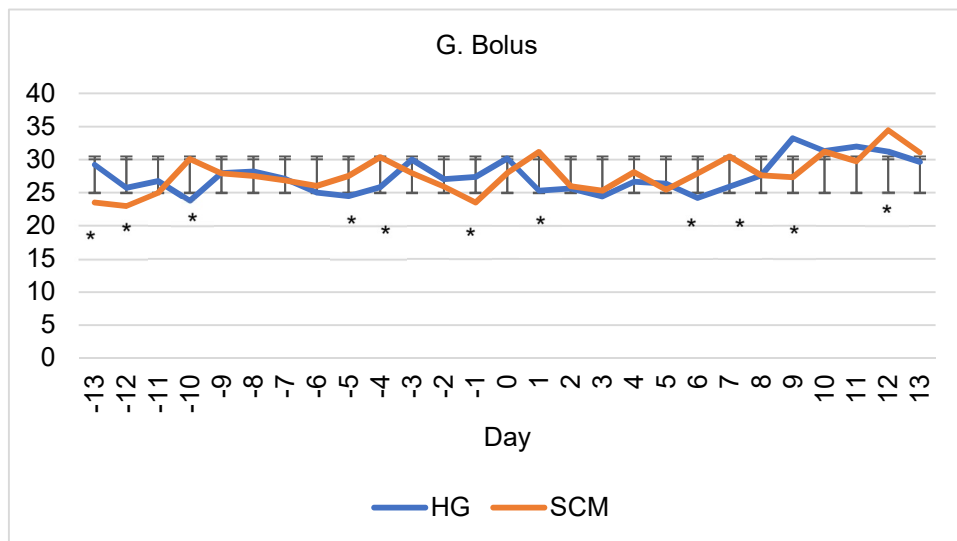
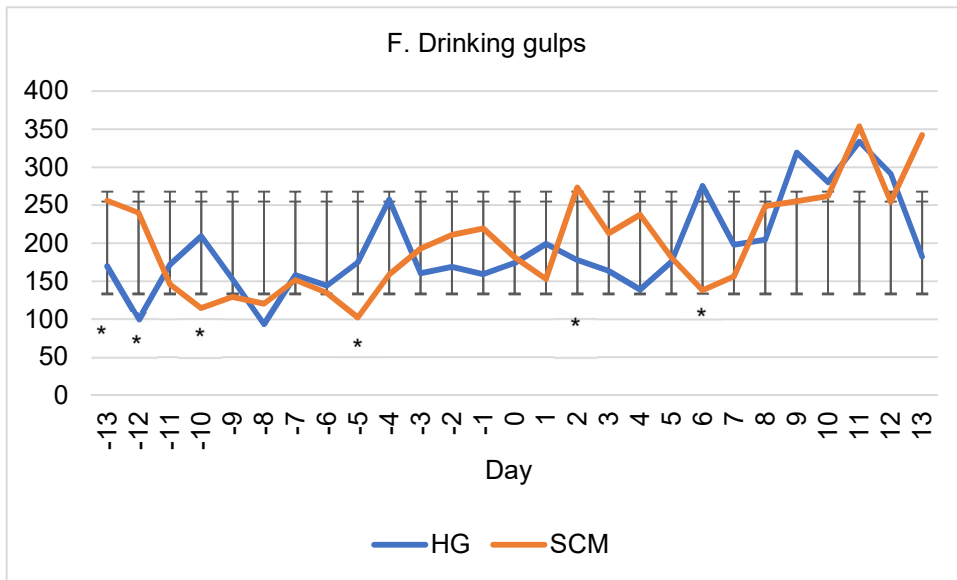
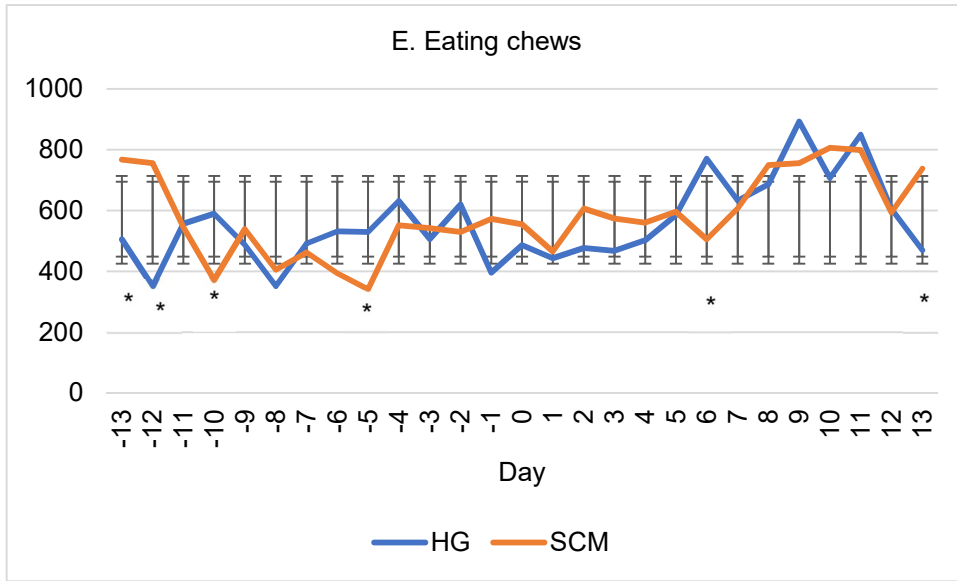


Figure 1. Indicators of cow groups by days of experiment. HG - Clinically healthy group; SCM - Subclinical mastitis group (SCM). Error bars indicate standard deviation.

Table 3. Pearson's correlation between biomarkers registered with real-time sensors measured by the RumiWatch nose-band sensor by group.

| Indicators | Rumination time | Eating time | Drinking time | Rumination chews | Eating chews | Drinking gulps | Bolus |
|------------------|-----------------|-------------|---------------|------------------|--------------|----------------|----------|
| Rumination time | × | 0.276** | -0.100** | 0.933** | 0.318** | 0.340** | 0.935** |
| Eating time | -0.021 | × | -0.176** | 0.205** | 0.798** | 0.687** | 0.328** |
| Drinking time | -0.066* | -0.037** | × | -0.087* | -0.099** | -0.151** | -0.164** |
| Rumination chews | 0.779** | -0.037 | -0.119** | × | 0.259** | 0.249** | 0.881** |
| Eating chews | 0.007 | 0.960** | -0.075* | -0.016 | × | 0.875** | 0.330** |
| Drinking gulps | 0.010 | 0.777** | -0.076* | 0.009 | 0.844** | × | 0.428** |
| Bolus | 0.766** | 0.025 | -0.150** | 0.699** | 0.031 | 0.133** | × |

The correlation coefficient is statistically significant: * = $p < 0.05$, ** = $p < 0.01$.

Above the diagonal are the correlation coefficients of the HG group, below the diagonal is the group of SCM cows (Table 3).

The rumination time of the cows of both groups was strongly positively correlated with rumination chews and bolus, and the eating time with chewing ($p < 0.01$; Table 3).

Rumination chews showed a weak positively association with eating chews and drinking gulps in the HG group ($p < 0.01$), while no significant linear relationship was found between these variables in the SCM group.

A moderate positive correlation was detected between drinking gulps and bolus in the HG group and a very weak correlation coefficient in the SCM group ($p < 0.01$).

The drinking time of both groups of cows had a very weak association with all other studied indicators.

The odds ratios for the effect of the risk factors on mastitis are reported in Table 4. Mastitis was associated with rumination time, rumination chews and bolus ($p < 0.05$).

The chosen method of exploring associations in a multivariable table, where one of the dimensions was an outcome of interest with two categories (subclinical mastitis detected or not detected) showed that subclinical mastitis in cows in the herd was more likely at the level of rumination time ≤ 23.80 (OR = 18.271 times, $p = 0.007$), number of boluses per rumination ≤ 27.63 (OR = 15.976, $p = 0.013$) and less likely when level of chews during rumination (for mechanical breakdown of the regurgitated materials into finer particles using the molars) ≤ 1627.88 (OR = 0.266, $p = 0.030$) (Table 4).

Table 4. Analysis of mastitis risk indicators using a multivariable logistic regression model.

| Risk indicators | Classes of explanatory variables | B | S.E. | Wald χ^2 | df | <i>p</i> | OR (95% CI OR) |
|------------------|----------------------------------|--------|-------|---------------|----|----------|------------------|
| Rumination time | G0 \leq 23.80 | 2.905 | 1.082 | 7.203 | 1 | 0.007 | 18.271 |
| | G1 $>$ 23.80 | | | | | | (8.756 - 31.117) |
| Rumination chews | G0 \leq 1627.88 | -1.323 | 0.609 | 4.714 | 1 | 0.030 | 0.266 |
| | G1 $>$ 1627.88 | | | | | | (0.081 - 0.879) |
| Bolus | G0 \leq 27.63 | 2.771 | 1.111 | 6.225 | 1 | 0.013 | 15.976 |
| | G1 $>$ 27.63 | | | | | | (7.248 - 30.223) |
| Constant | | -2.283 | 1.113 | 4.208 | 1 | 0.040 | 0.102 |

The classes of explanatory variables were formed according to the average values of herd indicators from the day "-4" to the day "0". B – unstandardized regression weight; S.E. – Standard error; Wald χ^2 - this is the test statistic for the individual predictor variable; df - degrees of freedom, *p* – *p*-value (statistically significant with a *p*-value < 0.05); OR - odds ratio, 95% CI OR – 95% confidence interval for odds ratios.

3. Discussion

There is an increasing interest in using behavior to detect disease early and, ideally, automatically [15,17]. During this study we have hypothesized that subclinical mastitis affects rumination behavior in cows (such as rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus) registered with sensor systems. According to our hypothesis the aim of the current study was to determine the relation between subclinical mastitis and rumination behavior such as rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus, which were registered by using online sensors. Based on the results of our study, we found that healthy cows spent more time for rumination and drinking ($p < 0.05$), and had more boluses per rumination. Also, it was ascertained that rumination time in the subclinical mastitis cows from day 4 to day 0 decreased by 60.91%, drinking time by 48.47%, and number of boluses per rumination by 8.67% ($p < 0.05$). Kaufman et al. [18] found that healthy cows spent an average of 459 min/d ruminating. Dairy cows spend approximately 7 hours each day with rumination (420 min) [19]. However, the majority of dairy cows that receive mixed diets ruminate for a shorter period of time [20]. Several studies have found a link between reduced rumination time and clinical and subclinical health problems [19,21]. It was discovered that the period of illness, RT was lowered by 5.33 % for subclinical mastitis (SM) and 14.85 % for clinical mastitis (CM), which could be attributed to the difficulty faced by the animals with mastitis [22]. A considerable difference in RT was identified across cows in one study that compared a group of healthy cows with a group of sick cows, and the RT began to diverge from the normal pattern until 2 weeks before the diagnosis of mastitis [23]. Other studies, however, have reported lower RT in sick animals when comparing cows with some form of health problem to healthy cows [24, 25]. When other investigators evaluated behavioral data prior to the manifestation of health problems, they discovered that the RT parameter aided in the early detection of diseases before the animals were clinically impacted [25]. When compared to healthy cows, cows with CM had a 397 min/d lower RT, according to some researchers [9]. As a result, the significant release of those inflammatory mediators, which regulate the release of inflammatory cells on the wounded tissue regulated by the hypothalamus, presumably activating the satiety center, could be a plausible explanation of the reduction in the animals' average RT [26]. Lower RT is a useful indication for providing health risk information and monitoring the metabolic states linked with illnesses [27]. The average RT of multiparous dairy cows was found to be effective in predicting SM and CM up to 2 days before disease onset.

Furthermore, its variability revealed that animals with subclinical cases had fewer fluctuations while being ill [24]. On the day of diagnosis we found that the rumination time of SCM cows was 39.79% and the drinking time was 16.95% longer, the number of boluses per rumination was 7.35% higher, but the eating time by 21.84% shorter, eating chews by 14.00% and drinking gulps by 4.34% less compared to the group of healthy cows ($p < 0.05$).

Feeding behavior changes have long been used to diagnose the onset of disease [28]. Although subclinical mastitis is recognized by the absence of external signs such as heat, swelling, and discoloration of the udder, abnormal secretion, and systemic reactions such as fever and loss of appetite, several studies have revealed behavioral changes several days before clinical signs of illness [29]. Changes in rumination patterns and daily rumination time, for example, were linked to metabolic problems and discovered several hours or days before clinical signs appeared [30].

Cows suffering from endotoxin-induced acute mastitis displayed normal illness behavior, such as decreased appetite. When the most severe clinical signs were present, the cows spent more time with eating and less time with rumination and drinking. Furthermore, in contrast to previously documented sickness behavior, cows increased their steps number [31]. According this in the following studies the values of RumiWatch noseband sensor and locomotion should be analyzed together. A poor appetite in sick cows has been viewed as a strategy of decreasing absorption of some micronutrients required for pathogen proliferation [31]. Cows ate for extended periods of time, most likely due to fever and illness, but we did not observe the normal sickness behavior associated with reduced appetite [31]. According to González et al. [32] some cows' eating length decreases with the development of mastitis, while others show no change. Feeding activity in barn-housed cattle is well coordinated, with major peaks in both feeding and social competition coinciding with fresh food delivery and lesser peaks following milking [33]. In cattle, water and feed intake are favorably connected [34]. However, drinking has a lower impact on health than feeding and water is more immediately necessary for body function, for example consumption of adequate feed and water is essential for maintaining healthy rumen function [35]. Furthermore, because drinking takes less time than feeding, it is less likely to be disrupted by social competition [36]. Also, it is important to the availability of a sufficient number and length of troughs, since dominant animals could hinder others to get sufficient amounts of water. Although water consumption in cows with mastitis has been observed to be reduced [37], King et al. [38] found reduced walking activity prior to mastitis clinical diagnosis. The quadratic associations described here between SCC and both behavioral changes and distance moved are of importance since activity might increase in cows with clinical mastitis [39]. Probably related to udder discomfort and a reduction in lying time. It can be because udder pain could hinder animals from lying down and getting up, that they rather avoid resting instead of actively intentionally being more active. According to Jadhav et al. [40] the threshold SCC value to differentiate subclinical mastitis from normal should be 310,000 cells/mL, rather than 200,000 cells/mL, as is currently used [41]. Also, the threshold of 200,000 cells/mL for detection of subclinical mastitis is used in other studies [42,43,44].

We found that the differences in the second period are less significant between the groups. It could be because environmental factors might have affected the results. For example heat stress, oestrus and other effects. Reith et al. [45] revealed that for oestrus identification, daily rumination time was reduced by 19.6 % (83 min/d) on the day of oestrus. It could be a possible explanation behind our diverse findings.

Changes in the amount of time a cow spends feeding and ruminating, for example, can reflect an underlying shift in cow contentment and welfare. Furthermore, it is well believed that variations in eating and rumination times can assist farmers in forecasting oestrus [46]. Davison et al. [47] discovered that cattle displayed indicators of heat stress for extended periods of time during the day when the ambient climatic conditions were favorable (in excess of 6 h). The study supports the possibility of incorporating heat stress

monitoring into neck-mounted collars via automatically reporting times. According to Weary et al. [28] changes in animal welfare, such as heat stress, can be recognized by a decrease in rumination time. Heat stress affects RT in moderate climates, such as Germany in Central Europe, and cows adjust their RT in response to heat stress thresholds, which are lower in moderate temperature zones than in hotter locations [48]. However, Abeni and Galli [49] discovered that the greatest reduction in daylight RT (from 0800 h to 2000 h) varied from 49 % for cows in the early stages of lactation to 45 % for cows in the plateau periods, although the range of decline in daily RT varied from 49 % to 45 %. In our past study we found, that on the day of lameness identification, reductions in rumination time, eating time, rumination chews, drinking time, and bolus count in the lame group may act as lameness markers [50].

A limitation of our study was that the groups are relatively small. We investigated subclinical mastitis group (n=10) and clinically healthy group (n=10). Subsequent studies recommend an increase in the number of cows. Also, factors such as heat stress, oestrus and other effects which have impact on rumination behavior biomarkers should be evaluated in a larger study with more animals.

Therefore, according to our knowledge, this is the first study that evaluates the relation between subclinical mastitis and rumination behavior such as rumination time, eating time, drinking time, rumination chews, eating chews, drinking gulps and bolus, which were registered by using online sensors.

4. Conclusions

We can conclude that subclinical mastitis effects rumination time and rumination chews registered with sensor systems. However, additional studies with a larger number of animals are required to confirm these results. Furthermore, impact of heat stress, oestrus and others effects on rumination behavior biomarkers should be evaluated.

From practical point, changes in rumination behavior could be possibly used to identify subclinical mastitis in dairy cows. It can be a great advantage for the farmers to recognize the affected cows and treat the udder inflammation at an early stage. Veterinarians and farmers can consider online sensor system which registers rumination behavior biomarkers for identification of subclinical mastitis. This would allow early identification of sick cows and reduce the economic losses to the farm due to this disease.

Further studies should focus more on the factors such as heat stress, oestrus and other effects which have impact on rumination behavior biomarkers with more animals. Also, in the following studies the values of RWS and locomotion should be analyzed together.

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