RUMINATION, ACTIVITY, MILK YIELD AND MILK COMPONENTS ANALYSIS FOR DISEASE DETECTION DURING THE TRANSITION PERIOD OF DAIRY COWS

By

Sushil Paudyal

A Thesis Submitted in Partial Fulfillment

of the Requirements of the Degree

MASTERS OF SCIENCE

Major Subject: Animal Science

West Texas A&M University

Canyon, Texas

December 2015

Approved:

Co-Chairman, Thesis Committee | Date

Co-Chairman, Thesis Committee | Date

Member, Thesis Committee | Date

Member, Thesis Committee | Date

Head, Major Department | Date

Dean, Graduate School | Date

ABSTRACT

Early detection of disease is the key to successful management of the transition dairy cows, leading to timely treatment and prevention of costs associated with prolonged treatment and prolonged milk yield reduction. Electronic systems that allow for monitoring rumination, activity and milk yield of individual cows are now available. Our objective was to determine the association between changes in rumination behavior, activity milk yield and milk components with health disorders in the peripartum and early lactation cows.

Three weeks before the estimated calving date, 198 multiparous Holstein cows housed at the University of Florida (UF) dairy unit were affixed with neck collars containing rumination loggers and activity (Hr-Tag rumination monitoring system, SCR Engineers Ltd., Netanya, Israel) providing rumination time (RT) and activity in 2-h intervals. Blood was collected 12-72 h after calving for non-esterified fatty acid (NEFA), beta-hydroxy butyraic acid (BHBA), and calcium(Ca) determination (n=136). Occurrence of clinical health disorders [mastitis (MAS), metritis (MET), clinical hypocalcemia (HYC), depressed and dehydrated (DEP), digestive disorders (DIG), lameness (LAM), and ketosis (KET)] was assessed until 60 DIM by UF veterinarians and farm personnel and this was regarded as gold standard for the experiment. For the blood analysis cows were considered in negative energy balance (NEB) if serum NEFA concentration were

> 0.5 mmol/L; positive for subclinical ketosis (SCK) if serum BHBA cocentrations were ≥ 1.4 mmol/L; and hypocalcemic (HYC) if serum Ca concentrations were < 8.0 mg/dl.

Two indexes were developed to explore the potential associations between the proposed parameters and health disorders.

i. CowIndex (CIx) that considered the difference in RT between the day of diagnosis (d0) and the daily average RT from d -3 to -5 relative to diagnosis (0vs-3to-5RT) divided by the daily average RT from d -3 to -5 (-3to-5RT) in the affected cow.

ii. MatesIndex (MIx) that considered the difference between the affected cow 0vs-3to-5RT and the pen mates 0vs-3to-5RT relative to the average d0 RT for healthy pen mates.

Using a CIx index value -0.1 as the cut off value for the change in rumination, we set a cow alarm (CAL) and marked the cow as flagged for a health disorder. Similarly, whenever the MIx was less than -0.1, we set a herd alarm (HAL) and marked the cow as flagged for a health disorder. A combined alarm (CombA) was created using the parallel combination of the HAL and CAL.A separate analysis was done to detect disorders one day prior to disease diagnosis.

The same procedure for calculation of CIx and MIx and the corresponding alarms were applied for activity, milk yield, and milk components (fat %, protein%, lactose%, and fat/lactose ratio) analyses.

The average rumination CIx in healthy cows was 0.049 while CIx in sick cows were -0.165, -0.029, -0.513,-0.048, -0.022, -0.098, -and 0.081 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Average rumination MIx in healthy cows ranged from 0.0001 to 0.001 and MIx in sick cows were -0.183, -0.101, -0.424,-0.114 -

0.101, -0.148, -0.147 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Sensitivity/specificity (%) of rumination CAL were 56/77, 39/77, 100/77, 47/77, 44/77, 67/77, and 61/77, for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Sensitivity/specificity (%) of rumination HAL were 63/77, 42/77, 100/77, 50/77, 48/77, 56/77, and 67/77 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for rumination CAL ranged from 0.55 to 0.86 whereas the AUC values for rumination HAL ranged from 0.57 to 0.86.

Sensitivity/specificity of activity CAL were 34/81, 50/81, 71/81, 31/81, 48/81, 44/81, 48/81 % to detect each disease on the day of diagnosis and area under the curve (AUC) resulting from receiver operating characteristic (ROC) curve analyses for our proposed cut-off value were 0.58, 0.65, 0.76, 0.56, 0.64, 0.70 and 0.65 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively.

Sensitivity/specificity (%) of milk CAL was 59/77, 67/77, 100/77, 59/77, 61/77, 27/77, and 61/77 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Area under the curve for milk CAL on our proposed cut-off value (-0.1) ranged from 0.52 to 0.88. Sensitivity/specificity (%) of milk MAV was 53/82, 46/82, 75/83, 52/83, 49/83, 20/83, and 49/83 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Area under the curve for milk MAL on our proposed cut-off value ranged from 0.51 to 0.78. Sensitivity/specificity (%) for fat CAL were 61/73, 48/73, 67/73, 35/73, 47/73, 10/73, and 46/73 and for fat MAL were 70/75, 57/75, 67/65, 30/75, 45/75, 19/75, 54/75 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for fat CAL ranged from 0.53 to 0.69 and that for fat HAL ranged from 0.53 to 0.72. The sensitivity/specificity (%) of protein CAL were 42/89, 44/89, 100/89, 22/89, 20/89, 10/89, and 19/89 and the protein HAL were 42/90, 43/90, 100/90, 22/90,22/90, 10/100, and 22/90 for MAS, MET, HYC, DEP, DIG, LAM, and KET respectively. The AUC values for protein CAL ranged from 0.50-0.95 and the AUC values for protein HAL ranged from 0.51-0.95. The sensitivity/specificity (%) of lactose CAL were 35/95, 17/95, 100/95, 13/95, 8/95, 5/95, and 5/95 and the sensitivity/ specificity (%) for lactose HAL were 35/96, 4/96, 100/96, 4/96, 8/95, 5/100 and 5/96 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for lactose CAL ranged from 0.50 to 0.98 and the lactose HAL ranged from 0.50 to 0.98 for different disorders. The sensitivity/specificity (%) of fatbylactose CAL were 78/59, 57/59, 80/59, 52/59, 58/59, 58/59, and 61/59 and the sensitivity/specificity (%) for fatbylactose HAL were 80/60, 77/60, 80/60, 48/60, 63/60, 58/60 and 71/60 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for fatbylactose CAL ranged from 0.55 to 0.69 and the lactose HAL ranged from 0.54 to 0.72 for different disorders. In conclusion consistent negative changes in rumination time, activity, and milk yield were observed on the day of clinical diagnosis of disease and could be used to assist in the early detection of periparturient cow disorders.

ACKNOWLEDGEMENTS

This thesis is one of the significant milestones in my academic career. To be able to be here and write down this thesis, several people have given their contribution from their side. I would remember each and every person and thank them from the core of my heart. I am greatly honored and privileged to have worked with Dr. Pablo Pinedo as my advisor. I am most grateful to him for his help guidance, kind encouragement and stimulating suggestions during the research period. Also, I am much indebted to my advisors Dr. John T. Richeson, Dr. Samuel E. Ives and Dr. Fiona Maunsell for their constant support and suggestions during the research period. The co-operation is much indeed appreciated.

My gratitude is with Ruminant Animal Health Laboratory, Texas A&M Agrilife Research and especially to Ms. Kelsey Bryan for her help during this period. UF FARMS team deserves a big thank you for collecting the samples for my research and helping me analyze the blood samples. I would like to thank Dr. Albert De Vries from Department of Animal Science, UF for helping me with the farm backup data and herd information during the research period. Finally, I would appreciate the help from SCR technology for providing us with the SCR Tags to be used in the research.Very special thanks to Pramod, Mahendra, Ram, Shravan for their constant support and for making my stay here memorable. Above all I would like to thank my parents and my family for trusting in my ideas and confiding in me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Early detection of signs of illness and consequently an early start of therapy is a key to successful management of disease in dairy cattle. In the last two decades there has been a consistent increase in the average US dairy herd size which is leading to less labor available per cow. Thus, the systematic evaluation of clinical parameters of animals at risk of disease on a regular basis becomes important to assure herd health and helps to select animals for clinical examination. During the transition phase, defined as the period from 3 week before to 3 week after calving, dairy cows are highly susceptible to metabolic and infectious diseases. Thus, monitoring cows during this period would help timely clinical examination, early implementation of therapy and prevention of potential production losses.

The overall objective of this study was to evaluate rumination activity as a cow-side parameter to detect developing disease in dairy cows at an early stage. To achieve improved accuracy, rumination is used in conjunction with activity, milk yield and milk components.

We explored rumination at different physiological stages of animals and how this parameter changes in sick animals. Also, we analyzed the rumination pattern during summer and winter seasons. Further, taking rumination, activity, milk yield and components, we have created different health index alarms that would suggest a significant decrease in rumination, activity, milk yield and fluctuation (either decrease or increase) in milk components at the day of clinical diagnosis and day previous to clinical diagnosis.

Finally, we studied the relationship between rumination time around calving and calcium, BHBA levels, and NEFA concentrations.

CHAPTER II

LITERATURE REVIEW

Rumination

Rumination can be defined as the process of regurgitation of fibrous ingesta from rumen to mouth, remastication, and reinsalivation followed by swallowing and returning of the material back to rumen (Welch et al., 1982). Rumination is an important step in the digestion process of ruminant animals with its primary role being the physical breakdown of coarse material to facilitate its passage from the rumen into the small intestines (Sjaastad et al., 2003). The phenomenon of "chewing the cud" or rechewing rumen contents ingested previously is a unique feature of ruminants (Ruckebusch, 1993).

Rumination is induced through mechanical stimulation of nerve endings by the coarse and ridged feed particles in the region of the esophageal opening. The re-mastication activity during rumination reduces particle size and enables the particles to pass on through to the reticulo-omasal orifice. The passage is also affected by particle shape, density and digestibility which are altered during the rumination process (Sjaastad et al., 2003). The chewing activity during rumination stimulates the secretion of saliva, which facilitates swallowing and possesses high concentrations of bicarbonate and phosphate buffers that aid in sustaining the ruminal pH at a level (5.5-6.5) that is suitable for rumen microbial activity (Ruckebusch, 1993). Regurgitation is initiated with a reticular contraction which is distinct from the primary contraction. This contraction, in association with relaxation of the distal esophageal sphincter, allows a bolus of ingesta to enter the esophagus. The bolus is carried into the mouth by reverse peristalsis. The fluid in the bolus is squeezed out with the tongue and reswallowed, and the bolus itself is remasticated, and then swallowed (Ruckebusch, 1993). The traffic of bolus in esophagus is complex such that upward traffic includes regurgitated digesta bolus, downward traffic includes excess regurgitated digesta swallowed immediately after mouth is full and intermediary swallows of partially ruminated digesta.

During the process of ruminal digestion, the uppermost layer of the rumen consists of gas produced particularly during fermentation of carbohydrates (Sjaastad et al., 2003). Below the gas layer occurs a stratification of feed particles according to their difference in density. Uppermost are partially degraded long fibrous materials floating on top of more fluid layers that create a “mat” layer. As fermentation proceeds, the organic matter which serves as fermentation substrates gets depleted and hence the gas production decreases. The particles now lose buoyancy due to loss of entrapped gas. Thus, the particles become small and dense enough to sink through the rumen mat to ventral parts of rumen. Larger particles are found to sink more slowly than smaller particles with the same density. Contractions of the reticulum and rumen provide mixing of fore stomach contents and a transfer of particles to the omasum. The contraction also helps in regurgitation and aids in eructation of gases. The water content of digesta is absorbed in the omasum prior to its transfer to abomasum where further digestion takes place due to enzymatic action. The digesta regurgitated is largely derived from contents that were in the cavity of the relaxed reticulum. Opening of the cardia during regurgitation and its closure at the end of swallowing depend upon action of the same but quantitatively different, esophageal muscle layers (Ruckebusch, 1993). When returned to the rumino reticulum, the ruminated digesta does not immediately pass to the omasum; but is deposited in the dorsal part of the cranial sac of the rumen.

Duration of rumination and the rumination pattern

Different authors report different RTs and patterns. Welch (1982) reported a basic circadian pattern in rumination with cattle normally spending 8-9 hours per day ruminating. However, the circadian pattern can be altered due to feeding frequency, feeding time; and ration composition (Lindgren, 2009). Rumination activity primarily occurs at night and during resting periods in the afternoon (Lindgren, 2009). Cattle spend 25-80 minutes ruminating per kg of roughage consumed (Sjaastad et al., 2003), and adult dairy cows ruminated 7 to 8 h per d in a recently published study (Adin et al., 2009). An average RT in dairy cows without disease and stress is found to be 463 min/d in primiparous and 522 min/d in pluriparous cows (Soriani et al., 2012).

As reported by Lindgren (2009) most cattle ruminate about ½ to 1 hour for 10-17 periods per day and during each period of rumination they produce 30-60 boluses. Each cycle lasts for approximately 40 seconds and contains 30 to 60 chewings with a minor variation in number of chewings per minute. An inter-cycle period of 4-8 seconds occurs between the two boluses during which there is no chewing.

Rumination is found to have voluntary control by the animal and the animal will therefore cease to ruminate if it is disturbed, for example at milking (Lindgren, 2009).

Any events that result in pain, hunger, maternal anxiety or illness also cause decrease in rumination.

Cows can ruminate while standing, but preferably ruminate lying down and commonly lie laterally on the left side to optimize positioning of the rumen (Albright., 1993; Acatincai et al., 2010). Considering the entire time spent ruminating, cows ruminate lying down 63.4% of the time and only 36.5% of the time in standing position. However these patterns can be altered by environmental conditions, and, during summer, cows ruminate in standing position more often (56% of the time) (Acatinacai et al., 2010).

A breed difference in rumination time has been reported. Among a total of 108 animals grazing on grass, Holstein cows spent more time ruminating and had more mastications during rumination than Jersey cows. However, when expressed per unit of body weight, RT was greater for Jersey cows and they had more ruminating mastications compared with Holstein cows (Prendiville et al., 2010). Aikman (2008) also reported that Holsteins spent more time ruminating per day compared with Jerseys but Jerseys spent more time eating and ruminating per unit of ingested feed. Similarly, Welch et al. (1982) concluded that RT per gram of cell wall constituent was increased for Jersey versus Holstein cows. However, Gregorini et al. (2013) studied three hundred and twenty lactating dairy cows and concluded that daily rumination time was only associated with age but not with breed or genetic merit of the cow.

Rumination is considered to be an indicator of feeding and lying behavior of cows. Schirmann et al. (2012) studied 42 mature Holstein dairy cows for their feeding and rumination behavior in the early dry period and observed that cows spent more time ruminating after periods of high feed intakes.

RT is also found to be affected by diet. Diets containing 11.7% NDF resulted in 12.7% less rumination time than diets with 14.1% NDF; with 23.5% increase in RT per kilogram of roughage ingested (Adin et al., 2009). Beauchemin and Yang (2005) also support the fact that RT linearly increases as dietary physically effective NDF. Furthermore, total RT was increased with increased saturated fat supplementation.

Bender et al. (2014) evaluated daily variation in body weight, milk production, and rumination activity in dairy cows and observed rumination to average 461.1 min/day, with a standard deviation of 6.1 min among days within a pen, 128.0 min among individual cows within a pen and 43.6 min among days within individual cows. De Vries and Chevaux (2014) supplemented dairy cows with live yeast and found that the supplemented cows ruminated longer (570.3 vs 544.9 min/d; SE ± 0.04 min). Sjostrom et al. (2014) concluded that the daily rumination time was greater during September (402 min/d) compared to July (361 min/d). Clement et al. (2014) stated that the rumination time estimate has a significant role in the DMI prediction model.

Measuring Rumination Time

Rumination is typically monitored through visual observation of individual animal (Schirmann et al., 2009). However, visual observation is labor intensive, time consuming, with only a small number of cows monitored at a time, and with limited accuracy (Schirmann et al., 2009; Carraway et al., 2014; Kononoff et al., 2002). Automating the monitoring of rumination is beneficial because it removes the influence of observers and may reduce the cost of obtaining information (Schirmann et al., 2009; Kononoff et al., 2002).

Indirect methods of monitoring rumination are based on jaw motion detecting devices that utilize strain or pressure gauges attached to or built in a halter (Kononoff et al., 2002; Braun et al., 2013; Schirmann et al., 2009). Braun et al. (2013) evaluated the rumination behavior using a noseband pressure sensor and found a significant correlation between visual observation and results of the noseband pressure sensor. Bikker et al. (2014) evaluated an ear-mounted movement sensor and recommends this device to be used for rumination monitoring. Buchel and Sundrum (2014) assessed the jaw movement-based monitoring system and concluded an adequate agreement of the results with visual observation. These devices provided useful information but the equipment had several limitations and was cumbersome. Most devices required full head halters that include moveable devices located under the jaw. These devices may be uncomfortable for the animals and may have affected their eating or rumination behavior but numerous studies have shown that they were effective in differentiating jaw movements associated with chewing and ruminating behavior (Schirmann et al., 2009). Earlier versions of these devices used cables to connect to a computer and hence had limited utility on cows housed in tie stalls (Bauchemin et al., 1989). Memory capacities of these devices for data storage were limited and furthermore, halter removal was required to retrieve the data for download to a computer. These challenges limited the collection of continuous rumination data more than 21 days from free stall housed cows (Schirmann et al., 2009). Elischer et al. (2013) also reported that improper placement of the sensor on cows neck may interfere with the data collection through automatic milking system rumen monitors.

The recently developed HR-Tag® rumination monitoring system (SCR Engineers Ltd., Netanya, Israel) provides output data for rumination time; intervals between regurgitation of boluses and chewing rate. The system consists of rumination loggers, stationary or mobile readers and software for processing the electric records (Data flow software, SCR Engineers Ltd.).The logger is positioned on the left side of the neck by a neck collar (Schirmann et al., 2009). The regurgitation and rumination produces distinctive sounds that are recorded by a microphone and then it is processed and digitally recorded. The calculated data are summarized in 2-h intervals and stored in the memory of the logger for up to 22h. The data are downloaded via readers positioned at locations within the barn (Schirmann et al., 2009). The only drawback of this system is if some problem prevents the data from being downloaded, the results are lost and overwritten (Schirmann et al., 2009).

The beginning of a rumination event as defined by the software occurs when the system detects the sound associated with regurgitation. The algorithm considers rumination events to be separate if successive regurgitations are separated by at least 30s. The rumination logger includes a microphone, a microprocessor, and a transponder that is encapsulated into a plastic casing (size 100 mm x 80mm x 35mm; weight 120g) and are fitted into a nylon collar. The collar consists of an adjustable nylon strap fitted with a weight (size = 70 mm x 70mm x 30mm; weight = 540gm) that hang below the neck to ensure that the rumination logger retains its position on the left side of the neck. The collar including all components weight 920g. The correct placement of the logger is approximately 20 cm behind the left ear and 5 to 10 cm ventral on the left side of the neck (Buchel, 2013).

The system has been validated in multiple studies. Schirmann et al. (2009) validated the system using 47 Holstein cows by comparing it to direct observation and suggested that this technology would be useful for research as well as commercial purposes such as detecting cows close to parturition and sick cows. The system was also evaluated by Burfeind et al. (2011) for the monitoring of rumination in heifers and calves. The system was found to be an accurate tool monitoring rumination in the calves more than 9 months of age. Byskov et al. (2014) stated that the rumination time recorded by rumination monitoring system correlated well with the jaw movement observation. Elischer et al. (2013) validated the system for dairy cows housed in a pasture-based automatic milking system. They also reported that improper placement of the sensor on cows neck may interfere with the data collection. This system was used in beef cattle by Goldhwk et al. (2013), who concluded that more research is required to refine the functioning and utilization in beef cattle.

Rumination time (RT) and calving

Cows spend relatively less time ruminating when parturition approaches. There is a distinct rumination behavior during the first week after calving; RT dramatically decreases at the day of calving and recovers quickly in the following week (Bar, 2010; Schirmann et al., 2013). In a study by Buchel and Sundrum (2014), 15 out of 17 (88%) of the dairy cows analyzed showed reduction in RT by a mean of 27% (25.6 min/6h) during the last 6 h of calving. Similarly, Pahl et al. (2014) studied the rumen activity of dairy cows 24 h before and after calving in a total of 17 cows and found that the RT decreased in the last 4 h antepartum and in the first 8h postpartum. Brochers et al. (2014) suggested that using activity measurement and RT was useful in predicting impending calving without any other new technology or parameter being used. In another study by Calamari et al. (2014), the average RT before calving was 479 min/d which reached a minimum value at calving (i.e. 30% of RT before calving). The relationship demonstrated between the RT and calving time constitute a new opportunity for predicting the timing of calving (Schirmann et al., 2013; Buchel and Sundrum, 2014).

Rumination time and estrus

Reith and Hoy (2012), studied 265 verified estrus cycles from 224 dairy cows with artificial insemination leading to conception. In the estrous cows RT was significantly reduced. The average decrease in RT was 17% (74 min/d) ranging from -71 to +16 % among animals and between 14 (60 min/d) and 24% (94 min/d) among herds with the decrease in RT more pronounced in primiparous than in multiparous cows.

Reith et al. (2014), furthered research to analyze the activity and RT in the peri-estrus period and confirmed that cows in estrus spent significantly less time ruminating and the activity level was significantly increased during the period.

Pahl et al. (2015) evaluated the changes in RT of 62 dairy cows around estrus. The study found that the RT was significantly decreased on d -1 and 0 with the RT of 77 min (day -1) and 75 min (day 0) less than on the reference day. The extent to which rumination time decreased did not differ among primiparous and multiparous cows in this study.

Rumination time and disease

Feed intake, feeding and RT are considered as important parameters for the identification of suboptimal feeding conditions, and can be used to indicate possible health disorders (Buchel, 2013). RT is associated with the metabolic condition and disease state of dairy cattle around parturition. Therefore, rumination monitoring may be helpful to quickly obtain information on the health status of animals in a critical period like the transition phase (Soriani et al., 2012; Siivonen et al., 2011). Monitoring RT around calving and in particular during first week of lactation has been proposed to be an effective means to identify the cows that are at a greater risk of developing disease in early lactation (Calamari et al., 2014). The early detection of clinical and subclinical disease through rumination monitoring would allow producers to more rapidly begin remedial therapies that could reduce the costs associated with later treatment and more severe production loss (Caraway et al., 2014). Furthermore, the time required for normalization of eating and rumination behavior in a sick animal has prognostic value and may be taken as a parameter of effectiveness of the applied treatment (Braun et al., 2013).

Increased rumination time is associated with increased saliva production and improved rumen health because of the buffering capacity of saliva (Schirmann et al., 2009). Decreased rumination time has been associated with the stress, anxiety and diseases (Welch et al., 1982; Hansen et al., 2003).

Rumination time before calving may be an indicator of health during early lactation. Soriani et al. (2012) monitored the rumination pattern during the transition period to investigate its relationships with metabolic conditions, milk yield and health status and reported that the rumination time was positively correlated with milk yield ($r = 0.36$). Cows with reduced rumination time before calving maintained reduced RT after calving and suffered a greater frequency of disease than cows with greater RT in late pregnancy. Cows with mild inflammatory conditions or without health disorders during parturition showed a greater average rumination time (>520 min/d) during the10 d after parturition. On the other hand, decreased RT (< 450 min/d) during the first few days of lactation was observed in cows with subclinical diseases or health disorders (Soriani et al., 2012). Calamari et al. (2014) observed that more than 90% of the cows that had low RT before parturition had clinical illness in early lactation; whereas only 42% of the high ruminating cows had clinical illness.

Rumination time in cattle decreases during rumen acidosis. DeVries et al. (2009) observed that high-risk cows (early lactation cows fed 45:55, forage: concentrate diet) spent less time ruminating (491 vs. 555 min/d) than low risk cows. In the acidosis challenged cattle, rumination time decreased the first day after the challenge (436 min/d) as compared to the baseline (533 min/d), but increased the day after the challenge. Fewer cows were observed to be ruminating at a given time on the first day following the challenge as compared with the baseline period. Thus they concluded that an acute episode of acidosis alters rumination patterns of lactating dairy cows (DeVries et al., 2009).

Cows affected by clinical mastitis demonstrated a reduction of RT and a change in its variability some days before antimicrobial treatment (Soriani et al., 2012). Chapinal et al. (2014) studied the effect of flunixin meglumine on rumination in dairy cows with endotoxin induced mastitis. Cows challenged with intramammary infusion of *Escherichia coli* lipopolysaccharide (LPS) and not treated with the drug (control group) ruminated less than treated cows 5-8 h and 11-12 h after LPS infusion. Thus, experimentally induced mastitis has an effect of reducing the rumination time. In another similar study, Fitzpatrick et al. (2013) studied the effect of meloxicam on rumination time in dairy cows with endotoxin induced mastitis. Neither the LPS infusion nor the meloxicam treatment had a significant effect on daily RT but the rumination diurnal pattern on the day of LPS infusion showed an overall deviation from the baseline pattern. Cows spent less time ruminating in the hours following LPS infusion and more time ruminating later in the day. Thus, mastitis can be related to the altered RT.

Van Hertem et al. (2013) investigated the utility of continuous monitoring of milk production and rumination activity for lameness detection. The investigators found that the highest correlation of lameness with a rumination variable was on d 6 before diagnosis for the nighttime RT and the correlation coefficient was 0.21 ± 0.007 for RT related behaviors.

Rumination can also be an asset to determine the heat stress level of dairy cattle. Acatincai et al. (2009) concluded that when temperature exceeds the upper limit of the thermal comfort of a particular breed, rumination process is severely affected. Temperatures beyond 27-28^{o} C reduce the overall rumination process, including both frequency and duration of this activity. Soriani et al, (2013b) observed that in dairy cows suffering mild to moderate heat stress there was a negative relationship between daily maximum temperature-humidity index (THI) and RT ($r = -0.32$), with a reduction of 2.2 min of RT for every daily maximum THI unit over the threshold of daily maximum THI of 76. Rumination time throughout the trial was negatively related to breathing rate and positively related to milk yield.

Rumination time and disease during the transition period

Dairy cows are most likely to become ill during the transition period around calving (i.e. 3wk before to 3 wk after calving). The transition period in cattle is typically characterized by a decline in feed intake beginning 3 wk before calving, depression of certain immune functions both before and after calving, a negative energy balance, and decline in serum calcium and glucose at the onset of lactation. Animals after calving sacrifice immune function for the sake of maintaining lactation (von Keyserlingk et al., 2009). Most postpartum diseases are complex and they have multiple causation. For instance, many infectious diseases diagnosed during transition occur as secondary illnesses to metabolic diseases such as ketosis or hypocalcemia (von Keyserlingk et al., 2009).Transition period cows are also subjected to regrouping as they move into pre-calving groups and then into the lactating herd, and there is evidence of decreased rumination in pre-partum cows that were moved to a new social group (Schirmann et al., 2011).

Cows that experienced metritis in the transition period demonstrated different feeding behavior and spent less time feeding during both pre- and post-calving periods (von Keyserlingk et al., 2009). Cows developing metritis also ate less than healthy cows in the pre-partum period (Huzzey et al. in von Keyserlingk et al., 2009). Goldhawk et al. (2009) found that cows with low pre-partum intakes were also at more at risk for subclinical ketosis after calving. Cows that later developed ketosis ate less and spent less time eating. Thus, feeding behavior of cattle during transition can be used to predict metabolic disease.

Similarly, there is evidence that knowledge of rumination behavior can help identify transition dairy cows at risk for metritis, subclinical ketosis and lameness. This information could also be used to guide the development of management practices that can help detect diseases early and help to prevent disease by addressing management challenges during transition (von Keyserlingk et al., 2009).

Soriani et al. (2013a) observed highly significant differences in rumination behavior between cows affected by severe metritis and healthy cows during the first week of lactation. The presence of severe ketosis or mild metritis or retained placenta affected the RT during the 6^{th} days in milk (DIM). Severe ketosis and retained placenta also affected the rumination time on the 5^{th} DIM and cows affected by retained placenta demonstrated reduction of daily RT during the 2^{nd} DIM (Soriani et al., 2013a). When daily rumination time was used to detect the cows with severe metritis it was possible to define thresholds of rumination time during the first six days of lactation to detect the cows with health disorders (Soriani et al., 2013a).

Schirmann et al. (2013b) studied rumination behavior before calving and its association with metritis and subclinical ketosis after calving. As compared to healthy cows, the cows with subclinical ketosis or metritis and subclinical ketosis together, spent less time ruminating in the pre-partum period. However, there was no difference between healthy and any of sick groups in time spent ruminating after calving. Thus, RT information before calving show promising results in identifying cows at risk for metritis and subclinical ketosis after calving.

Liborerio et al. (2014) studied peri-partum health events and RT and concluded that cows with retained placenta had reduced cud chewing time. These investigators identified an interaction effect of subclinical hypocalcemia and days relative to calving on RT. Similarly another interaction of ketosis and days relative to calving was observed on RT. Serum concentrations of calcium and beta hydroxybutyrate were also related with RT.

In a similar study (Sterrett et al., 2014) observed no differences in RT between subclinical hypocalcemia (HYC) and non- HYC cattle and also in subclinical ketosis and non-subclinical ketosis cattle.

Bar and Solomon (2010) compared average daily RT of milking cows on days without any event to days with either nutritional changes, mastitis, calving or estrus and observed a clear significant decrease in RT on days with these days. This supports the usefulness of RT to track potential individual cow health problems, deviation from normal behavior and to monitor the effects of intentional or accidental nutritional changes in herd.

Rumination time and milk yield and components

Soriani et al. (2012) also found that the RT was positively correlated with milk yield (r = 0.36). Norring et al. (2012) studied effects of milk yield on time budgets of dairy cows and concluded that higher yielding cows spent more time ruminating while standing and less overall time lying than lower yielding cows. Similarly, Reith et al. (2014) concluded that daily RT was greatest for low yielding cows and least for high yielding cows. Rumination time of high yielding cows was reduced to a greater extent on the day of estrus than that of herd mates with low milk production.

Lessire et al. (2014) studied milking production and milk components in grazing dairy cows and reported that the daily RT and milk fat to protein ratio was decreased during the heat stress. Bouraoui et al. (2002) studied milk production in dairy cows and reported decreased milk yield, milk fat and milk protein percentages during hot period. A negative correlation between the THI and milk yield and feed intake was observed. Smith et al. (2013) observed that milk yield in Holsteins decreased during moderate and severe heat stress and milk yield in Jersey decreased during the severe heat stress period. Holstein fat percentage was less during moderate and severe heat stress compared with milk fat percentage during mild heat stress.

Activity and milk yield for disease detection

Edwards and Tozer (2004) studied the association of activity and milk yield to fresh cow disorders and concluded that cows with ketosis, and digestive disorders could be detected 5 to 6 d earlier then clinician diagnosis based on changes in daily walking activity and milk yield. Stangaferro et al. (2015) were able to detect ketosis, mastitis and metritis using the activity and rumination monitoring. Chandler et al. (2015) concluded that milk components data could be used to detect and monitor herd level ketosis in dairy cows.

Rumination time, activity and milk monitoring provide opportunities for the early detection of health disorders in the transition period of the dairy cows. Different factors account for the variation in rumination time and the decreased rumination time could be an indicator of ill health of the transition cattle.

CHAPTER III

RUMINATION, ACTIVITY, MILK YIELD AND MILK COMPONENTS ANALYSIS FOR DISEASE DETECTION DURING THE TRANSITION PERIOD OF DAIRY COWS

ABSTRACT

Early detection of disease is the key to successful management of the transition dairy cows, leading to timely treatment and prevention of costs associated with prolonged treatment and prolonged milk yield reduction. Electronic systems that allow for monitoring rumination, activity and milk yield of individual cows are now available. Our objective was to determine the association between changes in rumination behavior, activity milk yield and milk components with health disorders in the peripartum and early lactation cows.

Three weeks before the estimated calving date, 198 multiparous Holstein cows housed at the University of Florida (UF) dairy unit were affixed with neck collars containing rumination loggers and activity (Hr-Tag rumination monitoring system, SCR Engineers Ltd., Netanya, Israel) providing rumination time (RT) and activity in 2-h intervals. Blood was collected 12-72 h after calving for non-esterified fatty acid (NEFA), beta-hydroxy butyraic acid (BHBA), and calcium(Ca) determination (n=136).

Occurrence of clinical health disorders [mastitis (MAS), metritis (MET), clinical hypocalcemia (HYC), depressed and dehydrated (DEP), digestive disorders (DIG), lameness (LAM), and ketosis (KET)] was assessed until 60 DIM by UF veterinarians and farm personnel and this was regarded as gold standard for the experiment. For the blood analysis cows were considered in negative energy balance (NEB) if serum NEFA concentration were > 0.5 mmol/L; positive for subclinical ketosis (SCK) if serum BHBA cocentrations were $\geq$ 1.4 mmol/L; and hypocalcemic (HYC) if serum Ca concentrations were < 8.0 mg/dl.

Two indexes were developed to explore the potential associations between the proposed parameters and health disorders.

i. CowIndex (CIx) that considered the difference in RT between the day of diagnosis (d0) and the daily average RT from d -3 to -5 relative to diagnosis (0vs-3to-5RT) divided by the daily average RT from d -3 to -5 (-3to-5RT) in the affected cow.

ii. MatesIndex (MIx) that considered the difference between the affected cow 0vs-3to-5RT and the pen mates 0vs-3to-5RT relative to the average d0 RT for healthy pen mates.

Using an CIx index value -0.1 as the cut off value for the change in rumination, we set a cow alarm (CAL) and marked the cow as flagged for a health disorder. Similarly, whenever the MIx was less than -0.1, we set a herd alarm (HAL) and marked the cow as flagged for a health disorder. A combined alarm (CombA) was created using the parallel combination of the HAL and CAL.A separate analysis was done to detect disorders one day prior to disease diagnosis.

The same procedure for calculation of CIx and MIx and the corresponding alarms were applied for activity, milk yield, and milk components (fat %, protein%, lactose%, and fat/lactose ratio) analyses.

The average rumination CIx in healthy cows was 0.049 while CIx in sick cows were -0.165, -0.029, -0.513,-0.048, -0.022, -0.098, -and 0.081 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Average rumination MIx in healthy cows ranged from 0.0001 to 0.001 while MIx in sick cows were -0.183, -0.101, -0.424,-0.114 -0.101, -0.148, -0.147 for MAS, MET, HYC, DEP, DIG, LAM, and KET respectively. Sensitivity/specificity (%) of rumination CAL were 56/77, 39/77, 100/77, 47/77, 44/77, 67/77, and 61/77, for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Sensitivity/specificity (%) of rumination HAL were 63/77, 42/77, 100/77, 50/77, 48/77, 56/77, and 67/77 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for rumination CAL ranged from 0.55 to 0.86 whereas the AUC values for rumination HAL ranged from 0.57 to 0.86.

Sensitivity/specificity of activity CAL were 34/81, 50/81, 71/81, 31/81, 48/81, 44/81, 48/81 % to detect each disease on the day of diagnosis and area under the curve (AUC) resulting from receiver operating characteristic (ROC) curve analyses for our proposed cut-off value were 0.58, 0.65, 0.76, 0.56, 0.64, 0.70 and 0.65 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively.

Sensitivity/specificity (%) of milk CAL was 59/77, 67/77, 100/77, 59/77, 61/77, 27/77, and 61/77 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Area under the curve for milk CAL on our proposed cut-off value (-0.1) ranged from 0.52 to 0.88. Sensitivity/specificity (%) of milk MAV was 53/82, 46/82, 75/83, 52/83, 49/83, 20/83, and 49/83 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. Area under the curve for milk MAL on our proposed cut-off value ranged from 0.51 to 0.78. Sensitivity/specificity (%) for fat CAL were 61/73, 48/73, 67/73, 35/73, 47/73, 10/73, and 46/73 and for fat MAL were 70/75, 57/75, 67/65, 30/75, 45/75, 19/75, 54/75 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for fat CAL ranged from 0.53 to 0.69 and that for fat HAL ranged from 0.53 to 0.72. The sensitivity/specificity (%) of protein CAL were 42/89, 44/89, 100/89, 22/89, 20/89, 10/89, and 19/89 and the protein HAL were 42/90, 43/90, 100/90, 22/90, 22/90, 10/100, and 22/90 for MAS, MET, HYC, DEP, DIG, LAM, and KET respectively. The AUC values for protein CAL ranged from 0.50-0.95 and the AUC values for protein HAL ranged from 0.51-0.95. The sensitivity/specificity (%) of lactose CAL were 35/95, 17/95, 100/95, 13/95, 8/95, 5/95, and 5/95 and the sensitivity/specificity (%) for lactose HAL were 35/96, 4/96, 100/96, 4/96, 8/95, 5/100 and 5/96 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for lactose CAL ranged from 0.50 to 0.98 and the lactose HAL ranged from 0.50 to 0.98 for different disorders. The sensitivity/ specificity (%) of fat:lactose CAL were 78/59, 57/59, 80/59, 52/59, 58/59, 58/59, and 61/59 and the sensitivity/specificity (%) for fat:lactose HAL were 80/60, 77/60, 80/60, 48/60, 63/60, 58/60 and 71/60 for MAS, MET, HYC, DEP, DIG, LAM, and KET, respectively. The AUC values for fat:lactose CAL ranged from 0.55 to 0.69 and the lactose HAL ranged from 0.54 to 0.72 for different disorders. In conclusion consistent negative changes in rumination time, activity, and milk yield were observed on the day of clinical diagnosis of disease and could be used to assist in the early detection of periparturient cow disorders.

Materials and methods

Animal and Management conditions

The research protocol and the animal care were approved by the University of Florida (UF) animal care and use committee. The animals involved in this study were housed in a free stall barn at the UF Dairy Unit, Gainesville, Florida, US (29° 47' N, 82° 24'W and 52.72 m above sea level). The farm milked approximately 500 Holstein cows milked twice daily with a rolling herd average of approximately 10,000 kg milk/cow. The free stall beds and walking alleys were cleaned twice daily. Twice weekly, clean dry sand was added on the top of free stall beds. Fans with misters and sprinklers over the feed line were present in the barns and activated when environmental temperature was above 18^o C. Dry cows and early lactating cows were kept in separate pen. Cows were dried off 60 days prior to expected calving date, or earlier if production was very low. Dry cows were placed in a far off dry-period pen at dry off. The cows were moved from the far off dry-period pen to a late-pregnancy pen about 15 day d before expected calving. The cows calved in the pen holding late pregnant cows. After parturition, healthy cows were moved to a post-partum pen consisting of free stall facilities with a concrete floor. Cows remained in the post-partum pen until 3-4 weeks after calving when they were moved to an early lactation pen. Sick cows were moved to an open hospital facility with shade and a sand-bedded pack. Fresh potable water and ad libitum feeding was available to all cows on the study. Each pen was equipped with axial-flow fans installed in the barn. Sprinklers over the feedlane sprinklers were placed perpendicular to air-flow fans. The fans and sprinklers were thermostatically controlled, constant management conditions (similar to feed delivery, milking frequency and working routine) and were maintained to provide homogeneity to the experiment. Cows were milked four times a day while in the post-partum pen and then twice daily after they were moved to the early lactation pen (A.M. and P.M. milking time). Milk yield was recorded daily using Afi Milk® meters and milk components were analyzed in line for each cow using the Afi lab system (S.A.E. Afikim; Kibbutz Afikim, Israel). The Afi lab system has been validated for milk yield and components analysis by Karp and Peterson-Wolfe (2010). Prepartum cows were fed a total mixed ration designed to meet or exceed NRC requirements for late gestation cows,a and the ration was formulated to have a negative calculated dietary cation-anion difference by limiting the amount of sodium and potassium in the ration and increasing the amount of supplemental chlorine. Lactating cows in early lactation were fed a total mixed ration designed to meet or exceed NRC requirements for lactating cows at 90 lb/d milk yield. The total mixed ration was formulated to meet or exceed the requirements of lactating cows weighing ~680 kg and producing 45 kg of 3.5% FCM as recommended by the National Research Council. Feed was delivered twice a day and feed was 'pushed up' at least twice a day between each feeding.

Health monitoring program

All cows went through a routine postpartum health monitoring protocol that consisted of clinical evaluation on days 4, 7 and 12 after calving, as performed by trained farm personnel or veterinarians from the UF. The protocol included the assessment of attitude, rectal temperature, rectal palpation, and examination of vaginal discharge, udder inspection, assessment of urine ketone bodies (Ketostix®, Bayer Corporation Elkhart, IN), monitoring of rumen motility, and checking for evidence of abomasum displacement. In addition, automatic health reports were created for every milking event based on individual milk production and milk component levels provided by the AfiMilk® meters (S.A.E. Afikim). Cows with deviations from pre-established ranges on at least two parameters (milk yield and milk components) within two consecutive milkings were automatically sorted for a complete health check.

Study population

The study involved 198 multiparous Holstein cows enrolled during the last month of pregnancy. Cows entered the study from November 2013 to August 2014. Cows were enrolled 15 day prior to the estimated calving date when in the dry off period and remained in the study until 60 days of lactation. The Hr-Tag® rumination monitoring device (HR tag®, previously described and validated by Schirmann et al., (2009) for the use of rumination monitoring) was affixed on the neck (collar) of the cows. Blood was collected 3-5 day post calving. Health of the cows in the study was monitored daily by UF veterinarians and farm staff and any adverse health events were recorded for each cow. The animals were housed in 10 groups (pens). Group 1 contained animals in 25-98 DIM, group 2 contained animals in 35-66 DIM, group 3 contained animals in 30-62 DIM, group 4 contained animals in 0-62 DIM, group 5 contained animals in 2-114 DIM, group 6 contained animals in 21-81 DIM, group 7 contained animals in 0-58 DIM, group 8 contained animals in 0-61 DIM, group 9 contained animals in 0-97 DIM and group 10 contained dry animals before parturition. Parameters obtained from cows were compared with their group mates to obtain the mates index and mates alarm values.

Measurement of rumination and activity data

The Hr-Tag® rumination monitoring system was used for measurement and collection of activity and rumination data. The Hr-Tag® is a herd management system with multiple components including activity and rumination monitoring system. The units are affixed as collars around the neck of animals. The rumination collars included a microphone, a microprocessor, and a transponder that are enclosed into a plastic case (size = 100 mm × 80 mm × 35 mm; weight = 120 g) and fitted onto a nylon collar. The nylon strap is adjustable and fitted with a weight (size = 70 mm × 70 mm × 30 mm; weight = 540 g) that hung below the neck to ensure that the rumination logger retains its position on the left side of the neck. The entire apparatus weighed approximately 920 g. Placement of the rumination logger was approximately 20 cm behind the left ear and 5 to 10 cm ventral on the left side of the neck.

The microphone records rumination and data is stored by the logger in 2 hour increments. The data is downloaded to a computer via a receiving antenna at least once per 24 hours when the cow is in the vicinity of the antenna. The activity data is collected by the sensor and calculated as an activity index for the two hour period which is sent to computer every 2 hour and downloaded every 24 hour period from the software.

The system uses sounds recorded by a built-in microphone to identify regurgitation and rumination. The beginning of a rumination event, as defined by the software, occurred when the system detected the sound associated with regurgitation. As boluses are typically masticated for 30 to 60 s (Beauchemin, 1991), the algorithm considered rumination events separate if successive regurgitations were separated by at least 30 s.

For measurement of activity, the acceleration sensor in the HR-Tag continuously records individual cow activity and calculates a general activity index in “activity units”. According to Elischer et al. (2013) and information from manufacturer, the tag collected only horizontal accelerations related to upward movements of cows head and neck during walking and mounting each other. Vertical movements of neck during eating were not considered. Raw activity data were analyzed in a microprocessor by proprietary algorithms. The data stored in 2-h intervals were read by a wireless receiver and automatically transferred on a real time basis to the herd management software on a farm computer. The 2-h values were arithmetically averaged to one value per day for further analysis.

Collection of milk and milk component data

The AfiFarm® program was used for measurement and collection of milk component data. The AfiFarm® program is a herd management system with multiple components including milk meters and milk component lab at every milking unit in the milking parlor and a scale for body weight measurement in the exit lanes from the milking parlor. AfiFarm® records the cow’s milk production (lb) at each milking. AfiLab® measures and records the cow's milk components (at each milking) including fat %, protein %, lactose %, conductivity and somatic cell count; from these data, the fat to protein ratio is calculated. The total daily milk yield was calculated as sum of AM and PM milking whereas the milk components were the average of the AM and PM milkings.

Serum Concentrations of Ca, NEFA and BHBA

A subset of cows (n=132) were used to investigate the relationship of serum Ca, NEFA and BHBA with rumination activity. Blood samples from the coccygeal vein were collected into evacuated tubes without an anticoagulant (BD vacutainer, Frankling Lakes, NJ) at 12-48 h and 5-7 days after calving. Samples were allowed to clot and were then placed on ice until processing. Within 8 h of collection, samples were centrifuged and serum was harvested and frozen at -20°C until analysis. Serum samples were analyzed for concentration of Ca using an atomic absorption spectrophotometer (A Analyst 200; Perkin-Elmer Inc., Waltham, MA), as previously described (Martinez et al., 2012). Commercial kits were used to determine serum concentrations of NEFA (NEFA-C Kit; Wako Diagnostics Inc., Richmond, Va., as modified by Johnson and Peters, (1993) and BHBA (Wako Autokit 3-HB; Wako Diagnostics Inc.).

Cows were considered in negative energy balance (NEB) if serum NEFA concentration > 0.5 mmol/L; positive for subclinical ketosis (SCK) if serum BHBA ≥ 1.4mmol/L; and hypocalcemic (HYC) if serum Ca concentration was < 8.0 mg/dl (Goff, J.P., 2008; LeBlanc et al., 2005; Chapinal et al., 2011).

Temperature and humidity data

Temperature and humidity data of the study site for the study period was obtained from archives of the weather channel (Weather underground) and accessed via: http://www.wunderground.com/history/airport/KGNV/2013/11/1/CustomHistory.html?dayend=1&monthend=12&yearend=2014&req_city=NA&req_state=NA&req_statename=NA .

Average daily temperature and average daily humidity was used to calculate the temperature humidity index THI for each day. The THI was calculated using the formula developed by Bohmanova et al. (2007):

$$\mathbf{THI = T\ (^{o}F) - (0.55 - (RH/100) \times 0.55) * (T - 58)}$$

A cut off value of 76.2 was considered for categorization of the weather as warm (> 76.2) or cold (< 76.2) based on previous research by Benzaquen et al. (2007) at the same study location.

The daily THI values ranged from 40.99 to 81.73. The greatest THI was recorded in August and the lowest THI was in January. During the overall study period, daily THI was greater than 76.2 for 97 days. The monthly average THI was greater than 76.2 during 4 months of the study period (June, July, August and September).

Data processing and statistical analysis

The rumination logger continuously recorded the duration of rumination in 2-h intervals, and these data were used to calculate the following variables: 1) Total daily RT, by adding the 2-h intervals values recorded from 20:00 to 20:00 h of the next day; 2) Total daily activity, by adding the 2-h intervals values recorded from 20:00 to 20:00 h of the next day. The health status data from the AFIFarm dairy management system indicated the health events of concern (MAS, MET, HYC, DEP, KET, DIG, LAM) for each cow in the study. A cow was considered "sick" if any of the health disorders included in the study occurred.

Two indices were created based on parameter dynamics:

i. CowIndex (CIx) that considered the difference in the affected cow RT between the day of diagnosis (d0) and her daily average RT from d -3 to -5 relative to diagnosis (0vs-3to-5RT) divided by the herself daily average RT from d -3 to -5 (-3to-5RT).

ii. MatesIndex (MIx) that considered the difference between the affected cow 0vs-3to-5RT and the pen mates 0vs-3to-5RT relative to the average d 0 RT for pen mates.

Consequently:

CIx = (d0 vs -3to-5RT) / -3to-5RT;

MIx = (0vs-3to-5RT - pen mates 0vs-3to-5RT)/pen mates d0 RT

Considering an index value of -0.1 as the cut off value for the change in rumination (whenever CIx index was less than -0.1), we set a cow alarm (CAL) and marked the cow as flagged for a health disorder. Similarly, taking an index lower than -0.1 as the cut off value for the change in rumination (whenever the MIx was less than -0.1), we set a Herd alarm (HAL) and marked the cow as flagged for a health disorder. A combined alarm (CombA) was created using the parallel combination of the HAL and CAL. We repeated the analysis to using 0.15 as alternative cutoff to get HAL and CAL respectively. A separate analysis was performed to detect disorders on day prior to disease diagnosis.

The same procedure for calculation of CIx and MIx were applied for milk yield and milk components (fat %, protein %, lactose %, and fat:protein) analysis.

For activity data, CIx and CAL were calculated similar to rumination data but the activity was not compared with the herd mates because of the lack of biological sense of this comparison. The CIx considered activity at d 0 - daily average acivity from d -3 to -5 relative to diagnosis divided by activity from d -3 to -5. The sensitivity and specificity of each cutoff value was determined by relating the diagnosed disease event with change in observed parameters. Sensitivity percentage was calculated as 100 × true positive/ (true positive + false negative) and specificity percentage as 100 × true negative/ (true negative + false positive). Additionally, analysis of the variables was performed dividing the events according to days in milk. Early milking 0-17 DIM, mid milking 17-53 DIM and peak milking >53 DIM were considered for the study.

Statistical analyses were performed using SAS (SAS Inst. Inc., Cary, NC). The FREQ procedure was used to evaluate the sensitivity and specificity of the alarms with respect to the observed clinical disease occurrence in the herd. The GLM procedure was used to evaluate the average indices of rumination with respect to the cow 3 to 5 day before clinical diagnosis. The LOGISTIC procedure was used to estimate the area under curve (AUC) values for the receiver operating characteristic (ROC) analysis. Herd alarm and cow alarm (for activity only cow alarm) generated by the calculation was tested with each other for convergence and compatibility.

Mixed models for repeated measures data were developed for the analysis of 2-h RT at the PRECLV and POSTCLV periods. Regression coefficients and the correlation between time relative to calving and 2-h rumination values was estimated for two periods: Pre calving (PRECLV: -7 to -1 d relative to calving); and post calving (POSTCLV: 1 to 7 d relative to calving) for both affected and healthy cows. In addition, the correlation between serum concentrations of Ca, NEFA or BHBA and total daily RT was measured for the day of blood sample collection.

Results

Of the total 210 cows enrolled, 198 cows successfully completed the study. The 12 cows that were removed from the study either were resistant to the tags or some of the tags stopped working. Blood was collected from only 132 cows within 12-72 hours after calving because of some limitations. Overall, 43 (21.7 %) cows were clinically diagnosed with mastitis, 32 (16.16 %) were clinically diagnosed with metritis, 7 (3.5 %) with milk fever, 32 (16.16 %) with symptoms of depression, dehydration and fever, 64 (32.32 %) with digestive problems, 25 (12.63 %) with foot problems and 45 (22.73 %) with ketosis during the study period. Overall, 136 (68.69 %) suffered from at least one disease mentioned in the study and 74 (37.38 %) healthy meaning they developed no disease in this study period.

Analysis of rumination data

Overall average daily RT of healthy cows pre-partum was 413.6 ± 1.6 min/d. Rumination time increased as lactation progressed, with the average daily RT in healthy milking cows being reduced for 0-17 DIM (458.2 ± 2.5), and for 17-53 DIM (458.3 ± 2.0) compared to 488.1 ± 2.4 min/d for >53 DIM (P <0.001). The average (± SE) 2-h rumination time for the cattle was 47.2 ± 0.5 at 0200 2 am in the morning, 46.7 ± 0.5 at 0400, 28.0 ± 0.5 at 0600, 26.9 ± 0.5 at 0800, 30.8 ± 0.5 at 1000 a.m., 23.4 ± 0.4 at 1200, 29.8 ± 0.5 at 1400, 23.1 ± 0.4 at 1600, 25.1 ± 0.4 at 1800, 38.9 ± 0.5 at 2000, 37.6 ± 0.5 at 2200, and 38.9 ± 0.5 at 0000. Similarly, group 8 had an average (± SE) rumination time of 487.51 ± 5.98, group 6 had 483.39 ± 5.44, group 3 had 480.11 ± 11.59, group 5 had 470.34 ± 3.35, group 1 had 457.4 ± 3.61, group 7 had 449.31 ± 3.99, group 2 had

446.54 ± 10.45, group 9 had 436.35 ± 3.81, group 4 had 409.51 ± 4.2, group 10 had 408.96 ± 2.02.

The average (± SE) daily rumination of cows pre-partum was 412.2 ± 2.78 min/d on the warm weather (THI > 76.2) and was 415.0 ± 1.71 min/d on the cold weather (THI < 76.2). Similarly, the RT of milking cows was lower ($P < 0.001$) i.e. 460.9 ± 1.97 min/d during warm weather and was 475.5 ± 2.29 min/d during cold weather. The average RT of healthy cows for each month of the study was found to be, January (429.2 ± 4.06), February (451.7 ± 3.78), March (451.0 ± 3.84), April (469.4 ± 3.99), May (455.1 ± 4.09), June (473.6 ± 3.83), July (480.7 ± 3.73), August (485.8 ± 3.92), September (483.1 ± 3.14), October (448.5 ± 3.36), November (404.4 ± 33.29), December (461.4 ± 5.33). The daily THI and daily RT of dry cows had correlation coefficient (r) = -0.02 ($P = 0.13$) and the coefficient of determination (r^2) = -0.004 (y = 429.02 - 0.22x; $P = 0.13$). Similarly, the daily THI and daily rumination of milking cows had r = -0.036 ($P < 0.0001$) and r^2 = 0.0012 (y = 435.15 + 0.35x; $P < 0.001$).

The average daily rumination time of the healthy cows was higher (459.17 ±1.73) than the average daily rumination time for sick animals on the day of clinical disease diagnosis (335.22 ± 6.9; $P < 0.0001$). Consistent negative changes in rumination activity were observed for each disease during the previous 5 days of the diagnosis of the disease (Fig. 2).

Results of rumination analysis on the day of diagnosis using -0.1 as cutoff value

The rumination cow alarm (CAL) created using a CIx index of -0.1 as the cutoff value detected 28.01% animals as sick. The CAL had 50% sensitivity and 77.6 % specificity to detect the animal as sick (as affected by any of the study disorders). The CAL was most sensitive to detect milk fever (sensitivity = 100 and specificity = 77.3 %) followed by lameness (66. 7 and 77.3%), ketosis (60.5 and 77.3%), mastitis (56.1 and 77.3%), depression (46. 7 and 77.3%), digestive disorders (43. 6 and 77.3%) and metritis (38.7 and 77.3%; Table 1).

The cow index (CIx) values estimated for sick cows were lower ($P < 0.05$) than those for healthy cows only in the case of sickness (Table 2). The AUC value from the ROC analysis was 0.61. This value was greatest for the milk fever (0.86) followed by lameness (0.69), ketosis (0.66), mastitis (0.64), depression (0.59), digestive disorders (0.57), and metritis (0.55). The herd alarm (HAL) created using a MIx index of -0.1 as the cutoff value detected 28.34% animals as sick. The alarm had 54.0 % sensitivity and 77.1 % specificity in detecting the animal as sick. The HAL method was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 76.7%) followed by ketosis (67.4 and 76.8%), mastitis (63.4 and 76.8%), foot problems (55.6 and 76.7%), depression (50 and 76.7%), digestive (48.4 and 76.8%), and metritis (41.9 and 76.7%) (Table1).

The Mates Index (MIx) was lower ($P < 0.05$) in sick cows than healthy cows with ketosis, digestive disorder, milk fever and mastitis being most prominent. The AUC value from the ROC analysis for the sick animals was 0.63. This value was greatest for milk fever (0.85) followed by ketosis (0.69), mastitis (0.67), lameness (0.63), depression (0.61), digestive disorder (0.60), and metritis (0.56) (Table 2).

A combined alarm (CombA) was created using the parallel arrangement of rumination CAL and rumination HAL. The alarm detected 33.58 % of the animals as sick. This alarm was found to have 56.7 % sensitivity and 66.7 % specificity to detect any illness. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 100 and specificity= 66.4%) and was followed by ketosis (72.1 and 66.5%), foot problems (66.7 and 66.5%), mastitis (63.4 and 66.5%), digestive (50 and 66.5%), depression (50 and 66.5%), and metritis (45.2 and 66.4%). The ROC analysis of the CombA had AUC value of 0.62. The AUC value was greatest for milk fever (0.83) followed by ketosis (0.69), lameness (0.67), mastitis (0.65), digestive (0.58), depression (0.58), and metritis (0.56; Table 1).

Results of rumination analysis on the day of diagnosis using -0.15 as cutoff value

The rumination CAL created for -0.15 as the cutoff value for the CIx detected 20.68 % animals as sick. The alarm had 41.5 % sensitivity and 85.4 % specificity to detect the animal as sick (as affected by any of the study-defined health disorders). The rumination CAL was most sensitive to detect milk fever (sensitivity = 100 and specificity = 77.3%) followed by mastitis (51.2 and 85.1%), ketosis (46.5 and 85.1%), foot problems (44.4 and 85.1 %), digestive disorders (40.3 and 85.1 %) depression (40.0 and 85.1%), and metritis (29.1 and 85.1%; Table 1).

The values estimated for the CIx in sick cows were lower ($P < 0.05$) than those for healthy cows only in the case of sickness (Table 2). The AUC value from the ROC analysis was 0.64. This value was greatest for the milk fever (0.93) followed by mastitis (0.68), ketosis (0.66), lameness (0.65), digestive disorders (0.63), depression (0.63), and metritis (0.57).

The rumination HAL created considering -0.15 as cutoff value for MIx detected 20.65 % animals as sick. This alarm had 44.3 % sensitivity and 85.3 % specificity in detecting the animal as sick. The rumination HAL was most sensitive in detecting milk fever (sensitivity= 100 and specificity= 84.98 %) followed by ketosis (58.1 and 85.1 %), lameness (50 and 85 %), mastitis (48.8 and 85.1 %), digestive (41.2 and 85.1%), depression (40 and 85%), and metritis (20.1 and 85%) (Table1).

The MIx was less ($P < 0.05$) in all cases with ketosis, digestive disorder, milk fever and mastitis being the most prominent. The AUC values from the ROC analysis was 0.64. This value was greatest for milk fever (0.92) followed by ketosis (0.72), lameness (0.68), mastitis (0.67), digestive disorder (0.64), depression (0.63), and metritis (0.57).

A CombA index was created using the parallel combination of CAL and HAL for the -0.15 cutoff value. This alarm detected 24.56 % animals as sick. The CombA alarm was found to have 46.5 % sensitivity to detect the cow as sick and had 81.3 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 100 and specificity = 80.9%) and was followed by ketosis (62.8 and 80.9%), lameness (55.6 and 80.9%), mastitis (51.2 and 80.9%), digestive (41.9 and 80.9%), depression (40 and 80.9%), and metritis (29.1 and 80.9%). The ROC analysis of the CombA had AUC value of 0.63 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.91) followed by ketosis (0.72), lameness (0.68), mastitis (0.66), digestive (0.61), depression (0.60), and metritis (0.55; Table 1).

Results of rumination analysis on the day prior to diagnosis using -0.1 as cutoff value

The rumination CAL created using -0.1 CIx as cutoff value detected 26.37 % animals as sick. The alarm had 38.1 % sensitivity and 79.0 % specificity to detect the animal as sick (as affected by any of the study-defined health disorders). The rumination CAL was most sensitive to detect milk fever (sensitivity = 100 and specificity = 78.8%) followed by mastitis (64.1 and 78.3%), foot problems (44.4 and 78.8%), ketosis (34.1 and 78.8%), depression (33.3 and 78.8%), digestive disorders (32.8 and 78.8%) and metritis (27.6 and 78.8 %; Table 1).

The values estimated for the CIx in cows with milk fever and mastitis only were lower ($P < 0.05$) than those for healthy cows when considering rumination on the day prior to diagnosis (Table 2). The AUC value from the ROC analysis was 0.59. This value was greatest for the milk fever (0.86) followed by mastitis (0.71), foot problems (0.62), digestive disorders (0.58), ketosis (0.56), depression (0.56), and metritis (0.53). The rumination HAL created for -0.1 index as cutoff value for the MIx detected 26.27 % animals as sick. The alarm had 54.0 % sensitivity and 77.1 % specificity in detecting an animal as sick. The rumination HAL was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 78.1%) followed by mastitis (66.7 and 78.2%), ketosis (45.5 and 78.2%), foot problems (44.4 and 78.1%), digestive (42.6 and 78.1%), depression (36.7 and 78.1%), metritis (20.7 and 78.1%; Table 1).

The MIx was lower ($P < 0.05$) in cases with ketosis, digestive disorder, milk fever, and mastitis being most prominent. The area under the curve value from the ROC analysis was 0.63. This value was highest for milk fever (0.85) followed by ketosis

(0.69), mastitis (0.67), foot problems (0.63), depression (0.61), digestive disorder (0.60), and metritis (0.56) (Table 2).

A CombA was created using the parallel combination of CAL and HAL for rumination on the day prior to disease diagnosis. The alarm detected 31.73 % animals as sick. The alarm had 45.7 % sensitivity to detect the cow as sick and had 73.3 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity= 73.1%) and was followed by mastitis (66.7 and 73.1%), ketosis (50 and 73.1%), foot problems (50 and 73.1%), digestive (42.6 and 73.1%), depression (40 and 73.1%), and metritis (31.1 and 73.1%). The ROC analysis of the CombA had AUC value 0.63 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.91) followed by ketosis (0.72), foot problems (0.68), mastitis (0.66), digestive (0.61), depression (0.60), and metritis (0.55).

Results of rumination analysis on day prior to diagnosis using -0.15 as cutoff value

The rumination CAL created for a 15 % drop in rumination with respect to its -3 to -5d average as cutoff value detected 18.9 % animals as sick. The alarm had 38.1 % sensitivity and 83 % specificity to detect the animal as sick (as affected by any of the study defined disorders) on a day ahead of diagnosis. The rumination CAL had greatest sensitivity to detect milk fever (sensitivity= 100 and specificity= 82.8%) followed by mastitis (56.4 and 82.8%), foot problems (44.4 and 82.8%), ketosis (38.6 and 82.8%), depression (33.3 and 82.8%), digestive disorders (36.1 and 82.8%) and metritis (27.6 and 82.8%; Table 1).

The values estimated for the CIx in cows with sick cows, milk fever, mastitis, ketosis, foot problems and digestive disorders were lower ($P < 0.05$) than those for healthy cows (Table 2). The AUC value from the ROC analysis for the sick animal was 0.60. This value was greatest for the milk fever (0.91) followed by mastitis (0.69), foot problems (0.63), digestive disorders (0.59), ketosis (0.60), depression (0.58), and metritis (0.55). The rumination HAL created using -0.15 as cutoff value for the MIx on the day prior to diagnosis detected 19.11 % animals as sick. The alarm had 34.1 % sensitivity and 86.6 % specificity in detecting the animal as sick. The rumination HAL was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 86.4%) followed by mastitis (53.9 and 86.5%), ketosis (34.1 and 86.4%), foot problems (33.3 and 86.4%), digestive (34.4 and 86.4%), depression (30 and 86.4%), metritis (17.2 and 86.4% ; Table1).

The MIx was lower ($P < 0.05$) in cases with sickness, ketosis, digestive disorder, milk fever, and mastitis. The area under the curve values from the ROC analysis for the sick animal was 0.60. This value was highest for milk fever (0.93) followed by mastitis (0.7), ketosis (0.60), digestive disorder (0.60), foot problems (0.59), depression (0.58), and metritis (0.52).

A CombA was created using the parallel combination of CAL and HAL. The CombA detected 22.58 % animals as sick on the day prior to clinician diagnosis using -0.15 as the indexes cutoff value. The alarm was found to have 38.1 % sensitivity to detect the animal as sick and had 83 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity= 82.8%) and was followed by mastitis (56.4 and 82.8%), foot problems (44.4 and 82.8%), ketosis (38.6 and 82.8%), digestive (36.1and 82.8%), depression (33.3 and 82.8%), and metritis (27.6 and 82.8%). The ROC analysis of the CombA had AUC value 0.61 to diagnose a cow as sick. The

AUC value was highest for milk fever (0.91) followed by mastitis (0.70), ketosis (0.61), foot problems (0.64), digestive (0.59), depression (0.58), and metritis (0.55).

Analysis of activity data

The average (± SE) activity for dry cows was 478.8 ± 1.59 units. The average daily activity of milking cows was reduced (*P* < 0.001) during 0-17 DIM (483.2 ± 2.63), intermediate (*P* < 0.001) at 17-53 DIM (521.4 ± 2.12) and lowest (*P* < 0.001) after 53 DIM (497.5 ± 1.85 units). The average (± SE) 2-h activity for the cattle was 29.72 ± 0.23 at 0200 in the morning, 26.96 ± 0.19 at 0400, 36.47 ± 0.33 at 0600, 39.04 ± 0.38 at 0800, 37.91 ± 0.34 at 1000, 41.8 ± 0.33 at 1200, 38.3 ± 0.34 at 1400, 40.9 ± 0.35 at 1600, 41.9 ± 0.36 at 1800, 35.1 ± 0.34 at 2000, 34.9 ± 0.35 at 2200, and 33.9 ± 0.29 units at 0000.

Similarly, group 4 had an average (± SE) activity time of 513.24 ± 4.29, group 2 had 509.33 ± 10.77, group 9 had 508.11 ± 3.92, group 1 had 500.18 ± 3.73, group 3 had 495.84 ± 11.96, group 5 had 492.47 ± 3.45, group 6 had 483.47 ± 5.61 group 7 had 482.45 ± 4.12, group 10 had 475.67 ± 1.99 units, group 8 had 435.77 ± 6.16

The average (± SE) activity of cows pre-partum was 493.1 ± 2.71 during the warm weather (THI > 76.2) and was 464.5 ± 1.67 during the cooler weather (THI < 76.2). Similarly, the activity of milking cows was greater (*P* < 0.001) i.e. 538 ± 2.63 during warm weather and was 494.3 ± 2.25 during the cooler weather. The average (± SE) activity of healthy cows for each month of the study was found to be, January (432.4 ± 4.18), February (452.9 ± 3.89), March (473.3 ± 3.96), April (499.5 ± 4.12), May (508.8 ± 4.21), June (508.9 ± 3.94), July (508.6 ± 3.85), August (534.6 ± 3.39), September (542.6 ± 3.23), October (524.28 ± 3.46), November (463.62 ± 34.32), and December (444.91 ± 5.49). The daily THI and daily activity of dry cows had r = -0.173 (*P* < 0.0001) and r^2 = 0.03 (y = 347.14 + 1.86x, P = 0.001). Similarly, the daily THI and daily activity of milking cows had r = 0.37 (P < 0.0001) and r^2 = 0.14 (y = 224.36 + 3.99x; P < 0.0001).

The average daily activity of the healthy cows was greater (508.5 ± 1.81) than the average daily activity for the sick animals (445.9 ±7.18; P < 0.0001).

Consistent negative changes in activity was observed both on the day of diagnosis and during the 5 days prior to diagnosis for each post-partum disease (Fig. 1).

Results of activity analysis on the day of diagnosis using -0.1 indices as cutoff value

The activity CAL created using -0.1 CIx as cutoff value detected 18.85 % animals as sick. The alarm was had 41.8 % sensitivity and 81.4 % specificity to detect the animal as sick. The activity CAL was most sensitive to detect milk fever (sensitivity = 71.4 and specificity = 81.2%) followed by metritis (50 and 81.2%), ketosis (47.7 and 81.3%), digestive disorder (47.6 and 81.3%), foot problems (44.4 and 81.18%), mastitis (33.3 and 81.18%), and depression (31.3 and 81.17%; Table 3).

The CIx calculated was lower (P < 0.05) than the healthy days in the case of sickness, ketosis, digestive disorders, depression and milk fever only (Table 4). The area under the curve values from the ROC analysis for the sick animal was 0.63. This value was highest for the milk fever (0.76) followed by foot problems (0.70), ketosis (0.65), metritis (0.65), digestive disorders (0.64), mastitis (0.58), depression (0.56; Table 3).

Results of activity analysis on the day of diagnosis using -0.15 as cutoff value

The activity CAL created using -0.15 activity CIx as cutoff value detected 11.85 % animals as sick. The alarm had 33.2 % sensitivity and had 88.5 % specificity to detect the animal as sick on the day of diagnosis. The activity CAL was most sensitive to detect milk fever (sensitivity = 71.4 and specificity = 88.2%) followed by metritis (46.9 and 88.2%), foot problems (38.9 and 88.2%), ketosis (36.6 and 88.2%), digestive disorder (33.3 and 88.3%), mastitis (28.6 and 88.2%), and depression (28.1 and 88.2%; Table 3).

The CIx calculated was significantly lower ($P < 0.05$) than the healthy cows in case of sickness, ketosis, digestive disorders, depression and milk fever only (Table 4). The AUC value from the ROC analysis for the sick animal was 0.62. This value was highest for the milk fever (0.8) followed by foot problems (0.72), ketosis (0.63), metritis (0.68), digestive disorders (0.61), mastitis (0.59), and depression (0.58; Table 3).

Results of activity analysis on a day previous to diagnosis using -0.1 as cutoff value

The activity CAL created using -0.1 activity CIx as cutoff value detected 17.34 % animal as sick. It had 38.4 % sensitivity and 82.9 % specificity to detect the animal as sick on a day ahead of diagnosis. The activity CAL was most sensitive to detect milk fever (sensitivity = 42.9 and specificity = 82.7%) followed by metritis (43.8 and 82.7%), foot problems (33.3 and 82.7%), ketosis (40.9 and 82.7%), digestive disorder (46.1 and 82.8%), mastitis (33.3 and 82.7%), and depression (34.4 and 17.3%; Table 3).

The CIx calculated was lower ($P < 0.05$) than the healthy days in case of sickness, metritis, digestive disorders, depression only (Table 4). The AUC value from the ROC analysis for the sick animal was 0.62. This value was highest for the foot problems (0.67), followed by digestive disorders (0.65), metritis (0.63), ketosis (0.62), milk fever (0.62), mastitis (0.59), and depression (0.58; Table 3).

Results of activity analysis on the day prior to diagnosis using -0.15 as cutoff value

The activity CAL created using -0.15 activity indices as cutoff value detected 10.46 % animals as sick. The alarm had 28.5 % sensitivity and 89.8 % specificity to detect the animal as sick on a day ahead of diagnosis. The activity CAL was most sensitive to detect metritis (40.6 and 89.6%) followed by digestive disorder (36.5 and 89.6%), ketosis (28.5 and 89.6%), milk fever (sensitivity = 28.6 and specificity = 89.5%), foot problems (27.8 and 89.6%), ketosis (29.6 and 89.6%), mastitis (26.2 and 89.6%), depression (25 and 89.6%) (Table 3).

The CIx calculated was significantly lower ($P < 0.05$) than the healthy days in case of sickness, metritis and digestive disorders only (Table 4). The AUC value from the ROC analysis for the sick animal was 0.61. This value was greatest for the foot problems (0.69), followed by digestive disorders (0.64), metritis (0.63), ketosis (0.60), milk fever (0.59), mastitis (0.58), and depression (0.57) (Table 3).

Analysis of milk yield data

The average (± SE) daily milk yield of cows was reduced ($P < 0.0001$) at 0-17 DIM i.e. 75.5 ± 0.79 lb, medium ($P < 0.0001$) at 17-53 DIM was 89.0 ± 0.69 lb and greatest ($P < 0.0001$) at > 53 DIM (92.2 ± 0.73) lb. Similarly, group 8 had an average (± SE) milk yield of 94.24±4.085, group 3 had 94.69 ± 2.58, group 6 yield of 93.52 ± 0.89, group 5 had 91.67 ± 0.37, group 1 yield had 88.07 ± 0.46, group 4 had 88.35 ± 0.496, group 2 had 81.72 ± 3.36, group 9 had 70.05 ± 0.595,and group 7 had 67.82 ± 0.51 lb. The average daily milk yield of milking cows was 85.6 ± 0.73 lb during warm weather (THI > 76.2) and was 85.0 ± 0.71 lb during the cold weather (THI < 76.2); this difference was not statistically significant. The average daily milk yield of healthy cows for each month of the study was found to be, January(88.8 ± 1.02), February (90.9 ± 0.95), March (94.4 ± 0.97), April (88.6 ± 1.03), May (88.0 ± 1.03), June(91.5 ± 0.99), July (88.2 ± 0.96), August (84.9 ± 0.86), September(79.1 ± 0.87), October (81.8 ± 1.11), November(65.4 ± 9.25), and December (90.6 ± 1.22) lb. The daily THI and daily milk yield of milking cows had correlation coefficient(r) = -0.115 ($P < 0.0001$) and the coefficient of determination (r^2) = 0.013 (y = 103.94-0.25x, $P < 0.0001$).

The average daily milk yield of the healthy animals was higher ($P < 0.0001$) i.e. 83.7 ± 0.65 whereas the average daily milk yield of the sick animals was 64.2 ± 1.56 on the day of diagnosis.

Consistent negative changes in total daily milk yield were observed both on the day of diagnosis and during the 5 days previous to diagnosis for each post-partum disease (Fig 3).

Results of daily milk yield analysis on the day of diagnosis using -0.1 as cutoff

The CAL created using -0.1 milk yield indices as cutoff value detected 21.68 % cows to be sick. The alarm had 58.6 % sensitivity and 77.5 % specificity to detect the animal as sick (as affected by any of the study disorders). The milk CAL was most sensitive to detect milk fever (sensitivity = 100 and specificity = 76.9%) followed by foot problems (26.67 and 76.9%), ketosis (60.5 and 77.1%), mastitis (59.4 and 77.1%), depression (59.3 and 77.0%), digestive disorders (61.4 and 77.1%) and metritis (66.7 and 77.1%; Table 5).

The values estimated for the CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, mastitis, milk fever, ketosis and digestive disorders only (Table 6). The AUC value from the ROC analysis was 0.68. This value was greatest for the milk fever (0.88) followed by foot problems (0.52), metritis (0.71), digestive disorders (0.69), ketosis (0.69), mastitis (0.68), and depression (0.68).

The milk HAL created using -0.1 milk index as cutoff value for the milk detected 16.8 % cows to be sick. The alarm had 46.3 % sensitivity and 83 % specificity in detecting the animal as sick. The milk HAL was most sensitive in detecting milk fever (sensitivity = 75 and specificity = 82.5%) followed by mastitis (53.1 and 82.6%), depression (51.9 and 82.6%), digestive (49.1 and 82.8%), ketosis (48.8 and 82.6%), metritis (46.7 and 82.6%), and foot problems (20 and 82.5%; Table 5).

The milk yield MIx was lower ($P < 0.05$) in cases with sickness, ketosis, digestive disorder, milk fever, metritis and mastitis than in healthy cows (Table 6). The AUC value from the ROC analysis for the sick animal was 0.65. This value was greatest for milk fever (0.79) followed by mastitis (0.68), depression (0.67), ketosis (0.66), digestive disorder (0.65), metritis (0.65), and foot problems (0.51).

A CombA was created using the parallel combination of milk yield CAL and HAL. The alarm detected 23.19 % cows as sick. The alarm was found to have 56.6 % sensitivity detect the disease as sick and had 66.7 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity= 66.4%) and was followed by ketosis (72.1 and 66.5%), foot problems (66.7 and 66.5%), mastitis (63.4 and 66.5%), digestive (50 and 66.5%), depression (50 and 66.5%), and metritis (45.2 and 66.4%). The ROC analysis of the milk yield CombA had AUC value of 0.68 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.87) followed by digestive (0.71), metritis (0.71), ketosis (0.68), mastitis (0.68), depression (0.67), and foot problems (0.54; Table 5).

Results of daily milk yield analysis on the day of diagnosis using -0.15 as cutoff

The milk CAL created using -0.15 milk yield CIx as cut off value detected 13.59 % animals as sick. The alarm had 47.8 % sensitivity and 86 % specificity to detect the animal as sick (as affected by any of the study defined disorders). The milk CAL was most sensitive to detect milk fever (sensitivity = 75 and specificity = 85.4 %) followed by foot problems (20 and 85.4%), ketosis (48.8 and 85.5%), mastitis (59.4 and 85.5%), depression (51.9 and 85.5%), digestive disorders (47.4 and 85.6%) and metritis (53.3 and 85.5%; Table 5).

The values estimated for the CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of sickness, mastitis, metritis, milk fever, ketosis and digestive disorders only (Table 6). The AUC value from the ROC analysis for the sick animal was 0.67. This value was highest for the milk fever (0.81) followed by mastitis (0.72), metritis (0.69), depression (0.69), ketosis (0.67), digestive disorders (0.66), and foot problems (0.52).

The milk HAL created using -0.15 milk index as cutoff value for the milk detected 10.32 % animals as sick. The alarm had 35 % sensitivity and 89.4 % specificity in detecting the animal as sick. The milk HAL was most sensitive in detecting milk fever (sensitivity = 75 and specificity = 89 %) followed by mastitis (53.1 and 89.1%), depression (37.1 and 89.1%), digestive (31.6 and 89.1%), ketosis (34.9 and 89.1%), metritis (36.7 and 89.1%), foot problems (13.3 and 89%; Table 5).

The milk MIx was significantly low ($P < 0.05$) than the healthy cows in cases with sickness, ketosis, digestive disorder, milk fever, metritis and mastitis (Table 6). The area under the curve values from the ROC analysis for the sick animal was 0.67. This value was highest for milk fever (0.8) followed by mastitis (0.72), metritis (0.71), depression (0.68), ketosis (0.67), digestive disorder (0.67), and foot problems (0.52).

A CombA was created using the parallel combination of CAL and HAL. The alarm detected 14.53 % animals as sick. The alarm was found to have 48.8 % sensitivity to detect the disease as sick and had 85.1 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 75 and specificity= 84.5%) and was followed by ketosis (48.8 and 84.6%), foot problems (20 and 84.5%), mastitis (59.4 and 84.6%), digestive (49.1 and 84.7%), depression (51.9 and 84.6%), and metritis (56.7 and 84.6%). The ROC analysis of the milk CombA had AUC value of 0.67 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.80) followed by digestive (0.67), metritis (0.71), ketosis (0.67), mastitis (0.72), depression (0.68), and foot problems (0.52; Table 5).

Results of daily milk yield analysis on day previous to diagnosis using -0.1 as cutoff

The milk yield CAL created using -0.1 milk index as cut off value detected 18.74 % animals as sick. The alarm had 42.44 % sensitivity and 80.63 % specificity to detect the animal as sick (as affected by any of the study defined disorders) on a day ahead of the diagnosis. The milk CAL was most sensitive to detect metritis (sensitivity = 48.3 and specificity = 80.3%) followed by mastitis (47.1 and 80.3%), ketosis (45.5 and 80.35%), digestive disorders (44.6 and 80.4%), depression (37.1 and 80.3%), foot problems (20 and 80.3%), and milk fever (20 and 80.3%; Table 5).

The values estimated for the milk CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of sickness, mastitis, and ketosis only (Table 6). The AUC value from the ROC analysis for the sick animal was 0.62. This value was highest for milk fever (0.50) followed by mastitis (0.64), metritis (0.64), depression (0.59), ketosis (0.63), digestive disorders (0.63), and foot problems (0.50).

The milk yield HAL created using -0.1 milk index as cutoff value for the milk detected 15.27 % animals as sick. The alarm had 31.2 % sensitivity and 84.5 % specificity in detecting the animal as sick on day previous to diagnosis. The milk yield HAL was most sensitive in detecting milk fever (sensitivity= 40 and specificity= 84.2%) followed by mastitis (35.3 and 84.2%), depression (25.9 and 84.2%), digestive (30.4 and 84.3%), ketosis (38.6 and 84.3%), metritis (27.6 and 84.2%), and foot problems (13.3 and 84.2%; Table 5).

The milk MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, ketosis, digestive disorder, milk fever, and mastitis. The AUC value from the ROC analysis for the sick animal was 0.62. This value was highest for metritis (0.65) followed by mastitis (0.63), ketosis (0.62), milk fever (0.59), depression (0.58), and foot problems (0.51).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 20.83 % animals as sick. The alarm was found to have 45.4 % sensitivity to detect the disease as sick and had 78.64 % specificity. On the individual disorders the sensitivity was highest for digestive (sensitivity = 50 and specificity= 78.4%) and was followed by ketosis (45.6 and 78.3%), foot problems (20 and 78.2%), mastitis (47.1 and 78.3%), milk fever (40 and 78.3%), depression (37 and 78.3%), and metritis (51.7 and 78.3%). The ROC analysis of the milk comb alarm had AUC value of 0.62 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.59) followed by digestive (0.64), metritis (0.65), ketosis (0.61), mastitis (0.63), depression (0.58), and foot problems (0.51).

Results of daily milk yield analysis on day prior to diagnosis using -0.15 as cutoff

The milk yield CAL for the day prior to diagnosis created using -0.15 milk yield CIx as cut off value detected 11.36 % animals as sick. The alarm had 36.1 % sensitivity and 88.3 % specificity to detect the animal as sick (as affected by any of the study defined health disorders) on a day ahead of the diagnosis. The milk yield CAL was most sensitive to detect mastitis (sensitivity = 44.1 and specificity = 88.1%) followed by metritis (41.4 and 88.0%), digestive disorders (37.5 and 88.1%), ketosis (34.1and 88.0%), depression (33.3 and 88%), foot problems (20 and 88%), and milk fever (20 and 88%; Table 5).

The values estimated for the milk yield CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, mastitis, and ketosis only (Table 6). The AUC value from the ROC analysis for the sick animal was 0.62. This value was greatest for mastitis (0.66) followed by, metritis (0.65), digestive disorders (0.63), depression (0.61), ketosis (0.61), foot problems (0.54), and milk fever (0.53).

The milk HAL created using -0.15 milk index as cutoff value detected 8.78 % animals as sick. The alarm had 21 % sensitivity and 91 % specificity in detecting the animal as sick on the day previous to diagnosis. The milk HAL was most sensitive in detecting milk fever (sensitivity = 40 and specificity = 91%) followed by mastitis (29.4 and 90.8%), ketosis (25 and 90.8%), digestive (17.9 and 90.8%), depression (14.8 and 90.8%), metritis (13.8 and 90.8%), foot problems (13.3 and 90.8%; Table 5).

The milk yield MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, ketosis, digestive disorder, milk fever, and mastitis. The AUC value from the ROC analysis for the sick animal was 0.56. This value was highest for milk fever (0.65) followed by mastitis (0.60), ketosis (0.58), metritis (0.53), depression (0.53), and foot problems (0.52).

A combined alarm (CombA) was created using the parallel combination of milk yield CAL and HAL. The milk combalarm detected 12.49 % animals as sick. The alarm was found to have 37.1% sensitivity to detect the disease as sick and had 87.2 % specificity. On the individual disorders the sensitivity was highest for mastitis (sensitivity = 44.1 and specificity= 86.9 %) and was followed by ketosis (34.1 and 86.8%), foot problems (20 and 86.8%), digestive (39.3 and 86.9%), milk fever (40 and 86.8%), depression (33.3 and 86.8%), and metritis (41.4 and 86.6%). The ROC analysis of the milk yield CombA had AUC value 0.62 to diagnose a cow as sick. The AUC value was greatest for mastitis (0.66) followed by metritis (0.64), milk fever (0.64), digestive (0.63), ketosis (0.61), depression (0.60), and foot problems (0.54).

Analysis of milk fat data

Milk fat percentage was highest ($P < 0.0001$) for cows at 0-17 DIM (4.22 ± 0.02 %), intermediate at 17-53 DIM (3.7 ± 0.01 %) and lowest ($P < 0.001$) at >53 DIM i.e. 3.5 ± 0.01%.

Similarly, group 8 had average percentage milk fat 4.001 ± 0.0133, group 5 had 3.87 ± 0.017, group 4 had 3.85 ± 0.104, group 3 had 3.81±0.0137, group 1 had 3.77 ± 0.01, group 9 had 3.75 ± 0.01, group 7 had 3.74 ± 0.066, group 2 had 3.64 ± 0.024 and group 6 had 3.41 ± 0.085.

The percentage milk fat of milking cows was reduced (*P* < 0.0001) (3.69 ± 0.019) during warm weather (THI > 76.2) and was higher (3.82 ± 0.018) during the cold weather (THI < 76.2). The average milk fat percentage of healthy cows for each month of the study was found to be, January(3.8 ± 0.027), February (3.9 ± 0.025), March (3.9 ± 0.025), April (3.9 ± 0.026), May (3.9 ± 0.027), June(3.9 ± 0.027), July (3.9 ± 0.026), August (3.7 ± 0.025), September(3.7 ± 0.023), October (3.6 ± 0.029), November(3.3 ± 0.235), and December (3.8 ± 0.033). The daily THI and average percentage daily milk fat of milking cows had r = -0.087 (*P* < 0.0001) and r^2 =0.0075 (y=4.0675-0.005x, *P* < 0.0001). The average daily milkfat percentage of the healthy animals was lower (3.7 ± 0.017) than the average daily milk fat percentage of the sick cows (4.3 ± 0.045; *P* < 0.0001) on the day of diagnosis.

The average daily milk fat percentage tended to increase in the in the sick animals when over the 5 days prior to diagnosis, and then significantly decreased from the day prior to diagnosis to the day of diagnosis (Fig 4).

Results of milk fat analysis on the day prior to diagnosis of disease using ±0.1 as cutoff

The milkfat CAL created using ±0.1 milkfat index as cutoff value detected 27.37 % cows as sick. The alarm had 43.2 % sensitivity and 72.8 % specificity to detect the animal as sick (as affected by any of the study-defined health disorders). The milkfat CAL was most sensitive to detect milk fever (sensitivity = 66.7 and specificity = 72.6%) followed by mastitis (60.9 and 72.7%), metritis (47.8 and 72.6%), digestive disorders (47.1 and 72.7%), ketosis (46 and 72.6%), depression (34.8 and 72.6%), and foot problems (9.1 and 72.6%; Table 7).

The values estimated for the milkfat CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of milk fever, digestive problems, and sickness only (Table 8). The AUC value from the ROC analysis for the sick animal was 0.58. This value was greatest for the milk fever (0.7) followed by mastitis (0.67), metritis (0.60), digestive disorders (0.60), foot problems (0.59), ketosis (0.59), and depression (0.54).

The milk HAL created using ±0.1 milkfat index as cutoff value for the milkfat detected 24.95 % animals as sick. The alarm had 45.6 % sensitivity and 74.9 % specificity in detecting the animal as sick. The milk HAL was most sensitive in detecting mastitis (sensitivity= 69.6 and specificity= 74.7%) followed by milk fever (66.7 and 74.6%), metritis (56.5 and 74.6%), ketosis (54.1 and 74.7%), digestive (45.1 and 74.7%), depression (30.4 and 74.6%), foot problems (18.2 and 74.6%; Table 7).

The milkfat MIx was reduced ($P < 0.05$) in cases with sickness, digestive disorder, and milk fever than with the healthy cows. The area under the curve values from the ROC analysis for sick animals was 0.60. This value was highest for mastitis (0.72) followed by milk fever (0.70), ketosis (0.64), digestive disorder (0.60), metritis (0.61), depression (0.53), and foot problems (0.53).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The milkfat CombA marked 23.19 % animals as sick. The alarm was found to have 48.5 % sensitivity and had 68.5 % specificity to detect the diseased animal as sick. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 66.7 and specificity = 68.3%) and was followed by metritis (66.7 and 68.3%), ketosis (56.8 and 68.3%), mastitis (47.8 and 68.3%), digestive (47.1 and 68.3%), depression (39.1 and 68.3%), and foot problems (18.2 and 68.2%). The ROC analysis of the milk fat CombA had AUC value of 0.58 to diagnose a cow as sick. The AUC value was greatest for mastitis (0.69) followed by milk fever (0.67), ketosis (0.63), metritis (0.62), digestive (0.58), foot problems (0.57), and depression (0.54).

Results of daily milk fat percentage analysis on the day of diagnosis using ±0.15 as cutoff

The milkfat CAL created using ±0.15 milk fat indices as cut off value detected 12.44 % animals as sick. The alarm had 21.9 % sensitivity and had 87.4 % specificity to detect the animal as sick (if affected by any of the study disorders). The milkfat CAL was most sensitive to detect milk fever (sensitivity= 67.7 and specificity= 87.3%) followed by mastitis (39.1 and 87.3%), digestive disorders (29.4 and 87.4%), depression (17.4 and 87.3%), metritis (13.1 and 87.3%), ketosis (10.8 and 87.3%), and foot problems (9.1 and 87.3%; Table 7).

The values estimated for the milkfat CIx in sick cows were reduced ($P < 0.05$) than those for healthy cows in the case of sickness, milk fever, and digestive disorders only (Table 8). AUC value from ROC analysis for the sickness was 0.55. This value was greatest for the milk fever (0.77) followed by mastitis (0.63), digestive disorders (0.58), depression (0.52), foot problems (0.52), ketosis (0.51), and metritis (0.50).

The milkfat HAL created using -0.15 or +0.15 milkfat MIx as cutoff value for the milk detected 11.16 % animals as sick. The alarm had 31.4 % sensitivity and 88.7 % specificity in detecting the animal as sick. The milkfat herd alarm was most sensitive in detecting milk fever (sensitivity = 66.7 and specificity = 88.4%) followed by mastitis (47.8 and 88.5%), depression (26.1 and 88.5%), digestive (37.3 and 88.5%), ketosis

(10.81 and 87.28%), metritis (13.1 and 87.3%), and foot problems (9.1 and 87.3%; Table 7).

The milkfat MIx was reduced ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, and milk fever only (Table 8). The AUC values from the ROC analysis was 0.60. This value was highest for milk fever (0.77) followed by mastitis (0.68), metritis (0.57), depression (0.57), ketosis (0.61), digestive disorder (0.63), and foot problems (0.51).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL for the +/- 0.15 cutoff values. The milkfat CombA detected 14.52 % animals as sick. The alarm was found to have 33.7 % sensitivity to detect the animal as sick and had 85.4 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 66.7 and specificity = 85.1%) and was followed by mastitis (52.2 and 85.1%), digestive (41.2 and 85.2%), ketosis (35.2 and 85.2%), depression (26.1 and 85.1%), metritis (26.1 and 85.1%) and foot problems (9.1 and 85.1%). The ROC analysis of the milkfat comb alarm had AUC value of 0.60 to diagnose a cow as sick. The AUC value was highest for milk fever (0.76) followed by mastitis (0.69), digestive (0.63), ketosis (0.60), metritis (0.56), depression (0.56), and foot problems (0.53; Table 7).

Results of daily milkfat analysis on the day prior to diagnosis using ±0.1 as cutoff

The milkfat CAL created using ±0.1 as cutoff as cut off value detected 25.05 % animals as sick. The alarm had 44.5 % sensitivity and 75.2 % specificity to detect the animal as sick (as affected by any of the study disorders) a day ahead of the diagnosis. The milkfat CAL was most sensitive to detect metritis (sensitivity = 46.2 and specificity = 75%) followed by mastitis (56 and 75%), ketosis (42.1 and 74.9%), digestive disorders

(43.1 and 75%), depression (41.7 and 75%), foot problems (33.3 and 75%), and milk fever (40 and 75%; Table 7).

The values estimated for the milk CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, digestive disorders, mastitis, and ketosis only (Table 8). The area under the curve values from the ROC analysis was 0.60. This value was highest for the mastitis (0.65) followed by, metritis (0.61), depression (0.59), ketosis (0.59), digestive disorders (0.59), milk fever (0.57), and foot problems (0.54).

The milkfat HAL created using ±0.1 milkfat index as cutoff value for the milkfat detected 23.12 % animals as sick a day ahead of diagnosis. The milkfat HAL had 48 % sensitivity and 77 % specificity. The milkfat HAL was most sensitive in detecting milk fever (sensitivity = 60 and specificity = 76.5%) followed by mastitis (56 and 76.6%), depression (33.3 and 76.5%), digestive (43.1 and 76.6%), ketosis (50 and 76.6%), metritis (57.7 and 76.6%), foot problems (33.3 and 76.5%; Table 7).

The milkfat MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, ketosis, digestive disorder, and mastitis. The AUC value from the ROC analysis for the sick animal was 0.63. This value was greatest for metritis (0.67) followed by milk fever (0.68), mastitis (0.66), ketosis (0.63), depression (0.55), and foot problems (0.55).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 28.73 % as sick on the day prior to diagnosis. The alarm was found to have 52.1 % sensitivity to detect the disease as sick and had 71.31 % specificity. On the individual disorders the sensitivity was greatest for metritis (sensitivity = 65.4 and specificity = 71.1%) and was followed by milk fever (60 and

71%), ketosis (50 and 71.1%), mastitis (56 and 71%), digestive (50 and 71.1%), depression (46 and 71%), and foot problems (33.3 and 71%). The ROC analysis of the milkfat combA had AUC value of 0.62 to diagnose a cow as sick. The AUC value was greatest for metritis (0.68) followed by milk fever (0.65), mastitis (0.65), ketosis (0.61), digestive (0.6), depression (0.58), and foot problems (0.52).

Results of daily milkfat analysis on the day prior to diagnosis using ±0.15 cutoff

The milkfat CAL created using ±0.15 milkfat index as cut off value detected 11.11 % animals as sick. The alarm had 27.8 % sensitivity and 89 % specificity to detect the animal as sick (as affected by any of the study defined health disorders) on a day ahead of the diagnosis. The milkfat CAL was most sensitive to detect mastitis (sensitivity = 44 and specificity = 88.8%) followed by metritis (30.8 and 88.8%), ketosis (29 and 88.8%), digestive disorders (29.4 and 88.8%), depression (16.7 and 88.8%), foot problems (16.7 and 88.8%), and milk fever (20 and 88.8%; Table 7).

The values estimated for the milkfat CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of sickness, digestive disorders, mastitis, and ketosis only (Table 8). The area under the curve values from the ROC analysis for the sick animal was 0.58. This value was highest for mastitis (0.66) followed by, metritis (0.60), ketosis (0.59), digestive disorders (0.59), milk fever (0.55), depression (0.53), and foot problems (0.53).

The milkfat HAL created for ±0.15 milkfat index as cutoff value for the milk detected 10.23 % animals as sick. The alarm had 27.8 % sensitivity and 89.7 % specificity in detecting the animal as sick a day ahead of the diagnosis. The milkfat HAL was most sensitive in detecting mastitis (sensitivity = 40 and specificity = 89.4%)

followed by ketosis (34.2 and 89.5%), digestive (27.5 and 89.5%), metritis (26.9 and 89.46%), milk fever (20 and 89.4%) depression (20.8 and 89.4%), and foot problems (16.7 and 89.4%; Table 7).

The milkfat MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, ketosis, digestive disorder, and mastitis. The AUC value from the ROC analysis for the sick animal was 0.58. This value was greatest for mastitis (0.64) followed by ketosis (0.62), metritis (0.58), milk fever (0.54), depression (0.55), and foot problems (0.53).

A combined alarm (CombA) was created using the parallel combination of milkfat CAL and HAL. The alarm detected 12.49 % animals as sick a day ahead of clinician diagnosis. The alarm was found to have 31.2 % sensitivity to detect the disorders and had 86.8 % specificity. On the individual disorders the sensitivity was highest for mastitis (sensitivity = 44 and specificity= 86.6%) and was followed by metritis (38.5 and 86.6%), ketosis (34.2 and 86.6%), digestive (31.4 and 86.6%), depression (20.8 and 86.6%), milk fever (20 and 86.6%), and foot problems (16.7 and 86.6%). The ROC analysis of the milk-fat CombA had AUC value of 0.59 to diagnose a cow as sick. The AUC value was highest for mastitis (0.65) followed by metritis (0.62), ketosis (0.61), digestive (0.59), milk fever (0.53), depression (0.53), and foot problems (0.52).

Analysis of milk protein data

The average percentage milkprotein of cows at 0-17 DIM was greater (3.33 ± 0.01) compared to 17-53 DIM (3.11 ± 0.01) and > 53 DIM (3.12 ± 0.01; $P < 0.0001$).

Similarly, group 9 had average percentage milkprotein of 3.29 ± 0.008, group 7 had 3.25 ± 0.007, group 6 had 3.19 ± 0.012, group 5 had 3.16 ± 0.047, group 3 had 3.15 ± 0.484, group 1 had 3.15 ± 0.006, group 4 had 3.13 ± 0.006, group 8 had 3.12 ± 0.048, group 2 had 3.03 ± 0.039,

The percentage milkprotein of milking cows was higher ($P < 0.0001$) i.e. 3.178 ± 0.01 during warm weather (THI > 76.2) than during cooler weather (3.15 ± 0.0094). The average (± SE**)** milk fat percentage of healthy cows for each month of the study was found to be, January(3.1 ± 0.01), February (3.2 ± 0.01), March (3.2 ± 0.01), April (3.1 ± 0.01), May (3.2 ± 0.01), June(3.2 ± 0.01), July (3.2 ± 0.01), August (3.2 ± 0.01), September(3.2 ± 0.01), October (3.2 ± 0.02), November(3.9 ± 0.1),and December (3.1 ± 0.02). The daily THI and average percentage daily milk protein of cows had r = 0.068 ($P < 0.0001$) and $r^2 = 0.0045$ (y = 3.03 + 0.00163x, $P < 0.0001$).

The average daily milkprotein percentage of the healthy cows was reduced (3.19 ± 0.009) compared to the average daily milk protein percentage of the sick animals (3.26 ± 0.023; $P = 0.0008$) on the day of diagnosis.

A consistent increase in average daily milk protein percentage was observed both on the day of diagnosis and day previous to diagnosis for each post-partum disease (Fig 5).

Results of average milkprotein analysis on the day of diagnosis using ±0.1 as cutoff

The milkprotein CAL created using ±0.1 as cutoff value detected 11.04 % animals as sick. The alarm had 23.7 % sensitivity and 89.3 % specificity to detect the animal as sick (as affected by any of the study defined health disorders). The milkprotein CAL was most sensitive to detect milk fever (sensitivity= 100 and specificity = 89.1%) followed by metritis (43.5 and 89.2%), mastitis (41.7 and 89.2%), depression (21.7 and 89.1%), digestive disorders (20 and 89.2%), ketosis (18.9 and 89.2%), and foot problems (9.1 and 89.1%; Table 9).

The values estimated for the milkprotein CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of milk fever, metritis, digestive problems, and sickness only (Table 10). The AUC value from the ROC analysis for the sick animal was 0.56. This value was greatest for the milk fever (0.94) followed by metritis (0.66), mastitis (0.65), digestive disorders (0.55), depression (0.55), ketosis (0.54), and foot problems (0.51). The milk protein HAL created using ±0.1 milkprotein indices as cutoff value for the milkprotein detected 10.1 % animals as sick. The alarm had 23.7 % sensitivity and 90.4 % specificity in detecting the cow as sick. The milk protein HAL was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 90.2 %) followed by mastitis (41.7 and 90.3%), metritis (43.5 and 90.3%), ketosis (21.6 and 90.2%), digestive (22 and 90.3%), depression (21.7 and 90.2%), foot problems (9.8 and 100%; Table 9).

The milk protein MIx was significantly lower ($P < 0.05$) in cases with sickness, digestive disorder, milk fever than with the healthy cows. The AUC value from the ROC analysis for the sick animal was 0.57. This value was highest for milk fever (0.95) followed by mastitis (0.66), metritis (0.61), digestive disorder (0.60), ketosis (0.56), depression (0.53), and foot problems (0.53).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 13.05 % animals as sick. The alarm was found to have 25.4 % sensitivity and 87.4 % specificity to detect the diseased animal as sick. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity = 87.3%) and was followed by metritis (43.5 and 87.3%), ketosis (21.6 and 87.3%), mastitis (41.7 and 87.3%), digestive (24 and 87.3%), depression (21.8 and 87.3%), and foot problems (9.1 and 87.2%). The ROC analysis of the milkprotein CombA had AUC value of 0.56 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.93) followed by mastitis (0.65), ketosis (0.54), metritis (0.65), digestive (0.56), foot problems (0.52), and depression (0.55).

Results of daily milk protein analysis on the day of diagnosis using ±0.15 as cutoff

The milk protein CAL created using ±0.15 milk protein indices as cut off value detected 4.26 % animals as sick. The alarm had 11.8 % sensitivity and had 95.7 % specificity to detect the animal as sick (if affected by any of the study defined health disorders). The milk protein CAL was most sensitive to detect milk fever (sensitivity = 100%, specificity = 95.7%) followed by metritis (30.4 and 95.7%), mastitis (20.8 and 95.7%), depression (13.1 and 95.6%), ketosis (8.1 and 95.6%), digestive disorders (8 and 95.6%), and foot problems (4.4 and 100%; Table 9).

The values estimated for the milk protein CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of sickness, milk fever, metritis and digestive disorders only (Table 10). Area under the curve values from ROC analysis for the sickness was 0.54. This value was greatest for the milk fever (0.98) followed by metritis (0.63), mastitis (0.58), depression (0.54), digestive disorders (0.52), foot problems (0.52), and ketosis (0.52).

The milkprotein HAL created using ±0.15 milk protein indices as cutoff value detected 3.69 % animals as sick. The alarm had 10.7 % sensitivity and 96.4 % specificity in detecting the animal as sick. The milk protein HAL was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 96.3%) followed by metritis (26.1 and 96.3%), mastitis (25 and 96.3%), depression (8.7 and 96.3%), ketosis (8.1 and 96.3%), digestive (6 and 96.3%), foot problems (3.7 and 100%; Table 9).

The milk protein MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, metritis and milk fever only (Table 10). The area under the curve values from the ROC analysis for the sick animal was 0.53. This value was highest for milk fever (0.98) followed by mastitis (0.58), metritis (0.63), depression (0.54), ketosis (0.52), digestive disorder (0.52), and foot problems (0.52).

A combined alarm (CombA) was created using the parallel combination (OR-combination) of milk protein CAL and HAL. The alarm detected 4.74 % animals as sick. The alarm was found to have 13.6 % sensitivity to detect the animal as sick and had 95.3 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity = 95.2%) and was followed by metritis (34.8 and 95.2%), mastitis (25 and 95.2%), depression (17.4 and 95.2%), ketosis (10.2 and 95.2%), digestive (8 and 95.2%), and foot problems (4.8 and 100%). The ROC analysis of the milk protein CombA had AUC value of 0.54 to diagnose a cow as sick. The AUC value was highest for milk fever (0.97) followed by metritis (0.65), mastitis (0.60), depression (0.56), ketosis (0.53), foot problems (0.53), and digestive disorders (0.52).

Results of daily milk protein analysis on day prior to diagnosis using ±0.1 as cutoff

The milk protein CAL created for ±0.1 as cut off value detected 9.62 % animals as sick. The alarm had 25.7 % sensitivity and 91.0 % specificity to detect the animal as sick (as affected by any of the study defined health disorders) on a day ahead of the diagnosis. The milk protein CAL was most sensitive to detect metritis (sensitivity = 80 and specificity = 90.8%) followed by mastitis (36 and 75%), ketosis (21.6 and 90.8%), digestive disorders (24 and 90.8%), depression (8.3 and 90.8%), foot problems (33.3 and 90.8%), and milk fever (80 and 90.8%; Table 9).

The values estimated for the milk protein CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, digestive disorders, mastitis, metritis, and milk fever only (Table 10). The AUC value from the ROC analysis for the sick animal was 0.58. This value was highest for the milk fever (0.85), followed by mastitis (0.63), metritis (0.62), foot problems (0.62), digestive disorders (0.57), ketosis (0.56), and depression (0.50).

The milk protein HAL created using ±0.1 milk protein indices as cutoff value detected 8.96 % animals as sick on the day prior to diagnosis. The alarm had 22.2 % sensitivity and 91.6 % specificity. The milk protein herd alarm (HAL) was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 91.4 %) followed by mastitis (32 and 91.5%), metritis (26.9 and 91.5%), foot problems (25 and 91.5%) depression (12.5 and 91.4%), ketosis (18.9 and 91.5%), and digestive (14 and 91.5%; Table 10).

The milk protein MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, metritis, milk fever, digestive disorder, and mastitis. The area under the curve values from the ROC analysis was 0.57. This value was highest for mastitis (0.62) followed by metritis (0.59), milk fever (0.58), foot problems (0.58), ketosis (0.55), and depression (0.52).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 11.26 % animals as sick on the day prior to diagnosis. The alarm was found to have 27.5 % sensitivity and had 89.5 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity= 89.3%) and was followed by metritis (38.5 and 89.3%), mastitis (36 and 89.3%), foot problems (33.3 and 89.3%), ketosis (24.3 and 89.3%), digestive (24 and 89.3%), and depression (12.5 and 89.2%). The ROC analysis of the milk protein CombA had AUC value 0.59. The AUC value was highest for milk fever (0.94) followed by metritis (0.63), mastitis (0.63), foot problems (0.61), ketosis (0.57), digestive (0.57), and depression (0.51).

Results of daily milk protein analysis on the day prior to diagnosis ±0.15 as cutoff

The milk protein CAL created using ±0.15 milk protein CIx as cut off value detected 3.30 % animals as sick (as affected by any of the study defined health disorders) a day ahead of diagnosis. The alarm had 9.4 % sensitivity and 96.8 % specificity. The milk protein CAL was most sensitive to detect milk fever (sensitivity= 40 and specificity= 96.7%) followed by mastitis (20 and 96.7%), digestive disorders (12 and 96.7%), depression (8.3 and 96.7%), foot problems (8.3 and 96.7%), metritis (7.7 and 96.7%) and ketosis (2.7 and 96.7%; Table 9).

The values estimated for the milk protein CIx in sick cows were significantly lower (P < 0.05) than those for healthy cows in the case of sickness, digestive disorders, metritis, mastitis, and milk fever only (Table 10). The area under the curve values from the ROC analysis was 0.53. This value was highest for milk fever (0.68) followed by mastitis (0.58), digestive disorders (0.54), metritis (0.52), depression (0.52), foot problems (0.52), and ketosis (0.50).

The milk protein HAL created using ±0.15 milkprotein MIx as cutoff value detected 3.01 % animal as sick. The alarm had 8.8 % sensitivity and 97.1 % specificity in detecting the animal as sick on a day ahead of the diagnosis. The milkprotein HAL was most sensitive in detecting milk fever (sensitivity = 40 and specificity = 96.1%) followed by mastitis (24 and 96.2%), digestive (12 and 96.1%), metritis (11.5 and 96.1%), depression (8.3 and 96.1%), foot problems (8.3 and 96.1%), and ketosis (2.7 and 96.1%; Table 9).

The milk protein MIx was significantly lower (P < 0.05) than the healthy cows in cases with sickness, digestive disorder, milk fever, metritis, and mastitis. The area under the curve values from the ROC analysis for the sick animal was 0.53. This value was highest for milk fever (0.69) followed by mastitis (0.59) metritis (0.54), depression (0.53), foot problems (0.53), and ketosis (0.50).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 3.88 % animal as sick a day prior to diagnosis. The alarm was found to have 10.5 % sensitivity to detect the disease as sick and had 96.2 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 40 and specificity = 96.2%) and was followed by mastitis (24 and 96.2%), metritis (11.5 and 96.1%), digestive (12 and 96.1%), foot problems (8.3 and 96.1%), depression (8.3 and 96.1%), and ketosis (2.7 and 96.1%). The ROC analysis of the milk protein CombA had AUC value of 0.53 to diagnose a cow as sick. The AUC value was highest for milk fever (0.68), followed by mastitis (0.60), metritis (0.53), digestive (0.54), depression (0.52), foot problems (0.52), and ketosis (0.51).

Analysis of milklactose data

The average percentage milklactose of milking animals at 0-17 DIM was lowest ($P < 0.0001$) i.e. 4.54 ± 0.007, at 17-53 DIM was 4.68 ± 0.0043 and highest ($P < 0.001$) at > 53 DIM i.e. 4.69 ± 0.0057.

Similarly, Group 5 had average percentage milklactose of 4.87 ± 0.005, group 4 had 4.72 ± 0.007, group 1 had 4.66 ± 0.007, group 9 had 4.62 ± 0.01, group 8 had 4.56 ± 0.057, group 6 had 4.41 ± 0.013, group 7 had 4.35 ± 0.008, group 2 had 4.32 ± 0.046, and group 3 had 4.04 ± 0.036.

The percentage milk lactose of milking cows was reduced (4.464 ± 0.01) during warm weather (THI > 76.2) than during cooler weather (4.54 ± 0.0098) ($P < 0.0001$). The average milk lactose percentage of healthy cows for each month of the study was found to be, January (4.89 ± 0.012), February (4.87 ± 0.0113), March (4.82 ± 0.011), April (4.785 ± 0.0117), May (4.73 ± 0.0119), June(4.72 ± 0.0116), July (4.58 ± 0.011), August (4.47 ± 0.0102), September(4.22 ± 0.01), October (4.116 ± 0.013), November (4.33 ± 0.11), December (4.89 ± 0.015). The daily THI and average percentage daily milk lactose of milking cows had correlation coefficient(r) = -0.449 ($P < 0.0001$) and the coefficient of determination (r^2) = 0.202 (y = 5.73-0.015x, $P < 0.0001$).The average daily milk lactose percentage of the healthy animals was higher (4.48 ± 0.01) compared to the average daily milk lactose percentage of the sick animals (4.38 ± 0.025; $P < 0.0001$) on the day of diagnosis.

Consistent negative changes in average daily percentage milk lactose were observed on the day of diagnosis and 5 days previous to diagnosis for each post-partum disease (Fig 6).

Results of average milklactose analysis on the day of diagnosis using ±0.1 as cutoff

The milklactose CAL created to detect ±0.1 milklactose CIx as cutoff value detected 4.92 % cows as sick. The alarm had 12.5 % sensitivity and 94.7 % specificity to detect the animal as sick (as affected by any of the study defined health disorders). The milk lactose CAL was most sensitive to detect milk fever (sensitivity = 100 and specificity = 95%) followed by mastitis (34.8 and 95.1%), metritis (17.4 and 95%), depression (13.1 and 95%), digestive disorders (8 and 95%), ketosis (5.4 and 95%), and foot problems (5.1 and 100%; Table 11).

The values estimated for the milklactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of milk fever, metritis, metritis, digestive problems, and sickness only (Table 12). The area under the curve values from the ROC analysis was 0.54. This value was highest for the milk fever (0.97) followed by metritis (0.56), mastitis (0.65), digestive disorders (0.55), depression (0.55), and ketosis (0.54) and foot problems (0.51). The milklactose HAL created using ±0.1 milk MIx as cutoff value detected 5.38 % cows as sick. The alarm had 10.1 % sensitivity and 95.6 % specificity in detecting the animal as sick. The milk lactose HAL was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 95.6%) followed by mastitis

(34.8 and 95.6%), digestive (8 and 95.6%), ketosis (5.4 and 95.6%), foot problems (4.5 and 100%), depression (4.4 and 95.6%), and metritis (4.4 and 95.6%; Table 11).

The milk lactose MIx was reduced ($P < 0.05$) in cases with sickness, digestive disorder, milk fever, metritis, and mastitis than with the healthy cows. The AUC value from the ROC analysis for the sick animal was 0.53. This value was greatest for milk fever (0.98) followed by mastitis (0.66), metritis (0.61), digestive disorder (0.60), ketosis (0.56), depression (0.53), and foot problems (0.53).

A combined alarm (CombA) was created using the parallel combination of milklactose CAL and HAL. The alarm detected 5.36 % of the animals as sick. The alarm was found to have 12.5 % sensitivity and was 94.7 % specificity to detect the diseased animal as sick. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity = 94.6%) and was followed by mastitis (34.8 and 94.7%), metritis (17.4 and 94.6%), depression (13.1 and 94.6%), digestive (8 and 94.6%), ketosis (5.4 and 100%), and foot problems (5.4 and 100%). The ROC analysis of the milklactose combalarm (CombA) had AUC value of 0.54 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.98) followed by mastitis (0.65), metritis (0.56), depression (0.54), foot problems (0.53), digestive (0.51), and ketosis (0.5).

Results of milklactose analysis on the day of diagnosis using ±0.15 as cutoff

The milk lactose CAL created using ±0.15 milk lactose indices as cut off value detected 2.24% animals as sick. The alarm had 8.3% sensitivity and had 97.6% specificity to detect the animal as sick (if affected by any of the study disorders). The milk lactose cow alarm was most sensitive to detect milk fever (sensitivity= 100 and specificity= 95.6%) followed by mastitis (26.1 and 97.6%), digestive disorders (6 and

97.6%), ketosis (5.4 and 97.6%), depression (4.4 and 97.6%), metritis (4.4 and 97.6%), and foot problems (2.5 and 100%; Table 11).

The values estimated for the milk lactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, milk fever, metritis, mastitis and digestive disorders only (Table 12). Area under the curve values from ROC analysis was 0.53. This value was highest for milk fever (0.99) followed by mastitis (0.62), digestive disorders (0.52), metritis (0.51), depression (0.51), foot problems (0.51), and ketosis (0.51).

The milk lactose HAL created using ±0.15 milk lactose indices as cutoff value for the milk lactose detected 1.85 % of the animals as sick. The alarm had 7.7 % sensitivity and 98.1 % specificity in detecting the animal as sick. The milk lactose herd alarm was most sensitive in detecting milk fever (sensitivity = 100 and specificity = 97.8%) followed by mastitis (26.1 and 98%), ketosis (5.4 and 98%), metritis (4.4 and 97.6%), depression (4.4 and 98%), digestive (4 and 98%), foot problems (2.1 and 100%; Table 11).

The milk lactose MIx was reduced ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, metritis, mastitis, and milk fever only. The area under the curve values from the ROC analysis for the sick animal was 0.53. This value was greatest for milk fever (0.99) followed by mastitis (0.62), ketosis (0.52), metritis (0.51), depression (0.51), digestive disorder (0.51), and foot problems (0.51).

A combined alarm CombA was created using the parallel combination of CAL and HAL. The alarm detected 2.4 % of animals as sick. The alarm was found to have 8.33 % sensitivity to detect the animal as sick and had 97.6 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 100 and specificity = 97.5%) and was followed by metritis (4.4 and 97.5%), mastitis (26.1 and 97.5%), depression (4.4 and 97.5%), ketosis (5.4 and 97.5%), digestive (6 and 97.5%), and foot problems (2.5 and 100%). The ROC analysis of the milk lactose (CombA) had AUC value of 0.53 to diagnose a cow as sick. The AUC value was highest for milk fever (0.99) followed by mastitis (0.62), ketosis (0.52), digestive (0.52), metritis (0.51), depression (0.51), and foot problems (0.51).

Results of daily milk lactose analysis on the day prior to diagnosis using ±0.1as cutoff

The milk lactose CAL created using ±0.1 milk lactose index as cut off value detected 4.15 % of the animals as sick (as affected by any study defined health disorder). The alarm had 11.18 % sensitivity and 95.89 % specificity to detect the animal as sick (as affected by any of the study defined health disorders) on a day ahead of the diagnosis. The milk lactose CAL was most sensitive to detect milk fever (sensitivity = 60 and specificity = 95.8%) followed by metritis (19.2 and 95.8%), ketosis (13.9 and 95.8%), mastitis (13.1 and 95.8%), digestive disorders (12 and 95.8%), depression (8.3 and 95.8%), and foot problems (4.2 and 100%; Table 11).

The values estimated for the milk lactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, ketosis, depression, digestive disorders, metritis, mastitis, and milk fever only (Table 12). The area under the curve values from the ROC analysis for the sick animal was 0.54. This value was highest for the milk fever (0.78) followed by metritis (0.58), ketosis (0.55), mastitis (0.54), digestive disorders (0.54), depression (0.52), and foot problems (0.52).

The milk lactose HAL created using ±0.1 milk lactose index as cutoff value for the milk lactose detected 3.89 % animals as sick. The alarm had 9.4 % sensitivity and 96.2 % specificity in detecting the animal as sick on a day ahead of the diagnosis. The milkfat HAL was most sensitive in detecting milk fever (sensitivity = 40%, specificity = 96.1%) followed by metritis (19.2%, 96.1%), mastitis (13.1%, 96.1%), ketosis (11.1%, 96.1%), digestive (9.8%, 96.1%), depression (8.3%, 96.1%), and foot problems (3.9%, 100%; Table 11).

The milk lactose MIx was significantly lower ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, ketosis, milk fever, metritis, and mastitis only. The area under the curve values from the ROC analysis for the sick animal was 0.53. This value was highest for milk fever (0.68) followed by mastitis (0.55) metritis (0.58), depression (0.52), foot problems (0.52), and ketosis (0.52).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 4.79 % animals as sick. The alarm was found to have 11.2 % sensitivity to detect the disease as sick and had 95.3 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 60 and specificity= 95.2%) and was followed by metritis (19.2 and 95.2%), ketosis (13.9 and 95.2%), mastitis (13.1 and 95.2%), digestive (11.8 and 95.2%), depression (8.3 and 95.2%), and foot problems (4.8 and 100%). The ROC analysis of the milk lactose CombA had AUC value of 0.53 to diagnose a cow as sick. The AUC value was highest for milk fever (0.77), followed by metritis (0.57), ketosis (0.55), digestive (0.54), mastitis (0.54), depression (0.52), and foot problems (0.52).

Results of daily milk lactose analysis on the day prior to diagnosis using ±0.15 as cutoff

The milk lactose CAL using ±0.15 milk lactose indices as cut off value detected 1.74 % animals as sick. The alarm had 4.12 % sensitivity and 98.2 % specificity to detect the animal as sick (as affected by any of the study defined health disorders) on a day ahead of the diagnosis. The milk lactose CAL was most sensitive to detect milk fever (sensitivity = 20 and specificity = 98.2%) followed by depression (8.3 and 98.2%), ketosis (5.6 and 98.2%), mastitis (4.4 and 98.2%), metritis (3.9 and 98.2%), digestive disorders (3.9 and 98.2%), and foot problems (1.8 and 100%) (Table 11).

The values estimated for the milk lactose cow index (CIx) in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, ketosis, depression, digestive disorders, metritis, mastitis, and milk fever only (Table 12). The area under the curve values from the ROC analysis for the sick animal was 0.52. This value was highest for the milk fever (0.59) followed by depression (0.54), ketosis (0.52), mastitis (0.52), digestive disorders (0.52), metritis (0.51), and foot problems (0.51).

The milk lactose HAL created using ±0.15 milk lactose index as cutoff value for the milk lactose detected 1.67 % animals as sick and had 4.1 % sensitivity and 98.2 % specificity in detecting the animal as sick on a day ahead of the diagnosis. The milkfat HAL was most sensitive in detecting milk fever (sensitivity = 20%, specificity = 98.2%) followed by depression (8.3 and 98.2%), ketosis (5.6 and 98.2%), mastitis (4.4 and 98.2%), metritis (3.9 and 98.2%), digestive (3.9 and 98.2%), and foot problems (1.8 and 100%; Table 11).

The milk lactose MIx was significantly lower ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, ketosis, milk fever, metritis, and mastitis only. The area under the curve values from the ROC analysis was 0.51. This value was highest for milk fever (0.59) followed by depression (0.53), ketosis (0.52), mastitis (0.51) metritis (0.51), and foot problems (0.51).

A combined alarm was created using the parallel combination of cow alarm and herd alarm. The alarm detected 2.0 % animals to be sick. The alarm was found to have 4.1% sensitivity to detect the disease as sick and had 97.9 % specificity. On the individual disorders the sensitivity was highest for milk fever (sensitivity = 20 and specificity= 97.9%) and was followed by metritis (3.9 and 97.9%), ketosis (5.6 and 97.9%), mastitis (4.4 and 97.9%), digestive (3.9 and 97.9%), depression (8.3 and 97.9%), and foot problems (2.1 and 100%). The ROC analysis of the milk lactose CombA had AUC value of 0.51 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.59), followed by metritis (0.51), ketosis (0.52), digestive (0.51), mastitis (0.51), depression (0.53), and foot problems (0.51).

Analysis of fat to lactose ratio

The average milk fat: lactose of milking animals at 0-17 DIM was greatest (0.94 ± 0.003), intermediate at 17-53 DIM (0.79 ± 0.002) and lowest (0.76 ± 0.003) at > 53 DIM ($P < 0.0001$).

Similarly, Group 3 had average fat: lactose of 0.92 ± 0.016, group 7 had 0.89 ± 0.004, group 9 had 0.86 ± 0.004, group 8 had 0.86 ± 0.034, group 4 had 0.85 ± 0.004, group 6 had 0.83 ± 0.007, group 1 had 0.79 ± 0.003, group 2 had 0.79 ± 0.023, and group 5 had 0.78 ± 0.003.

The average daily milk fat: lactose of milking cows was reduced (0.84 ± 0.005) during the warm weather (THI > 76.2) than during the cooler weather (0.86 ± 0.005; $P < 0.0001$). The average milk fat: lactose of healthy cows for each month of the study was found to be, January (0.8 ± 0.071), February (0.83 ± 0.007), March (0.83 ± 0.007), April (0.85 ± 0.007), May (0.86 ± 0.007), June (0.84 ± 0.007), July (0.83 ± 0.007), August (0.84 ± 0.006), September (0.89 ± 0.007), October (0.88 ± 0.002), November (0.78 ± 0.063), and December (0.797 ± 0.009). The daily THI and daily milk fat: lactose of milking cows had r = 0.108 ($P < 0.0001$) and $r^2 = 0.012$ (y = 0.696 + 0.00156x; $P < 0.0001$). The average milk fat: lactose of the healthy animals was lower (0.85 ± 0.005) as compared to the average fat: lactose of the sick animals (0.99 ± 0.012; $P < 0.0001$).Consistent increase in average daily milk fat: lactose, were observed both on the day of diagnosis and 5 days previous to diagnosis for each post-partum disease (Fig 7).

Results of fat to lactose ratio analysis on the day of diagnosis using ±0.1 as cutoff

The fat: lactose CAL created using ±0.1 fat by lactose indices as cutoff value detected 43.3% animals as sick. The alarm had 60.4 % sensitivity and 59.1 % specificity to detect the animal as sick (as affected by any of the study defined health disorders). The fat: lactose CAL was most sensitive to detect milk fever (sensitivity= 80 and specificity= 58.8%) followed by mastitis (77.8 and 58.9%), ketosis (61.4 and 58.8%), foot problems (57.9 and 58.8%), digestive disorders (57.6 and 58.8%), metritis (56.7 and 58.8%), depression (51.7 and 58.8%; Table 13).

The values estimated for the fat: lactose CIx in sick cows were lower ($P < 0.05$) than those for healthy cows in the case of milk fever, mastitis, digestive problems, and sickness only (Table 14). The AUC value from the ROC analysis for the sick animal was

0.6. This value was highest for the milk fever (0.69) followed by mastitis (0.68), ketosis (0.60), digestive disorders (0.58), foot problems (0.58), metritis (0.57), and depression (0.55). The fat: lactose HAL created using ±0.1fat: lactose index as cutoff value detected 41.49% animals as sick. The alarm had 65.9 % sensitivity and 60.65 % specificity in detecting the animal as sick. The herd alarm was most sensitive in detecting milk fever (sensitivity = 80 and specificity = 60.2%) followed by mastitis (80.3 and 60.3%), metritis (76.7 and 60.3%), ketosis (70.5 and 60.3%), digestive (62.7 and 60.3%), foot problems (57.9 and 60.2%) and depression (48.3 and 60.2%; Table 13).

The fat: lactose MIx was significantly lower ($P < 0.05$) in cases with sickness, digestive disorder, milk fever and mastitis than with the healthy cows. The AUC value from the ROC analysis for the sick animal was 0.63. This value was greatest for milk fever (0.7) followed by mastitis (0.72), metritis (0.68), digestive disorder (0.62), ketosis (0.65), depression (0.54), and foot problems (0.59).

A combined alarm (CombA) was created using the parallel combination of cow alarm and herd alarm. The alarm detected 48.4 % animals as sick. The alarm was found to have 68.7 % sensitivity and had 53.6 % specificity to detect the diseased animal as sick. On the individual disorders the sensitivity was highest for mastitis (sensitivity = 83.3 and specificity= 53.2%) and was followed by metritis (80 and 53.2%), milk fever (80 and 53.1%), ketosis (75 and 53.2%), digestive (66.1 and 53.2%), foot problems (57.9 and 53.1%) and depression (55.2 and 53.1%). The ROC analysis of the fat:lactose CombA had AUC value 0.61. The AUC value was highest for mastitis (0.68) followed by milk fever (0.67), metritis (0.67), ketosis (0.64), digestive (0.6), foot problems (0.56), and depression (0.54).

Results of daily fat to lactose ratio analysis on the day of diagnosis using ±0.15 cutoff

The fat: lactose CAL created using ±0.15 fat by lactose index as cut off value detected 30.1 % animals as sick. The alarm had 46.5 % sensitivity and had 72.52 % specificity to detect the animal as sick (if affected by any of the study defined health disorders). The CAL was most sensitive to detect milk fever (sensitivity = 80 and specificity = 72.2%) followed by mastitis (63.9 and 72.3%), digestive disorders (44.1 and 72.3%), ketosis (43.2 and 72.2%), depression (34.5 and 72.2%), metritis (46.7 and 72.2%), and foot problems (52.6 and 72.2%; Table 13).

The values estimated for the fat: lactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, milk fever, mastitis and digestive disorders only (Table 14). Area under the curve values from ROC analysis for the sickness was 0.59. This value was highest for the milk fever (0.76) followed by mastitis (0.68), foot problems (0.62), metritis (0.59), digestive disorders (0.58), ketosis (0.58), and depression (0.53).

The fat: lactose HAL created using ±0.15 fat by lactose index as cutoff value detected 28.76% animals as sick. The alarm had 52.1 % sensitivity and 73.76 % specificity in detecting the animal as sick. The HAL was most sensitive in detecting milk fever (sensitivity = 80 and specificity= 73.3%) followed by mastitis (72.2 and 73.4%), ketosis (56.8 and 73.4%), metritis (56.7 and 73.4%), foot problems (52.6 and 73.3%), digestive (47.55 and 73.4%), and depression (37.9 and 73.3%; Table 13).

The fat: lactose MIx was significantly lower ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, mastitis, and milk fever only. The AUC value from the ROC analysis for the sick animal was 0.63. This value was greatest for milk fever (0.76) followed by mastitis (0.73), ketosis (0.65), metritis (0.65), foot problems (0.63), digestive disorder (0.60), and depression (0.55).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 32.67 % animals as sick. The alarm was found to have 53.5 % sensitivity to detect the animal as sick and had 69.7 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 80 and specificity = 69.3%) and was followed by mastitis (72.2 and 69.4%), metritis (60 and 69.4%), ketosis (56.8 and 69.4%), digestive (50.9 and 69.4%), foot problems (52.6 and 69.4%), and depression (41.4 and 69.4%). The ROC analysis of the fat: lactose CombA had AUC value 0.62. The AUC value was greatest for milk fever (0.75) followed by mastitis (0.71), metritis (0.65), ketosis (0.63), foot problems (0.61), digestive (0.6), and depression (0.55).

Results of average fat to lactose analysis on day previous to diagnosis using ±0.1 as cutoff

The fat:lactose CAL created using ±0.1fat by lactose index as cutoff value detected 40.59 % animals as sick(as affected by any of the study defined health disorders) a day ahead of the diagnosis. The alarm had 61.3 % sensitivity and 62.1 % specificity. The fat: lactose cow alarm was most sensitive to detect mastitis (sensitivity = 69.4 and specificity = 61.8%) followed by metritis (66.7 and 61.8%), digestive disorders (62.7 and 61.8%), foot problems (63.2 and 61.8%), milk fever (60 and 61.8%), ketosis (59.1 and 61.8%), and depression (55.2 and 61.8%; Table 13).

The values estimated for the fat: lactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, ketosis, digestive disorders, metritis, mastitis, and milk fever only (Table 14). The AUC value from the ROC analysis for the sick animal was 0.62. This value was highest for the milk fever (0.61) followed by metritis (0.64), ketosis (0.60), mastitis (0.66), digestive disorders (0.62), depression (0.58), and foot problems (0.62).

The fat: lactose HAL created using ±0.1fatbylactose index as cutoff value detected 39.1 % as sick. The alarm had 64.1 % sensitivity and 63.2 % specificity in detecting the animal as sick on day previous to the diagnosis. The fat: lactose HAL was most sensitive in detecting milk fever (sensitivity= 80 and specificity= 62.7 %) followed by metritis (73.2 and 62.8%), mastitis (72.2 and 62.8%), ketosis (65.9 and 62.8%), digestive (59.3 and 62.8%), depression (55.2 and 62.8%), and foot problems (63.2 and 62.8%; Table 13).

The fat: lactose MIx was lower ($P < 0.05$) than the healthy cows in cases with sickness, digestive disorder, ketosis, milk fever, metritis and mastitis only. The AUC value from the ROC analysis for the sick animal was 0.64. This value was greatest for milk fever (0.71) followed by mastitis (0.68), metritis (0.68), ketosis (0.64), foot problems (0.63), and depression (0.59).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 45.11 % animals as sick. The alarm was found to have 69.6 % sensitivity to detect the disease as sick and had 57.3 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 80 and specificity = 56.9%) and was followed by metritis (80 and 57%), ketosis (70.5 and 57%), mastitis (72.2 and 56.9%), digestive (69.5 and 57%), depression (62.1 and 56.9%), and foot problems (68.4 and 56.9%). The ROC analysis of the fat: lactose CombA had AUC value of 0.63 to diagnose a cow as sick. The AUC value was highest for milk fever (0.68), followed by metritis (0.68), ketosis (0.64), digestive (0.63), mastitis (0.65), depression (0.59), and foot problems (0.63).

Results of daily fat to lactose analysis on day previous to diagnosis using ±0.15 as cutoff

The fat: lactose CAL created using ±0.15 fat by lactose indices as cut off value detected 28.34 % animals as sick (as affected by any of the study defined health disorders) a day ahead of the diagnosis. The alarm had 50.2 % sensitivity and 74.6 % specificity. The fat: lactose CAL was most sensitive to detect milk fever (sensitivity= 40 and specificity= 74.2%) followed by depression (37.9 and 74.2%), ketosis (54.6 and 74.3%), mastitis (66.7 and 74.3%), metritis (53.3 and 74.2%), digestive disorders (47.5 and 74.3%), and foot problems (47.4 and 74.2%; Table 13).

The values estimated for the fat: lactose CIx in sick cows were significantly lower ($P < 0.05$) than those for healthy cows in the case of sickness, ketosis, digestive disorders, metritis, mastitis, and milk fever only (Table 14). The area under the curve values from the ROC analysis for the sick animal was 0.62. This value was highest for the milk fever (0.57) followed by depression (0.56), ketosis (0.64), mastitis (0.7), digestive disorders (0.61), metritis (0.64), and foot problems (0.61).

The fat: lactose HAL created using ±0.15 fat by lactose indices as cutoff value detected 27.26 % animals as sick. The alarm had 51.6 % sensitivity and 75.4 % specificity in detecting the animal as sick on a day ahead of the diagnosis. The fat: lactose HAL was most sensitive in detecting milk fever (sensitivity= 60 and specificity= 75%) followed by depression (37.9 and 74.9%), ketosis (54.6 and 75.1%), mastitis (66.7 and

75.1%), metritis (60 and 75.1%), digestive (44.1 and 75.1%), and foot problems (52.6 and 74.9%; Table 13).

The fat: lactose MIx was reduced (P < 0.05) than the healthy cows in cases with sickness, digestive disorder, ketosis, milk fever, metritis, and mastitis only. The area under the curve values from the ROC analysis for the sick animal was 0.64. This value was highest for milk fever (0.68) followed by depression (0.56), ketosis (0.65), mastitis (0.71) metritis (0.67), and foot problems (0.64).

A combined alarm (CombA) was created using the parallel combination of CAL and HAL. The alarm detected 30.66 % animals as sick a day ahead of clinical diagnosis. The alarm was found to have 53.5 % sensitivity to detect the disease as sick and had 72.1 % specificity. On the individual disorders the sensitivity was greatest for milk fever (sensitivity = 60 and specificity= 71.6%) and was followed by metritis (63.3 and 71.6%), ketosis (54.6 and 71.7%), mastitis (66.7 and 71.7%), digestive (47.5 and 71.7%), depression (41.4 and 71.6%), and foot problems (52.6 and 71.6%). The ROC analysis of the fat: lactose CombA had AUC value of 0.63 to diagnose a cow as sick. The AUC value was greatest for milk fever (0.66), followed by metritis (0.68), ketosis (0.63), digestive (0.6), mastitis (0.69), depression (0.57), and foot problems (0.62).

Combination of alarms

The alarms of rumination CAL, milk yield CAL and activity CAL were used in series and parallel combination to develp rumination, activity and milk (RAM_serial) and RAM_parallel alarms. A parallel RAM alarm is positive (i.e. flag a cow as sick) if any one of the three CAL alarms are positive. A serial RAM alarm is positive only when all three of the CAL alarms (rumination, milk yield, activity) are positive.

Alarms on the day of diagnosis with -0.1 as cutoff value

The RAM_parallel detected 48% animals as sick. The RAM_parallel had 86.8 % sensitivity to detect the animal as sick (any one of the study defined health disorders) and had 49.7 % specificity. The alarm was most sensitive to detect milk fever (100 and 49.2%) followed by foot problems (95.8 and 49.2%), ketosis (93.2 and 49.3%), digestive disturbances (88.5 and 49.3%), metritis (83.9 and 49.2%), mastitis (81.6 and 49.2%), and depression (79.3 and 49.2%). The area under the curve value of ROC analysis for RAM_parallel was 0.68 to detect the animal as sick and was highest for milk fever (0.75) followed by foot problems (0.72), ketosis (0.71), digestive disorders (0.69), metritis (0.67), mastitis (0.65) and depression (0.64; Table 15).

The RAM_serial detected 1.42% animals as sick. The RAM_serial had 16.3 % sensitivity to detect the cow as sick (any of the study defined health disorder) and had 98.5 % specificity. The alarm was most sensitive to detect milk fever (33.3 and 98.27) followed by digestive disorder(21.3 and 98.3 %), mastitis (21.1 and 98.3 %), metritis(19.4 and 98.3 %), ketosis(18.2 and 98.3 %), depression(6.9 and 98.3%) and foot problems(4.2 and 98.3%).The Area under the curve value for ROC analysis for RAM_serial was (0.58) to detect a sick animal and was highest for milk fever (0.66), followed by mastitis (0.6), digestive (0.6), metritis (0.59), ketosis (0.58), depression (0.53), and foot problems (0.51).

Alarms on the day of diagnosis using -0.15 cutoff values

The RAM_parallel detected 34.25 % animals as sick. The RAM_parallel had 74.5 % sensitivity to detect the animal as sick (any one of the study defined disorders) and had 65.9 % specificity. The alarm was most sensitive to detect milk fever (100 and 65.35 %) followed by foot problems (87.5 and 65.4%), ketosis (86.4 and 65.4%), metritis (77.4 and 65.4%), digestive disturbances (73.8 and 65.5%), mastitis (68.4 and 65.4%), and depression (62.1 and 65.4%). The AUC of ROC analysis for RAM_parallel was 0.71 to detect the animal as sick and was highest for milk fever (0.83) followed by foot problems (0.76), ketosis (0.76), digestive disorders (0.69), metritis (0.71), mastitis (0.67) and depression (0.64) (Table 15).

The RAM_serial detected 0.73% animals as sick when using -0.15 cutoff values. The RAM_serial had 12.3 % sensitivity to detect the animal as sick (any of the study defined disorders) and had 99.3 % specificity. The alarm was most sensitive to detect milk fever (33.3 and 99.1%) followed by digestive disorder(11.5 and 99.1%), mastitis (21.1 and 99.1%), metritis(12.9 and 99.1%), ketosis(13.6 and 99.1%), depression(6.9 and 99.1%) and foot problems(4.2 and 99.1%).The AUC value for ROC analysis for RAM_serial was (0.56) to detect a sick animal and was highest for milk fever(0.66), followed by mastitis(0.6), metritis(0.56), ketosis (0.56), digestive(0.55), depression(0.53), and foot problems(0.52).

Alarms on the day prior to diagnosis using -0.1 as cutoff value

The RAM_parallel detected 45.38 % animals as sick. The RAM_parallel was 80.2 % sensitive to detect the animal as sick (any one of the listed disorders) and had 52.7 % specificity. The alarm was most sensitive to detect milk fever (100 and 52.3%) followed by ketosis (84.1 and 52.3%), metritis (83.9 and 52.3%), foot problems (83.3 and 52.3%), digestive disturbances (80.3 and 52.4%), depression (78.3 and 52.3%), and mastitis (76.3 and 52.3%). The area under the curve value of ROC analysis for RAM_parallel was 0.66 to detect the animal as sick and was highest for milk fever(0.76) followed by foot problems(0.68), ketosis(0.68), digestive disorders(0.66), metritis(0.68), mastitis(0.64), and depression(0.66).

The RAM_serial detected 1.26% animals as sick a day ahead of diagnosis. The RAM_serial was 8.4% sensitive to detect the animal as sick and had 98.6% specificity. The alarm was most sensitive to detect mastitis (13.2 and 98.5%) followed by digestive disorder(13.1 and 98.5%), ketosis(11.4 and 98.5%), foot problems (4.17 and 98.5%), metritis(1.54 and 100%), milk fever (1.5 and 100%), and depression(1.5 and 100%).The area under the curve value for ROC analysis for RAM_serial was (0.53) to detect a sick animal and was highest for mastitis (0.56) followed by ketosis (0.55), digestive (0.51), depression (0.51), metritis (0.51), foot problems (0.51), and milk fever (0.50).

Alarms on the day prior to diagnosis using a -0.15 cutoff

The RAM_parallel detected 31.35 % animals as sick a day ahead of diagnosis. The RAM_parallel was 67.4 % sensitive and had 69.16 % specificity. The alarm was most sensitive to detect milk fever (100 and 68.7%) followed by ketosis (65.9 and 68.8%), metritis (74.2 and 68.8%), foot problems (75 and 68.7%), digestive disturbances (67.2 and 68.8%), depression (69 and 68.8%), and mastitis (68.4 and 68.8%). The area under the curve value of ROC analysis for RAM_parallel was 0.68 to detect the animal as sick and was highest for milk fever(0.84) followed by foot problems(0.72), ketosis(0.67), digestive disorders(0.68), metritis(0.71), mastitis(0.69), and depression(0.69; Table 15).

The RAM_serial detected 0.60 % animal as sick (any of the study defined health disorders) a day ahead of diagnosis. The RAM_serial had 5.3 % sensitivity to detect the animal as sick and had 99.3 % specificity. The alarm was most sensitive to detect digestive disorder(11.5 and 99.3%) followed by, ketosis(6.8 and 99.3%), mastitis (5.3 and 99.3%), foot problems (4.2 and 99.3%), metritis(0.7 and 100%), milk fever(0.7 and 100%), and depression(0.7 and 100%).The Area under the curve value for ROC analysis for RAM_serial was (0.52) to detect a sick animal and was highest for digestive (0.55) followed by ketosis (0.53), mastitis (0.52) , foot problems (0.52) , depression (0.50), metritis (0.50), and milk fever (0.50).

Association of rumination with serum calcium, NEFA and BHBA

Overall, 48.52 % were diagnosed as hypo-calcemic cows by the total Ca analysis. The rumination CAL had 30 % sensitivity and 70 % specificity to detect the hypocalcemia detected by this test. The rumination HAL was 31 % sensitivity and 70 % specificity to detect the cow as hypo-calcemic as detected by this test. The CAL had 0.51 AUC and HAL had 0.51 AUC from the ROC curve analysis. There was decreasing trend in RT in the pre-calving (PREC) period whereas there was increasing trend in RT in the post-calving (POSTC) period for both the HYC and healthy cows. The 2 h RT was lower in case of HYC than in case of healthy cows in both PREC and POSTC period (Fig. 8). The repeated measures analysis shows that hypocalcemia, time denoted by 2h interval before/after calving and the interaction of hypocalcemia and time are all significant ($P < 0.0001$) in POSTC period whereas only time ($P < 0.001$) was significant on PREC period. The interaction effect enables us to compare healthy and sick cows within each day and the cows were found to be statistically different on day 1, 6, and 7 after calving.

On the PREC period, the 2-h RT of the sick cows had correlation coefficient of r = -0.1 (P < 0.0001) and the 2-h RT of the healthy cows had correlation coefficient of r = -0.05 (P < 0.0001). However, on the POSTC period the 2-h RT of the sick cows had correlation coefficient of r = 0.278 (P < 0.0001) and the 2-h RT of the healthy cows had correlation coefficient of r = 0.27 (P < 0.0001). The coefficient of regression for sick cows on PREC period (r^2) = 0.009, b = -0.07115 and for the healthy cows on PREC period (r^2) = 0.0026, b = -0.04. The coefficient of regression for healthy cows on POSTC period (r^2) = 0.07, b = 0.204 and for the sick cows PREC period (r^2) = 0.078, b = 0.205.

Overall 17.79 % of the enrolled cows were diagnosed as ketotic (KET) cows by the commercial BHBA test kits. The CAL had 30% sensitivity and 71% specificity to detect the cow as sick as detected by this test. HAL was 31% sensitivity and 71% specificity to detect the cow as ketotic. There was decreasing trend in RT in the PREC period whereas there was increasing trend in RT in the post-calving POSTC period for both the KET and healthy cows. The 2 h RT was lower in case of KET than in case of healthy cows in both PREC and POSTC period (Fig. 9). The repeated measures analysis shows that Ketosis (P = 0.0001) and time denoted by 2h interval before/after calving (P < 0.0001) were significant on PREC period and on POSTC period also ketosis (P = 0.0002) and time (P < 0.0001) were significant. On the PREC period, the 2-h RT of the sick cows had correlation coefficient of r = -0.08 (P = 0.0016) and the 2-h RT of the healthy cows had correlation coefficient of r = - 0.066 (P < 0.0001). However on the POSTC period the 2-h RT of the sick cows had correlation coefficient of r = 0.25 (P < 0.0001) and the 2-h RT of the healthy cows had correlation coefficient of r = 0.269 (P < 0.0001). The coefficient of regression for sick cows on PREC period (r^2) = 0.005, b = -0.056 and for the healthy cows on PREC period r^2 =0.004, b = -0.049. The coefficient of regression for healthy cows on POSTC period r^2 = 0.072, b = 0.2 and for the sick cows POSTC period r^2 = 0.061, b = 0.18.

Overall 56.4 % of the enrolled cows were diagnosed as negative energy balanced (NEB) cows by NEFA analysis. The rumination ACI had 31 % sensitivity and 71 % specificity to detect by this analysis. AMI had 32% sensitivity and 71 % specificity to detect the cow as NEB. There was decreasing trend in RT in the PREC period whereas there was increasing trend in RT in the post-calving POSTC period for both the NEB and healthy cows. The 2 h RT was lower in case of NEB cows than in case of healthy cows for both PREC and POSTC periods (Fig. 10). The repeated measures analysis shows that NEB (P = 0.0001) and time denoted by 2h interval before/after calving ($P < 0.0001$) in PREC period and time ($P < 0.0001$) and NEB ($P < 0.0001$) are significant POSTC. On the PREC period, the 2-h RT of the sick cows had correlation coefficient of r = -0.08 ($P <$ 0.0001) and the 2-h RT of the healthy cows had correlation coefficient of r = -0.056 ($P <$ 0.0001). However on the POSTC period the 2-h RT of the sick cows had correlation coefficient of r = 0.28 ($P < 0.0001$) and the 2-h RT of the healthy cows had correlation coefficient of r= 0.247($P < 0.0001$). The coefficient of regression for sick cows on PREC period r^2 = 0.006, b = -0.06 and for the healthy cows on PREC period r^2 = 0.0029, b = -0.042. The coefficient of regression for healthy cows on POSTC period r^2 = 0.059, b = 0.185 and for the sick cows POSTC period r^2 = 0.079, b = 0.21.

Discussion

Rumination Time (RT)

Automated monitoring of rumination has been proposed due to labor intensive and inaccurate measurements derived from visual observation. The automated monitoring devices are effective at measuring rumination (Kononoff et al., 2002; Braun et al., 2013; Schirmann et al., 2009).

The average daily RT in the precalving cows observed in our study was 413.58±1.63 which is lower than that reported by Soriani et al, (2012) (522 min/d) and Aikman et al. (2008) (477 min/d). This lower RT can be explained by differences in feed ingredients, environmental conditions, and animal factors. The data are within range as presented by Soriani et al., (2012), Beauchemin and Yang (2005) (340 and 540 min/d) and Rengman et al. (2013) with 425-575 min/day. The rumination increased to 458.16±2.5 at 17 day in milk (DIM), 458.33 ± 2.012 at 17-53 DIM and again increased to 488.06 ± 2.43 minutes after 53 days. This may be related to the cow returning to normal condition after the parturition stress. Schirmann et al. (2013) found post calving rumination to be 428±26.5 and found it to be reduced to 63.3 ± 30.6 min/d immediately before calving, as an indicator of stress during calving. The decrease in RT at the days near to calving is also supported by Clark et al. (2015) and Schirmann et al. (2013) who found 33 % and 31 % decrease in daily rumination around the time of calving. Also the reduced rumination during calving is related to the findings by Pahl et al. (2014) that RT was decreased in last 4hr pre-partum and in first 8hr post-partum. The 2h rumination time showed some consistent fluctuations during each 24 h period. Rumination was highest during morning (2 am), started to decrease until 8 am and again start to increase at 10 am, slightly reduced at 12 pm, increased at 2 pm but again decreased at 4 pm and after that keeps on increasing at 6 and 8 pm, slightly decreased at 10 pm and again increased at 12 am. This fluctuation of rumination during the day is likely related to the feed delivery time in barn during the day, time of milking and return from the parlor and the THI fluctuation in different parts of the day. Cattle have lower rumination during the feed delivery and the rumination increased after they have finished eating. The higher RT during night time and early morning suggests us that rumination time increases when the cattle are at rest. The results are in line with Clark et al. (2015) who reported that longest and most intensive rumination bouts occur during night time. Reith et al. (2014) and Pahl et al. (2015) also supported our finding and reported the maximum RT around 0200 and 0400h and minimum levels at 0800h and 1000h. Soriani et al. (2013) also concluded that most of the rumination occurred during the night time.

In our study, for pre-partum cows, the average daily rumination during warm weather was not significantly different than during cooler weather. But for postpartum cows the daily rumination time during warm weather was lower than during cold weather. The coefficient of correlation was negative (-0.04) for the daily THI and daily rumination time of milking cows which was similar to Soriani et al.(2013) who also obtained a negative correlation(r= -0.32) between THI and RT. Contrarily, Sterrett et al. (2013) identified an r = 0.03 between the variable which may be due to small number of cows they observed. In addition that study was conducted from October to January where there is not much THI variation. Similar results were obtained by Lessire et al., (2014) and Acatincai et al., (2009) who concluded that temperatures beyond comfort 27-28°C diminish the entire rumination process, frequency and duration. The monthly rumination pattern shows that the rumination was highest during August and lowest in the month of November. Brscic et al., (2007) also observed greater rumination time during warm months and related that with decreased activity.

The average daily RT was higher in healthy animals in comparison to sick animals on the day of diagnosis. This can be explained by altered rumination and feeding during sickness. Siivonen et al., (2011) and Fitz Patrick et al. (2013) found reduced rumination in cows with endotoxin-induced clinical mastitis cows. Sterrett et al. (2014) observed reduced rumination during subclinical milk fever and ketosis. Kaufman et al. (2015) found reduced RT in cows suffering from subclinical ketosis compared to healthy cows and suggested the monitoring in transition period. Stangaferro et al. (2015) evaluated the rumination monitoring system and concluded that the system is effective to detect cows suffering from metabolic and digestive disorders.

For the detection of a sick animal on the day of diagnosis -0.1 as a cutoff value, the rumination HAL (53.9%, 77.1%) had higher sensitivity and specificity then rumination CAL (50%, 77.6%). As expected when combining both the alarms the sensitivity was increased (56.6%) but the specificity was reduced (66.69%). Whereas, taking AUC values under consideration, rumination HAL had the highest AUC values (0.63). When the cut off was changed to -0.15, obviously the sensitivity was lower and the AUC values were increased in all three alarms with highest AUC values again for the rumination HAL (0.65). For detecting individual disease sensitivity, specificity and AUC values for each disease were mastitis (48.7%, 85.0%, 0.68) by (-0.1) HAL, metritis (29.0%, 85.1%,0.57) for (-0.15) CAL, milk fever (100%, 85.1%, 0.93) for (-0.1) CAL, depression (40%, 85.1%, 0.62) for (-0.15) CAL, digestive problems (41.9%, 85.1%, 0.64) for(-0.15) HAL, foot problems (66.7%, 77.3%, 0.69) for (-0.1) CAL and ketosis (62.8%, 81.0%, 0.72) for (-0.15) CombA. The sensitivity and specificity to diagnose metritis was lower than that obtained by Liboreiro et al. (2014) who found metritis with 75% sensitivity and 93.1% specificity and Sorianai et al., (2013) who found 82% sensitivity and 82.5% sensitivity with AUC 0.906. This may be because they only focused on diagnosis 72 h after parturition whereas we included metritis diagnosed at any time during the post-partum period. The sensitivity and specificity we obtained for milk fever was higher than they obtained (66.7% sensitivity and 61.3% specificity). Stangaferro et al. (2015) obtained greater sensitivity for detection of ketosis whereas reduced sensitivity to detect mastitis and metritis than obtained in this study. This difference can be accounted for health index they created combining both rumination and activity data whereas we created indices for individual rumination and activity data. The alarms created to detect the disease on a day ahead of diagnosis had lower AUC values, sensitivity and specificity than the alarms for the day of diagnosis, except for diagnosis of mastitis. The highest AUC value was observed for (-0.1) HA (66.7% sensitivity, 78.2% specificity and, 0.72 AUC).

Analysis of activity data

Although measuring activity by pedometers is thoroughly investigated, only a few studies of activity measurement by means of acceleration systems attached to neck collars are available. Most of the studies have been based on a particular disease or event detection such as estrus (Reith et al. 2014), calving (Clark et al., 2015) and lameness (Van Hertem et al, 2013). In our study, the average daily activity of cows pre-partum was

478.81±1.59 units and was increased from 483.24± 2.63 to 497.54±1.85 post-calving. Similar increase in postpartum activity was observed by Clark et al. (2015). The activity was lowest at 0200hrs and increased gradually to peak at 1200 hrs, slightly decreased after that and constantly decrease after it obtained high at 6 pm. The results are supported by Reith et al. (2014) who found the minimum activity around 0200 hrs.

The activity in case of milking animals was found to be higher in warm weather than in cold weather conditions. This is in agreement with Brscic et al. (2007) and can be explained by the increased water demand of the animals in warm climatic conditions. The average daily activity was highest in September and least in January. Contrary to dry cows where the THI and activity had a negative correlation, in milking cows the correlation was positive. This may be explained by the different housing condition of cattle pre-partum and postpartum.

For the detection of a sick animal on the day of diagnosis, the activity cow alarm (CA) with -0.15 cutoff had higher AUC value of its sensitivity and specificity (33.19%, 88.45%, 0 .62). To detect the individual disease, AUC values for sensitivity and specificity was higher for (-0.1) CA for Mastitis (28.57%, 88.2%, 0.59), Metritis (46.88%, 88.2%, 0.67), milk fever (71.43%, 88.21%, 0.79) depression (28.1%, 88.2%, 0.58), and foot problems (38.89%, 88.21%, 0.72). (-0.15)CA was higher for digestive (47.6%, 81.3%, 0.71) and ketosis (47.7%, 81.2%, 0.65). Liboreiro et al. (2014) did not find the activity data to be useful in diagnosis of any of the same health disorders that we investigated. Activity was not related to ketosis on days near to calving but was activity was reduced on the later days. The analysis to detect disease on day previous to diagnosis was more efficient only in the case of depression using the -0.1 cutoff value and only for digestive problems with the -0.15 cutoff value.

Analysis of Milk yield

Average total daily milk yield for first 17 days after parturition was 75.53lbs (34.26 kg) which increased to 89.01lbs (40.37 Kg) after 17 days and peaked to 92.19lbs (41.82 kg) after 53 days. The yield was not significantly different in the milking cows. Typically, lower milk yields are expected during hot weather due to reductions in dry matter intake during heat stress (Soriani et al., 2013). The fact that we did not find a difference in milk yield during hot weather may indicate that heat stress mitigation practices on the farm are being used successfully. Similar, non- significant difference in milk yield between hot and cold periods was reported by Lessire et al. (2014). The effect of heat stress in higher THI may have been not significant because of lower decrease in Milk yield in early lactation than in late lactation as reported by Abeni et al., (2007). Monthly milk yield pattern shows that the milk yield was highest in March and lowest in November. However there was a significant negative correlation between the daily THI and daily milk yield which is supported by Bouraoui et al. (2002).

The milk yield for healthy cows was significantly higher than the yield for sick animals on the day of diagnosis. Van Hertem et al. (2013) also found out a high positive correlation(r = 0.45) of lameness detection with milk yield variable.

For the detection of a sick animal on the day of diagnosis with -0.1 as a cutoff value, the milk CAL (58.6%, 77.5%, 0.68) had higher AUC then milk HAL (46.3%, 83%, 0.64).This suggests to us that individual change in milk yield are more predictive of disease occurrence than a comparison to herdmates. As expected, combining both the alarms increased the sensitivity (63.41%) but reduced the specificity (66.49%). This can be explained by the parallel combination we used in creating the CombA. This combination alarm will help in increasing the sensitivity and detecting more animals as sick so that we would not miss the sick animal without treatment. When the cut off was changed to -0.15, obviously the sensitivity was lower (47.78%, 85.97%, 0.67) since we were more strict to marking the alarm. The AUC values were decreased in all three alarms with highest AUC values again for the rumination self-alarm (0.66). This suggests that -0.1 cut off in milk is more powerful in detecting the disease than -0.15 cutoff value except for detecting mastitis. For detecting individual disease the highest AUC values for sensitivity and specificity for each disease was obtained as mastitis (59.4%,77.1%, 0.72) by (-0.15) CAL, metritis (66.7%, 77.1%,0.72) for (-0.1) CAL, milk fever (100%, 76.9%, 0.89) for -0.1(CAL), depressed (51.9%, 85.5%, 0.69) for -0.15(CAL), digestive problems (50%, 66.5%, 0.71) for (-0.1) CombA, foot problems (66.7%, 77.3%, 0.54) for (-0.1) CAL and Ketosis (60.5%, 77.1%, 0.69) for (-0.1) CAL. The analysis to detect the sickness on day previous to the diagnosis revealed that each of the disease had lower AUC values, sensitivity and higher specificity than at the day of diagnosis for their respective cutoff values.

Analysis of Milk fat

The milkfat percentage of milking cows was lower in during warm weather than cold weather. The fat percentage was highest in April and lowest in November. There was a significant negative coefficient of correlation between percentage milk fat and daily THI during the study period. This result was supported by Bouraoui et al. (2002) but different from that obtained by Smith et al. (2013) who found an increase in fat percentage in heat stress. Several factors such as stage of lactation, diet as well as heat abatement could account for the differences among these studies.

The average milk fat percentage of sick cows on the day of diagnosis was higher than when the cows were healthy. This may be due to reduced milk yield which increased the fat percentage even if the amount of fat was not changed.

For the detection of a sick animal on the day of diagnosis with -0.1 or +0.1milkfat index as a cutoff value, the milkfat HAL (45.6%, 74.9%, 0.72) had higher AUC then milk CombA (48.5%, 68.5%, 0.69) and milk CAL (43.2%, 72.8%, 0.58).This suggests us that herd alarm in milkfat is more predictive of disease occurrence than the individual cow and combined alarm. When combining both the alarms the sensitivity was increased (48.5%) but the specificity was reduced (74.9%). This can be explained by the parallel combination we used in creating the comb alarm. This combination alarm will help in increasing the sensitivity and detecting more animals as sick so that we would not miss the sick animal without treatment. As expected, when the cut off was changed to ±0.15, the sensitivity was lower (21.9%, 87.4%, 0.55) because we were more strict to marking the alarm. The AUC values were decreased in all three alarms except for milk fever, depression and digestive problems, in which the AUC values are increased than during ±0.1 cutoff. This suggests that -0.1 cut off in milk is more powerful in detecting the diseases than ±0.15 cutoff value except for detecting milk fever, depression and digestive problems in which case ±0.15 cutoff seems to be more predictive. For detecting individual disease the highest AUC values for sensitivity and specificity for each disease was mastitis (69.6%,74.7%, 0.72) by (±0.1) HAL, metritis (66.7%, 78.3%,0.62) for (±0.1) CombA, milk fever (66.7%,88.4%, 0.78) for (±0.15) HAL, depressed

(26.1%,88.5%, 0.57) for (±0.15) HAL, digestive problems (41.2%, 85.2%, 0.63) for (±0.15) CombA, foot problems (9.1%,72.6%, 0.59) for (±0.1) CAL and ketosis (54.05%, 74.7%, 0.64) for (±0.1) HAL. Duffield et al. (1997) obtained 54% sensitivity and 72% specificity to detect ketosis using test day fat percentage as diagnostic tool.

During the analysis to detect the sickness on day prior to diagnosis, each of the alarms had lower AUC values except when using the ±0.1 cutoff for milk fever and for foot problems. Also the ±0.15 cutoff values for depression, digestive, ketosis had higher AUC values for diagnosis of disease on the day prior to diagnosis than on the day of diagnosis. This means that these diseases could have been predicted by the respective alarms, earlier than the time they were diagnosed by the farm personnel.

Analysis of Milk protein

The sick animals had higher milk protein percentage on the day of diagnosis than animals that were healthy. This may be due to lower milk yield in sick animals without a change in the amount of milk protein secreted.

For the detection of a sick animal on the day of diagnosis with ±0.1milk protein index as a cutoff value, the HAL (23.6%, 90.4%, 0.57) had higher AUC value then CAL (23.7%, 89.3%, 0.57) and CombA (25.4%, 87.4%, 0.56).This suggests us that herd alarm of milk protein is more predictive of disease occurrence than the individual cow and comb alarm. When combining both the alarms the sensitivity was increased (25.4%) but the specificity was reduced (87.4%). This can be explained by the parallel combination we used in creating the comb alarm. This combination alarm will help in increasing the sensitivity and detecting more animals as sick so that we would not miss the sick animal without treatment. When the cut off was changed to ±0.15, obviously the sensitivity was lower because we were more strict to marking the alarm. The AUC values were decreased in all three alarms for all diseases. This suggests that ±0.1 cut off in milk protein is more powerful in detecting the disease than ±0.15 cutoff value. For detecting individual disease the highest AUC values for sensitivity and specificity for each disease was obtained as, mastitis (41.7%,90.3%, 0.66) by (±0.1) HAL , metritis (43.5%, 90.3%,0.67) for (±0.1) HAL, milk fever (100%, 96.3%, 0.98) for (±0.15) HAL, depressed (21.7%,90.2%, 0.56) for (±0.1) HAL, digestive problems (22%, 90.2%, 0.56) for (±0.1) HAL, foot problems (4.8%,100%, 0.52) for (±0.15) CombA and Ketosis (22%, 90.2%, 0.56) for (±0.1) HAL. Duffield et al. (1997) obtained 46% sensitivity and 80% specificity to detect ketosis using test day protein percentage as diagnostic tool. The analysis to detect the sickness a day ahead of the diagnosis, each of the disease had lower AUC values except for alarms by ±0.1 cutoff in detecting digestive, foot problems, ketosis and sickness. Also ±0.15 cutoff values in detecting digestive and foot problems had higher AUC values for diagnosis of disease on a day ahead of diagnosis than on the day of diagnosis. This means that these diseases could be predicted by the respective alarms, earlier than the time it is being diagnosed by farm personnel.

Analysis of milk lactose

For the detection of a sick animal on the day of diagnosis with -0.1 or +0.1 milk lactose index as a cutoff value, the herd alarm (HAL) (10.1%, 95.6%, 0.53) had lower AUC value then cow-alarm(CAL) (12.5%, 94.7%, 0.54) and CombA (12.5%, 94.7%, 0.54).This suggests us that mates alarm, self-alarm and comb alarm from milk lactose is not helpful in predicting disease because of their very low AUC values. When the cut off was changed to ±0.15, obviously the sensitivity was lower because we were more strict to marking the alarm. The AUC values were not decreased in all three alarms because of the values were already low and this change in cutoff did not have much effect. Thus, this suggests that all three alarms in milk lactose are not efficient in detecting the disease conditions. For detecting individual disease the highest AUC values for sensitivity and specificity for each disease was obtained as, Mastitis (34.8%,95.6%, 0.65) by (±0.1)HAL , metritis (17.4%, 95%,0.56) for (±0.1) CAL, milk fever (100%, 97.9%, 0.99) for ±0.15(HAL), Depression (13.0%,94.9%, 0.54) for (±0.1) CAL, digestive problems (8%, 95.6%, 0.52) for (±0.1) HAL, foot problems (5.0%,100%, 0.53) for (±0.1) CAL and Ketosis (11.1%, 95.6%, 0.52) for (±0.15) HAL. The analysis to detect the sickness a day ahead of the diagnosis, each of the disease had lower AUC values except for alarms by ±0.1cutoff in detecting digestive, Ketosis. Also ±0.15 cutoff values in detecting depression had higher AUC values for diagnosis of disease on a day ahead of diagnosis than on the day of diagnosis. This means that these diseases could be predicted by respective alarms, earlier than the time it is being diagnosed by farm personnel.

Analysis of fat to lactose data

The milk fat to lactose ratio was lower in the healthy cows than in the sick cows on the day of diagnosis.

For the detection of a sick animal on the day of diagnosis with -0.1 or +0.1 fat by lactose index as a cutoff value, the HAL (65.9%, 60.7%, 0.63) had lower AUC value then CAL (60.4%, 59.1%, 0.59) and CombA (68.7%, 53.6%, 0.61).This suggests us that mates alarm, was less predictive of sickness at this cutoff point. The Comb alarm for each disease has higher sensitivity than both of the alarms because of the OR function we used in creating the alarm. This alarm would be helpful in detecting the diseased cow so that sick cow would not be left behind. Also, Mates alarm had higher specificity which can be used if we only want to treat the diseased animal only and save the resources. When the cut off was changed to ±0.15, obviously the sensitivity was lower because we were more strict to marking the alarm. The AUC values were decreased in all three alarms in all diseases except for milk fever and foot problems. Thus, this suggests that all three alarms in milk lactose are efficient in detecting the disease conditions at ±0.1 except for milk fever and foot problems which would be better detected at 15% cutoff values. For detecting individual disease the highest AUC values for sensitivity and specificity for each disease was obtained as, mastitis (72.2%,73.4%, 0.73) by (±0.15) HAL, metritis (76.7%, 60.3%,0.69) for (±0.1) HAL, milk fever (80%, 73.3%, 0.77) for (±0.15) HAL, depression (37.9%,73.3%, 0.56) for (±0.15) HAL, digestive problems (62.7%, 60.3%, 0.62) for(±0.1) HAL, foot problems (52.6%,73.3%, 0.63) for (±0.15) HAL and ketosis (56.8%, 73.4%, 0.65) for (±0.15) HAL. The analysis to detect the sickness a day ahead of the diagnosis, each of the disease had lower AUC values except for alarms by 10% cutoff in detecting metritis, depression, digestive problems and ketosis. Also ±0.15 cutoff values in detecting mastitis, metritis, depressed, digestive problems, and ketosis had higher AUC values for the diagnosis of disease on a day ahead of diagnosis than on the day of diagnosis. This means that these diseases could be predicted by respective alarms, earlier than the time it is being diagnosed by the farm personnel.

Analysis of the combination of alarms

Rumination, Activity and Milk yield Alarms can be used together to detect a sick animal. Combining the alarms in the parallel (i.e. either of the alarms to be positive) we get a RAM_parallel alarm which has high sensitivity. The alarm could detect sick animals with 86.78% sensitivity and 49.65% specificity with AUC value of 0.68. On the other hand, combining the alarms in series (i.e. all of the alarms need to be positive) we get a RAM_serial alarm which has very high specificity. The alarm could detect sick animals with 16.30% sensitivity and 98.46% specificity with AUC value of 0.57. The decision to use the alarms in series or in parallel combination is dependent on the disease type, cost of medication, management strategies. For example, when we want to identify all the diseased animals (surveillance) from our herd we would like to have the parallel combination which could detect every animal most affected. On the other hand, if the cost of treatment and management (labor, moving cows) is high and we want that only the sick animal to be examined, we would go for serial combination.

Changing the cutoff value to -0.15 we get higher AUC values for Parallel combination of the alarms in all disease cases but we get higher AUC value for Milk fever and ketosis only for Serial combination. This concludes us that if we want Parallel combination in our farm i.e. surveillance, -0.15 cutoff value is more appropriate. Whereas, for the serial combination i.e. treatment of specific disease, -0.1 cutoff is appropriate.

The alarms created to detect diseases one day ahead of diagnosis had higher AUC values for metritis, milk fever, depression and digestive disorders for RAM_parallel at -0.1 cutoffs than at their respective counterpart on the day of diagnosis. Similarly, for the -0.15 cutoff value on day ahead of diagnosis, mastitis, metritis, milk fever and depression had higher AUC values than on the day of diagnosis. So, these diseases could be diagnosed on a day ahead of the farm personnel diagnosis.

Conclusions

Monitoring rumination, activity, milk yield, and milk components has the potential for detection of different disease conditions in transition dairy cows. Total daily rumination was high in healthy cows compared to sick cows. The sensitivity was high for herd alarm but the specificity was high for cow-alarm. As expected, combining these two, a higher sensitivity was obtained at expenses of specificity. Consequently, a combination alarm and herd alarm could be used for aggressive detection of disease whereas a cow-alarm could be used for more specific detection of health disorders. Considering AUC values, cut off of –0.15 was more effective in detecting the diseases even though the sensitivity was lower than that of -0.1 cut off. Similarly, an activity alarm at a -0.15 cutoff was more useful than that at a -0.1 cutoff. In contrast an individual change in milk was more suggestive of a disease condition because cow alarm had higher sensitivity and AUC values. The milk cow alarm was more effective at -0.1 cutoff value than at -0.15 cutoff value. For milk fat, mates alarm was more effective than cow alarm and combined alarm considering the AUC values. The -0.15 cut off was more effective to diagnose milk fever, depression, and digestive problems. For the protein percentage, herd alarm is more predictive of disease than cow-alarm. Furthermore, alarms creating using -0.1 cutoff value was more effective than a -0.15 cutoff value. Alarms created using milk lactose were not much effective in detecting a sick cow (AUC values ~ 0.5).The fat to lactose ratio was higher in sick cows than in healthy cows. CombA was more effective than cow-alarm and herd alarm. Alarms created using a -0.15 cutoff value were more predictive of milk fever and foot problems but for other diseases -0.1 was more predictive. For the parallel combination of rumination, activity, and milk yield alarms, -0.15 cutoff was more effective in predicting the diseases than -0.1 cutoff, whereas for series combination -0.1 cutoff was more effective to detect milk fever and ketosis .

To detect the diseases on the day prior to diagnosis, CAL, HAL and CombA all rumination alarms were able to detect mastitis with improved efficacy over the day of diagnosis, and (-0.1) HAL was most sensitive in the detection. Activity CAL was more efficient tin diagnosing on the day of diagnosis but depression could be detected by(-0.1) CAL and digestive disorder could be detected by (-0.15) on the day previous to diagnosis. Detection of disease on the day previous to diagnosis was not effective for any milk yield alarms. For milkfat, when using a -0.1 cutoff value milk fever and foot problems could be detected on the day previous to diagnosis whereas using -0.15 cutoff values depression, digestion and ketosis could be detected on the day previous to diagnosis. For milk protein, using -0.1 cutoff value digestive problems and ketosis could be detected on the day previous to diagnosis whereas -0.15 cutoff were effective in detecting digestive problems and foot problems on the day previous to diagnosis. For milk lactose, digestive disease and ketosis could be diagnosed a day previous to disease diagnosis using -0.1 cutoff whereas depression could be diagnosed on the day previous to disease using -0.15 with better efficacy. For fat to lactose ratio, -0.1 alarms were effective in detecting metritis, depression, digestive problems and ketosis whereas -0.15 cutoff alarms were effective in detecting mastitis, metritis, depression, digestive problems, and ketosis on the day previous to diagnosis.

In conclusion, for detection of sick cows on the same day that they were diagnosed by farm personnel or on the day prior to diagnosis, we found that an alarm based on a combination of deviations in rumination, activity and milk yield data was most efficient to detect sick cows.

LITERATURE CITED

Abeni, F., L. Calamari and L. Stefanini. 2007. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. Int J Biometeorol 52:87-96.

Acatincai, S., D. Gavojdian, G. Stanciu, L.T. Cziszter,I. Tripon, and S. Baul. 2010. Study regarding rumination behavior in cattle- position adopted by cows during rumination process. Animal Science and Biotechnologies. 43:199-202.

Acatincai, S., D. Gavojdian, L. T. Cziszter, I. Tripon, A. Alungel and C. Popian. 2009. Study regarding rumination behaviour in multiparous Romanian black and white cows during summer season. Lucrari stiintifice Zootehnie si Biotehnologii. 42:191-193.

Adin, G., R. Solomon, M. Nikbachat, A. Zenou, E. Yosef, A. Brosh, A. Shabtay, S.J. Mabjeesh, I. Halachmi, and J. Miron. 2009. Effect of feeding cows in early lactation with diets differing in roughage-neutral detergent fiber content on intake behavior, rumination, and milk production. J. Dairy Sci. 92:3364-3373.

Aikman, P.C., C.K. Reynolds and D.E. Beever. 2008. Diet digestibility, rate of passage, and eating and rumination behavior of jersey and Holstein cows. J. Dairy Sci. 91:1103-1114.

Albright, J.L. 1993. Feeding behavior of dairy cattle. J. Dairy. Sci. 76:485-498.

Bar, D. 2010. Daily rumination time and calving diseases. Proc. 26th world Buiatrics cong., Santiago Chile. http://www.kenes.com/buiatrics/cd/pdf/153.pdf. Accessed Nov. 23, 2013.

Bar, D., and R. Solomon. 2010. Rumination collars; What they tell us? Proc. 1st N. Amer. Conf. Precision Dairy Mgmt., Toronto, Canada.

Beauchemin, K.A. and W.Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. J. Dairy Sci. 88:2117-2129.

Benzaquen, M.E., C.A. Risco, L.F. Archbald, P. Melendez, M.J. Thatcher, and W.W. Tharcher. 2007. Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J. Dairy Sci. 90:2804-2814.

Bikker, J.P., H. van Laar, P. Rump, J. Doorenbos, K. van Meurs, G.M. Griffioen, and J. Dijkstra. 2014. Evaluation of an ear attached movement sensor to record cow feeding behavior and activity. J. Dairy Sci. 97:2974-2979.

Bohmanova, J., I. Misztal, and J.B. Cole. 2007. Temperature-Humidity Indices as Indicators of milk production losses due to heat stress. J. Dairy Sci. 90:1947-1956.

Borchers, M.R., A.E. Sterrett, B.A. Wadsworth, and J.M. Bewley. 2014. Predicting impending calving using automatically collected measures of activity and rumination in dairy cattle. J. Dairy Sci. 97.(E-Suppl. 1):179.

Bouraoui, R., M. Lahmar, A. Majdoub, M. Djemali, and R. Belyea.2002. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Animal Research 51:479-491.

Braun, U., L. Trosch, F. Nydegger, and M. Hassig. 2013. Evaluation of eating and rumination behaviour in cows using a noseband pressure sensor. BMC Vet. Res. 9:164.

Brscic. M., F. Gottardo, A. Mazzenga and G. Cozzi. 2007. Behavioral response to different climatic conditions of beef cattle in intensive rearing systems. POLJOPRIVREDA 13: 103-106.

Buchel, S. 2013. Sensor based control of chewing and rumination behavior of dairy cows. PhD Thesis. University of Kessel, Hasse, Germany.

Buchel, S., and A. Sundrum. 2014. Decrease in rumination time as an indicator of the onset of calving. J. Dairy Sci. 97:3120-3127.

Buchel, S., and A. Sundrum. 2014. Evaluation of a new system for measuring feeding behavior of dairy cows. Comput Electron Agr.108:12-16.

Burfeind, Q., K. Schirmann, M.A.G. von Keyserlingk, D.M. Veira, D.M. Weary, and W. Heuwieser. 2011. Evaluation of a system for monitoring rumination in heifers and calves. J. Dairy Sci. 94:426-430.

Byskov, M.V., A.K.S. Schulze, M. R. Weisbjerg, B. Markussen, and P. Norgaard. 2014. Recording rumination time by a rumination monitoring system in jersey heifers fed grass/clover silage ad hay at three feeding levels. J. Anim. Sci. 92:1110-1118.

Calamari, L., N. Soriani, G. Panella, F. Petrera, A. Minuti, and E. Trevisi. 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. J. Dairy Sci. 97:3635-3647.

Carraway, K., M. Brauneis, N. Engwall, and J. Fain. 2013. Rumination monitoring: A management tool for early detection of metabolic disorders. J. Dairy Sci. 96(E-Suppl. 1):180.

Chapinal, N., C.E. Fitzpatrick, K.E. Leslie, and S.A. Wagner. 2014. Automated assessment of the effect of flunixin meglumine on rumination in dairy cows with endotoxin-induced mastitis. Can. J. Anim. Sci. 94:21-25.

Chandler, T. L., R. S. Pralle, G. R. Oetzel, R. H. Fourdraine and H. M. White. 2015. Development of a ketosis prevalence tool in Holstein dairy cows based on milk component data and cow test-day information. J. Dairy Sci. 98, Suppl.2: 507.

Clark, C.E.F., N.A. Lyons, L. Millapan, S. Talukder, G.M. Cronin. 2015. Rumination and activity levels as predictors of calving for dairy cows. Animal. 9:691-695.

Clement, P., R. Guatteo, L. Delaby, B. Rouille, A. Chavallon, J.M. Philipot, and N. Bareille. 2014. Added value of rumination time for the prediction of dry matter intake in lactating dairy cows. J. Dairy Sci. 97:6531-6535.

DeVries T.J., and E. Chevaux. 2014. Modification of the feeding behavior of dairy cows through live yeast supplementation. J. Dairy Sci. 97(E-Suppl. 1):788.

DeVries, T.J., K.A. Beauchemin, F. Dohme, and K.S. Schwartzkopf-Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. J. Dairy Sci. 92:5067-5078.

Dollinger, J., and O. Kaufmann. 2013. Feeding behaviour in dairy cows with and without the influence of clinical diseases or subclinical disorders. Archiv Tierzucht. 56: 149-159.

Duffield, T.F., D.F. Kelton, K.E. Leslie, K.D. Lissemore, J.H. Lumsden. 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. Can Vet J. 38:713-718.

Fitzpatrick, C.E., N. Chapinal, C.S. Petersson-Wolfe, T.J. DeVries, D.F. Kelton, T.F. Duffield and K.E. Leslie. 2013. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. J. Dairy Sci. 96:2847-2856.

Edwards J.L. and P. R. Tozer. 2004. Using activity and milk yield as predictors of fresh cow disorders. J. Dairy Sci. 87:524-531.

Elischer, M.F., M.E. Arceo, E.L. Karcher, and J.M. Siegford. 2013. Validating the accuracy of activity and rumination monitor data from dairy cows housed in a pasture based automatic milking system. J. Dairy Sci. 96: 6412-6422.

Fitzpatrick, C.E., N. Chapinal, C.S. Petersson-Wolfe, T.J. DeVries, D.F. Kelton, T.F. Duffield, and K.E. Leslie. 2013. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. J. Dairy Sci. 96:2847-2856.

Goldhawk, C., K. Schwartzkopf-genswein, and K.A. Beauchemin. 2013. Validation of rumination collars for beef cattle. J. Anim. Sci. 91:2858-2862.

Gonzalez, L.A., X. Manteca, S. Calsamiglia, K.S. Schwartzkopf-Genswein, and A. Ferret. 2012. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior. Anim Feed Sci Tech.172:66-79.

Gregorini, P., B. Dela Rue, M. Pourau, C. Glassey, and J. Jago. 2013. Livestock Sci. 158:151-156.

Gregorini, P., B. DelaRue, K. McLeod, C.E.F. Clark, C.B. Glassey, and J. Jago. 2012. Rumination behavior of grazing dairy cows in response to restricted time at pasture. Livestock Sci. 146:95-98.

Johnson M.M. and J.P. Peters.1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. J Anim Sci. 71:753-756.

Karp, H.J. and C. Peterson-Wolfe. 2010. Use of Milk Lactose contentration as an indicator of mastitis following the validation of a Novel In-line milk analysis system designed to measure milk components. The First North American Conference on Precision Dairy Management 2010.

Kaufman, E. I., S. J. LeBlanc, B. W. McBride, T. F. Duffield, and T. J. DeVries. 2015. Monitoring rumination in transition dairy cows for the early detection of subclinical ketosis. J. Dairy Sci. 98, Suppl. 2: 506.

Kononoff, P.J., H.A. Lehman, and A.J. Heinrichs. 2002. A comparison of methods used to measure eating and ruminating activity in confined dairy cattle. J. Dairy Sci. 85:1801-1803.

Leblanc, S. 2010. Monitoring metabolic health of dairy cattle in transition period. J. Reprod. Dev. 56: S29-S35.

Lessire, F., J.L. Hornick and I. Dufrasne. 2014. Effects of mild heat stress periods on milk production, milking frequency and rumination time of grazing dairy cows milked by a mobile automatic system. Grassland science in Europe. 19:181-183.

Liboreiro, D.N., K.S. Machado, P. Basso Silva, M.M. Filho, G. Franco, A.E. Barreto, M.I Endres and R.C. Chebel. 2014. Use of peripartum period cud chewing and activity data for diagnosis of health disorders. J.Dairy Sci. Vol.97, E-Suppl.1 P. 400.

Liboreiro, D.N., K.S. Machado, P. Basso Silva, M.M. Filho, G. Franco, A.E. Barreto, M.I Endres and R.C. Chebel. 2014. Association among peripartum health parameters, cud chewing, and activity. J.Dairy Sci. Vol.97, E-Suppl.1 P. 412.

Lindgren, E. 2009. Validation of rumination measurement equipment and the role of rumination in dairy cow time budgets. Master's Thesis. Swedish university of agricultural sciences, Uppsala, Sweden.

Martinez N., C.A. Risco, F.S. Lima, R.S. Bissinotto, L.F. Greco, L.F. Ribeiro, F Maunsell, K.N. Galvão, J.E.P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile and neutrophil function of dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158-7172.

Mooney, C.S., and M.S. Allen. 2007. Effect of dietary strong ions on chewing activity and milk production in lactating dairy cows. J. Dairy Sci. 90:5610-5618.

Norring, M., A. Valros, and L. Munksgaard. 2012. Milk yield affects time budget of dairy cows in tie-stalls. J. Dairy Sci. 95:102-108.

Pahl, C., E. Hartung, A. Grothmann, K. Mahlkow-Nerge and A. Haeussermann. 2014. Rumination activity of dairy cows in the 24 hours before and after calving. J. Dairy Sci. 97:1-7.

Pahl, C., E. Hartung, K. Mahlkow-Nerge and A. Haeussermann. 2015. Feeding characteristics and rumination time of dairy cows around estrus. J. Dairy Sci. 98:1-7.

Prendiville, R., E. Lewis, K.M. Pierce, and F. Buckley. 2010. Comparative grazing behavior of lactating Holstein-Friesian, Jersey, and Jersey X Holstein-Friesian dairy cows and its association with intake capacity and production efficiency. J. Dairy. Sci. 93:764-774.

Rengman, S., B. Johansson, L. Karlsson, M. Murphy, A. Sterk and E. Weurding. 2013. Rumination times in balanced dairy cow rations. J. Dairy Sci. 96. (Esuppl. 1):509.

Reith, S., H. Brandt, and S. Hoy. 2014. Simultaneous analysis of activity and rumination time, based on collar- mounted sensor technology, of dairy cows over the peri-estrus period. Livestock Science. 170:219-227.

Reith, S., and S. Hoy. 2012. Relationship between daily rumination time and estrus of dairy cows. J. Dairy Sci. 95:6416-6420.

Ruckebusch, Y. 1993. Motility of the gastrointestinal tract. In : Church D.C. (Ed). The Ruminant Animal Digestive Physiology and Nutrition (Pp 64-107). Waveland press, Illinois, USA.

Schirmann, K., M.A.G. von Keyserlingk, D.M. Weary, D.M. Veira, and W. Heuwieser. 2009. Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052-6055.

Schirmann, K., N. Chapinal, D.M. Weary, L. Vickers, and M.A.G. von Keyserlingk. 2013a. Rumination and feeding behavior before and after calving in dairy cows. J. Dairy Sci.96:7088-7092.

Schirmann, K., N. Chapinal, D.M. Weary, W. Heuwieser, and M.A.G. von Keyserlingk. 2012. Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. J. Dairy Sci. 95:3212-3217.

Schirmann, K., N. Chapinal, W. Heuwieser, D.M.Weary, and M.A.G. von Keyserlingk. 2013b. Rumination and feeding behavior before calving can identify cows at risk for metritis and subclinical ketosis after calving. J. Dairy Sci. 96. (Esuppl. 1):676.

Siivonen, J., S. Taponen, M. Hovinen, M. Pastell, B.J. Lensink, S. Pyorala, L. Hanninen. 2011.Impact of acute clinical mastitis on cow behaviour. Applied Animal Behavior Science. 132:101-106.

Sjaastad, O.V., K. Hove, and O. Sand. 2003. Physiology of Domestic Animals, pp. 507-527. Scandinavian Veterinary Press. Oslo.

Sjostrom, L.S., B.J. Heins, M.I. Endres, R.D. Moon, and J. Paulson. 2014. Effect of organic grain supplementation on activity and rumination time of organic dairy cows. J. Dairy Sci. 97(ESuppl. 1):281.

Smith, D.L., T. Smith, B.J. Rude, and S.H. Ward. 2013. Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. J. Dairy Sci. 96:3028-3033.

Soriani, N., D. Bar, L. Calamari, and G. Tadini. 2013a. Rumination time: an indicator of health status and welfare condition. Proceedings of the precision dairy conference and expo, Rochester, Minnesota. June 26-27, 2013. Pp: 91-92.

http://precisiondairy.umn.edu/prod/groups/cfans/@pub/@cfans/@ansci/documents/asset/cfans_asset_463117.pdf .

Soriani, N., E. Trevisi, and L. Calamari. 2012. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. J. Anim. Sci. 90:4544-4554.

Soriani, N., G. Panella, and L. Calamari. 2013b. Rumination time during the summer season and its relationships with metabolic conditions and milk production. J. Dairy Sci. 96:5082-5094.

Stangaferro M.L., R. Wijma, C. E. Quinteros, M. M. Medrano, M. Masello, and J. O. Giordano. 2015. Use of rumination and activity monitoring for the identification of dairy cows with health disorders. J. Dairy Sci. 98 Suppl. 2: 506.

Sterrett, A.E., B.A. Wadsworth, R.J. Harmon, M. Arnold, J.D. Clark, E.P. Aalseth, D.L. Ray and J.M. Bewley. 2014. Detection of subclinical milk fever and ketosis in fresh dairy cows using rumination time, lying time, reticulorumen temperature, and neck activity. J. Dairy Sci. 97(E-Suppl. 1):574.

Van Hertem, T., E. Maltz, A. Antler, C.E.B. Romanini, S. Viazzi, C. Bahr, A. Schlageter-Tello, C. Lokhorst, D. Berckmans and I. Halachmi. 2013. Lameness detection based on multivariate continuous sensing of milk yield, rumination, and neck activity. J. Dairy Sci. 96:4286-4298.

Van Hertem, T., Y. Parmet, M. Steensels, E. Maltz, A. Antler, A.A. Schlageter-Tello, C. Lokhorst, C.E.B. Romanini, S. Viazzi, C. Bahr, D. Berckmans, and I. Halachmi. 2014. The effect of routine hoof trimming on locomotion score, ruminating time, activity, and milk yield of dairy cows. J. Dairy Sci. 97:4852-4863.

Von Keyserlingk M.A.G., K.L. Proudfoot, L. Vickers, and D.M.Weary. 2011. Using cow behaviour to predict disease. WCDS Advances in Dairy Technology. 23:61-69.

Von Keyserlingk, M.A.G., and D.M. Weary. 2011. Identifying transition cows at risk and how best to manage them. Proceedings of tri state dairy nutrition conference 2011. http://tristatedairy.osu.edu/Proceedings%202011/von%20Keyserlingk%20paper.pdf Assessed 18th Jan. 2015.

Welch J.G., and A.M. Smith. 1972. Milk fat depression and rumination stimulation. J. Dairy Sci. 58:678-681.

Welch, J.G., A.M. Smith, and K.S. Gibson. 1982. Rumination time in four breeds of dairy cattle. J. Dairy Sci. 53:89-91.

Table 1: Sensitivity and Specificity of rumination cow and mates alarms for different health disorders

	Cow alarm			Herd alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC[1]**
Analysis on the day of diagnosis: - 0.10 cutoff									
Mastitis	56.1	77.3	0.64	63.4	76.8	0.68	63.4	66.5	0.65
Metritis	38.7	77.3	0.55	41.9	76.7	0.57	45.2	66.4	0.56
Milk fever	100	77.3	0.86	100	76.7	0.86	100	66.4	0.83
Dep Dehy.[2]	46.6	77.3	0.59	50	76.7	0.61	50	66.5	0.58
Digestive	43.5	77.3	0.58	48.4	76.8	0.60	50	66.5	0.58
Lameness	66.7	77.3	0.69	55.6	76.7	0.64	66.7	66.4	0.67
Ketosis	60.5	77.3	0.66	67.4	76.8	0.70	72.1	66.4	0.69
Sick	**50**	**77.58**	**0.61**	**53.98**	**77.08**	**0.63**	**56.6**	**66.7**	**0.61**
Analysis on day previous to diagnosis: -0.10 cutoff									
Mastitis	64.1	78.8	0.71	66.7	78.2	0.72	66.7	73.1	0.69
Metritis	27.6	78.8	0.53	20.7	78.1	0.51	31.0	73.1	0.52
Milk fever	100	78.8	0.89	100	78.1	0.89	100	73.1	0.87
DepDehy.[2]	33.3	78.8	0.56	36.7	78.1	0.57	40	73.1	0.57
Digestive	32.8	78.8	0.58	42.6	78.2	0.60	42.6	73.1	0.58
Lameness	44.4	78.8	0.62	44.4	78.1	0.61	50.0	73.1	0.62
Ketosis	34.1	78.8	0.56	45.5	78.2	0.62	50.0	73.1	0.62
Sick	**38.12**	**78.96**	**0.59**	**43.1**	**78.4**	**0.61**	**45.7**	**73.3**	**0.59**
Analysis on the day of diagnosis: -0.15 cutoff									
Mastitis	51.2	85.1	0.68	48.8	85.0	0.67	51.2	81.0	0.66
Metritis	29.3	85.1	0.57	29.1	84.9	0.57	29.1	81.0	0.55
Milk fever	100	85.1	0.93	100	84.9	0.93	100	81.0	0.91
DepDehy.[2]	40.0	85.1	0.63	40	85.00	0.63	40	81.0	0.61
Digestive	40.3	85.1	0.63	41.9	85.1	0.64	41.9	81.0	0.62
Lameness	44.4	85.1	0.65	50.0	85.0	0.68	55.6	81.0	0.68
Ketosis	46.5	85.1	0.66	58.1	85.1	0.72	62.8	81.1	0.72
Sick	**41.6**	**85.4**	**0.64**	**44.3**	**85.3**	**0.65**	**46.5**	**81.3**	**0.60**
Analysis on day previous to diagnosis: -0.15 cutoff									
Mastitis	53.9	86.7	0.70	53.9	86.5	0.70	56.4	82.8	0.70
Metritis	27.6	86.7	0.58	17.2	86.4	0.52	27.6	82.8	0.55
Milkfever	100	86.7	0.93	100	86.4	0.93	100	82.8	0.91
DepDehy.[2]	33.3	86.7	0.60	30	86.4	0.58	33.3	82.8	0.58
Digestive	27.9	86.7	0.57	34.4	86.5	0.60	36.1	82.8	0.59
Lamenes	44.4	86.7	0.66	33.3	86.4	0.60	44.4	82.8	0.64
Ketosis	34.1	86.7	0.60	34.1	86.4	0.60	38.6	82.8	0.61
Sick	**34.1**	**86.9**	**0.61**	**34.1**	**86.6**	**0.61**	**38.1**	**83.0**	**0.61**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 2: Average rumination indices

	Cow index (CIx)			Mates index (MIx)		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day diagnosis						
Mastitis	0.049	-0.165	0.072	0.0004	-0.1832	<0.0001
Metritis	0.049	-0.029	0.568	0.0002	-0.1008	0.0031
Milk fever	0.049	-0.513	0.071	0.0001	-0.4245	<0.0001
Depressed dehydrated fever	0.049	-0.048	0.485	0.0002	-0.1136	0.0011
Digestive	0.049	-0.022	0.462	0.0014	-0.1015	<0.0001
Foot problems	0.049	-0.098	0.413	0.0001	-0.1485	0.0009
Ketosis	0.049	-0.081	0.264	0.0003	-0.1476	<0.0001
Analysis on a day previous to diagnosis						
Mastitis	0.039	-0.162	0.047	-0.001	-0.167	<0.0001
Metritis	0.039	0.121	0.480	-0.001	0.018	0.5616
Milk fever	0.039	-0.518	0.031	-0.001	-0.446	<0.0001
Depressed dehydrated fever	0.039	0.015	0.840	-0.001	-0.044	0.1736
Digestive	0.039	0.037	0.986	-0.001	-0.064	0.0058
Foot problems	0.039	-0.087	0.400	-0.001	-0.099	0.0151
Ketosis	0.038	-0.012	0.598	-0.001	-0.104	<0.0001

Table 3: Sensitivity and Specificity of activity cow alarms for different health disorders

	Cow alarms		
	Sensitivity	Specificity	AUC[1]
Analysis on the day of diagnosis: -0.10 cutoff			
Mastitis	33.3	81.2	0.58
Metritis	50	81.2	0.66
Milk fever	71.4	81.2	0.76
Dep. Dehy.[2]	31.3	81.2	0.56
Digestive	47.6	81.3	0.65
Foot problems	44.4	81.2	0.71
Ketosis	47.7	81.2	0.65
Sick	41.8	81.4	0.63
Analysis on a day previous to diagnosis: -0.10 cutoff			
Mastitis	33.3	82.7	0.59
Metritis	43.9	82.7	0.63
Milkfever	42.9	82.7	0.63
Dep.Dehy.[2]	34.4	17.3	0.59
Digestive	46.0	82.8	0.65
Footproblems	33.3	82.7	0.67
Ketosis	40.9	82.7	0.63
Sick	38.4	82.9	0.62
Analysis on the day of diagnosis: -0.15 cutoff			
Mastitis	28.6	88.2	0.59
Metritis	46.9	88.2	0.68
Milkfever	71.4	88.2	0.80
Dep.Dehy. [2]	28.1	88.2	0.58
Digestive	33.3	88.3	0.61
Footproblems	38.9	88.2	0.72
Ketosis	36.4	88.2	0.63
Sick	33.2	88.5	0.62
Analysis on the day previous to diagnosis: -0.15 cutoff			
Mastitis	26.2	89.6	0.59
Metritis	40.6	89.6	0.63
Milkfever	28.6	89.5	0.59
Dep.Dehy.[2]	25	89.6	0.57
Digestive	36.5	89.6	0.64
Footproblems	27.8	89.6	0.69
Ketosis	29.6	89.6	0.60
Sick	**28.5**	**89.8**	**0.61**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 4: Average activity indices

	Cow index		
	Healthy	**Sick**	**P value**
Analysis on the day diagnosis			
Mastitis	0.015	-0.004	0.567
Metritis	0.015	-0.057	0.065
Milk fever	0.015	-0.219	0.005
Depressed dehydrated fever	0.015	0.011	0.019
Digestive	0.016	-0.076	0.0012
Foot problems	0.015	-0.087	0.0521
Ketosis	0.015	-0.062	0.0208
Analysis on the day previous to diagnosis			
Mastitis	0.015	-0.036	0.113
Metritis	0.015	-0.0769	0.0137
Milk fever	0.015	-0.106	0.126
Depressed dehydrated fever	0.015	-0.028	0.245
Digestive	0.015	-0.076	0.0005
Foot problems	0.015	-0.038	0.276
Ketosis	0.015	-0.038	0.094

Table 5: Sensitivity and Specificity of milk cow and mates alarms for different health disorders

	Cow alarm			Herd alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC**[1]	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
Analysis on the day of diagnosis: -0.10 cutoff									
Mastitis	59.4	77.1	0.68	53.1	82.6	0.68	63.4	66.5	0.68
Metritis	66.7	77.1	0.72	46.7	82.6	0.65	45.2	66.4	0.71
Milk fever	100	77.0	0.89	75	82.5	0.79	100	66.4	0.88
Dep. Dehy.[2]	59.3	77.0	0.68	51.9	82.6	0.67	50	66.5	0.67
Digestive	61.4	77.1	0.69	49.1	82.7	0.66	50	66.5	0.71
lameness	26.7	77.0	0.52	20	82.5	0.51	66.7	66.5	0.54
Ketosis	60.5	77.1	0.69	48.8	82.6	0.66	72.1	66.5	0.68
Sick	58.6	77.5	0.68	46.3	83.0	0.65	56.6	66.7	0.68
Analysis on the day previous to diagnosis: -0.10 cutoff									
Mastitis	47.1	80.3	0.64	35.3	84.2	0.60	47.1	78.3	0.63
Metritis	48.3	80.3	0.64	27.6	84.2	0.56	51.7	78.3	0.65
Milkfever	20.0	80.3	0.50	40	84.2	0.62	40	78.3	0.59
Dep.Dehy.[2]	37.0	80.3	0.59	25.9	84.2	0.55	37.1	78.3	0.58
Digestive	44.6	80.4	0.63	30.4	84.3	0.57	50	78.4	0.64
lameness	20	80.3	0.50	13.3	84.2	0.51	20.0	78.2	0.51
Ketosis	45.5	80.4	0.63	38.6	84.3	0.62	45.5	78.3	0.62
Sick	42.4	80.6	0.62	31.2	84.5	0.58	45.4	78.6	0.62
Analysis on the day of diagnosis: -0.15 cutoff									
Mastitis	59.4	85.5	0.73	53.1	89.1	0.71	59.4	84.6	0.72
Metritis	53.3	85.5	0.69	36.7	89.0	0.63	56.7	84.6	0.71
Milkfever	75	85.4	0.80	75	89.0	0.82	75	84.5	0.80
Dep.Dehy.[2]	51.8	85.5	0.69	37.0	89.0	0.63	51.9	84.6	0.68
Digestive	47.4	85.6	0.67	31.6	89.1	0.60	49.1	84.7	0.67
lameness	20	85.4	0.53	13.3	89.0	0.51	20	84.5	0.52
Ketosis	48.8	85.5	0.67	34.9	89.1	0.62	48.8	84.6	0.67
Sick	47.8	86.0	0.67	35.0	89.4	0.62	48.8	85.1	0.67
Analysis on a day previous to diagnosis: -0.15 cutoff									
Mastitis	44.1	88.0	0.66	29.4	90.8	0.60	44.1	86.9	0.66
Metritis	41.4	88.0	0.65	13.8	90.8	0.52	41.4	86.6	0.64
Milkfever	20.0	88.0	0.54	40	90.8	0.65	40	86.8	0.63
Dep.Dehy.[2]	33.3	88.0	0.61	14.8	90.8	0.53	33.3	86.8	0.60
Digestive	37.5	88.1	0.63	17.9	90.8	0.54	39.3	86.9	0.63
lameness	20	88.0	0.54	13.3	90.8	0.52	20.0	86.8	0.53
Ketosis	34.1	88.0	0.61	25	90.8	0.58	34.1	86.8	0.61
Sick	**36.1**	**88.3**	**0.62**	**21.0**	**91.0**	**0.56**	**37.1**	**87.2**	**0.62**

[1]AUC =Area under the curve
[2] Dep. Dehy. = Depressed and/or dehydrated

Table 6: Average milk indices

	Cow index			Mates index		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day of diagnosis						
Mastitis	-0.014	-0.277	<0.001	-0.003	-0.175	<0.0001
Metritis	-0.014	-0.115	0.0346	-0.003	-0.070	0.0148
Milk fever	-0.014	-0.522	<0.0001	-0.003	-0.515	<0.0001
Depressed dehydrated fever	-0.015	-0.110	0.0554	-0.003	-0.053	0.0839
Digestive	-0.015	-0.113	0.0041	-0.003	0.080	0.0001
Foot problems	-0.015	-0.007	0.0907	-0.003	-0.035	0.4140
Ketosis	-0.015	-0.133	0.0028	-0.003	-0.084	0.0004
Analysis on the day previous to diagnosis						
Mastitis	-0.007	-0.144	0.001	-0.002	-0.079	0.001
Metritis	-0.007	-0.030	0.594	-0.002	-0.028	0.30
Milk fever	-0.007	-0.103	0.362	-0.002	-0.141	0.0192
Depressed dehydrated fever	-0.007	-0.033	0.561	-0.002	-0.021	0.466
Digestive	-0.007	-0.039	0.312	-0.002	-0.048	0.009
Foot problems	-0.007	0.010	0.785	-0.002	0.004	0.859
Ketosis	-0.007	-0.085	0.029	-0.002	-0.053	0.011

Table 7: Sensitivity and Specificity of milk fat cow and mates alarms for different health disorders

	Cow alarm			Herd alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
Analysis on the day of diagnosis: ±0.10 cutoff									
Mastitis	60.9	72.7	0.67	69.6	74.7	0.72	47.8	68.3	0.69
Metritis	47.8	72.6	0.60	56.5	74.6	0.61	66.7	68.3	0.62
Milk fever	66.7	72.6	0.70	66.7	74.6	0.71	66.7	68.3	0.68
Dep. Dehy.[2]	34.8	72.6	0.54	30.4	74.6	0.53	39.1	68.3	0.54
Digestive	47.1	72.7	0.60	45.1	74.7	0.60	47.1	68.3	0.58
lameness	9.1	72.6	0.59	18.2	74.7	0.54	18.2	68.2	0.57
Ketosis	46	72.6	0.59	54.1	74.7	0.64	56.7	68.3	0.63
Sick	43.2	72.8	0.58	45.6	74.9	0.60	48.5	68.5	0.59
Analysis on a day previous to diagnosis: ±0.10 cutoff									
Mastitis	56	75.0	0.66	56	76.6	0.66	56	71.0	0.64
Metritis	46.2	74.9	0.61	57.7	76.6	0.67	65.4	71.1	0.68
Milkfever	40	74.9	0.57	60	76.5	0.68	60	71.0	0.66
Dep.Dehy.[2]	41.7	74.9	0.58	33.3	76.5	0.55	45.8	71.0	0.58
Digestive	43.1	75.0	0.59	43.1	76.6	0.60	49.0	71.1	0.60
lameness	33.3	74.9	0.54	33.3	76.5	0.55	33.3	71.0	0.52
Ketosis	42.1	74.9	0.59	50	76.6	0.63	50	71.0	0.61
Sick	44.5	75.2	0.60	48.0	76.9	0.62	52.0	71.3	0.62
Analysis on the day of diagnosis: ±0.15 cutoff									
Mastitis	39.1	87.3	0.63	47.8	88.5	0.68	52.2	85.1	0.69
Metritis	13.0	87.3	0.50	26.1	88.5	0.57	26.1	85.1	0.56
Milkfever	66.7	87.3	0.77	66.7	88.4	0.78	66.7	85.1	0.75
Dep.Dehy.[2]	17.4	87.3	0.52	26.1	88.5	0.57	26.1	85.1	0.56
Digestive	29.4	87.4	0.58	37.2	88.5	0.63	41.2	85.2	0.63
lameness	9.1	87.3	0.52	9.1	88.4	0.51	9.1	85.1	0.53
Ketosis	10.8	87.3	0.51	32.4	88.5	0.61	35.1	85.1	0.60
Sick	21.9	87.4	0.55	31.4	88.7	0.60	33.7	85.4	0.60
Analysis on a day previous to diagnosis: ±0.15 cutoff									
Mastitis	44	88.8	0.66	40	89.5	0.65	44	86.6	0.65
Metritis	30.8	88.8	0.60	26.9	89.5	0.58	38.5	86.6	0.63
Milkfever	20	88.8	0.54	20	89.4	0.55	20	86.6	0.53
Dep.Dehy.[2]	16.7	88.8	0.53	20.8	89.4	0.55	20.8	86.6	0.54
Digestive	29.4	88.8	0.59	27.5	89.5	0.59	31.4	86.6	0.59
lameness	16.7	88.8	0.53	16.7	89.4	0.53	16.7	86.6	0.52
Ketosis	29.0	88.8	0.59	34.2	89.5	0.62	34.2	86.6	0.60
Sick	**27.8**	**89**	**0.58**	**27.8**	**89.7**	**0.59**	**31.2**	**86.8**	**0.59**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 8: Average milkfat indices

	Cow index			Mates index		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day of diagnosis						
Mastitis	0.014	0.039	0.245	0.001	0.029	0.209
Metritis	0.014	0.019	0.814	0.001	-0.006	0.728
Milk fever	0.014	-0.136	0.014	0.001	-0.194	0.001
Depressed dehydrated fever	0.014	0.017	0.887	0.001	-0.005	0.761
Digestive	0.013	0.086	<0.001	0.001	0.078	<0.0001
Foot problems	0.014	0.038	0.450	0.001	0.031	0.354
Ketosis	0.014	0.025	0.517	0.001	-0.002	0.868
Analysis on the day previous to diagnosis						
Mastitis	0.011	0.072	0.002	0.001	0.076	0.001
Metritis	0.011	0.031	0.301	0.001	0.001	0.445
Milk fever	0.011	0.017	0.883	0.001	0.107	0.715
Depressed dehydrated fever	0.011	0.015	0.815	0.001	0.010	0.667
Digestive	0.010	0.091	<0.001	0.001	0.086	<0.0001
Foot problems	0.011	0.039	0.325	0.001	0.034	0.254
Ketosis	0.010	0.093	<0.0001	0.001	0.079	<0.0001

Table 9: Sensitivity and Specificity of milk protein cow and mates alarms for different health disorders

	Cow alarm			Herd Alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
Analysis on the day of diagnosis: ±0.10 cutoff									
Mastitis	41.7	89.2	0.65	41.7	90.3	0.66	41.7	87.3	0.65
Metritis	43.5	89.2	0.66	43.5	90.3	0.67	43.5	87.3	0.65
Milk fever	100	89.1	0.95	100	90.2	0.95	100	87.3	0.94
Dep. Dehy.[2]	21.7	89.1	0.55	21.7	90.2	0.56	21.7	87.2	0.55
Digestive	20	89.2	0.55	22	90.2	0.56	24	87.3	0.56
Lameness	9.1	89.1	0.51	9.8	100	0.55	9.1	87.2	0.52
Ketosis	19.0	89.1	0.54	21.6	90.2	0.56	21.6	87.3	0.54
Sick	23.7	89.3	0.57	23.7	90.4	0.57	25.4	87.4	0.56
Analysis on a day previous to diagnosis: ±0.10 cutoff									
Mastitis	36	90.8	0.63	32	91.5	0.62	36	89.3	0.63
Metritis	34.6	90.8	0.63	26.9	91.5	0.59	38.5	89.3	0.64
Milkfever	80	90.8	0.85	100	91.5	0.96	100	89.3	0.95
Dep.Dehy.[2]	8.3	90.8	0.51	12.5	91.4	0.53	12.5	89.2	0.51
Digestive	24	90.8	0.57	14	91.5	0.53	24	89.3	0.57
Lameness	33.3	90.8	0.62	25	91.5	0.58	33.3	89.3	0.61
Ketosis	21.6	90.8	0.56	18.9	91.5	0.55	24.3	89.5	0.57
Sick	25.7	91.0	0.58	22.2	91.6	0.57	27.5	89.5	0.59
Analysis on the day of diagnosis: ±0.15 cutoff									
Mastitis	20.8	95.7	0.58	25	96.3	0.61	25	95.2	0.60
Metritis	30.4	95.7	0.63	26.1	96.3	0.61	34.8	95.2	0.65
Milkfever	100	95.7	0.98	100	96.3	0.98	100	95.2	0.98
Dep.Dehy.[2]	13.0	95.6	0.54	8.7	96.3	0.53	17.4	95.2	0.56
Digestive	8.0	95.6	0.52	6.0	96.3	0.51	8.0	95.2	0.52
Lameness	4.4	100	0.52	3.7	100	0.52	4.8	100	0.52
Ketosis	8.1	95.6	0.52	8.1	96.3	0.52	10.8	95.2	0.53
Sick	11.8	95.7	0.54	10.7	96.4	0.53	13.6	95.3	0.55
Analysis on a day previous to diagnosis: ±0.15 cutoff									
Mastitis	20	96.7	0.58	20	97.1	0.59	24	96.2	0.60
Metritis	7.7	96.7	0.52	11.5	97.1	0.54	11.5	96.1	0.54
Milkfever	40	96.7	0.68	40	97.1	0.69	40	96.1	0.68
Dep.Dehy.[2]	8.3	96.7	0.53	8.3	97.1	0.53	8.3	96.1	0.52
Digestive	12	96.7	0.54	8	97.1	0.53	12	96.1	0.54
Lameness	8.3	96.7	0.53	8.3	97.1	0.53	8.3	96.1	0.52
Ketosis	2.7	96.7	0.50	2.7	97.1	0.50	2.7	96.1	0.51
Sick	**9.4**	**96.8**	**0.53**	**8.8**	**97.1**	**0.53**	**10.5**	**96.2**	**0.53**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 10: Average milk protein indices

	Cow index			Mates index		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day of diagnosis						
Mastitis	0.014	0.042	0.063	0.001	0.032	0.031
Metritis	0.014	0.08	<0.0001	0.001	0.060	<0.0001
Milk fever	0.014	0.286	<0.0001	0.001	0.287	<0.0001
Depressed dehydrated fever	0.014	0.030	0.296	0.001	0.011	0.511
Digestive	0.014	0.041	0.007	0.001	0.027	0.009
Foot problems	0.014	0.014	0.991	0.001	-0.003	0.844
Ketosis	0.014	0.014	0.985	0.001	0.009	0.486
Analysis on the day previous to diagnosis						
Mastitis	0.010	0.069	<0.0001	0.0003	0.061	<0.0001
Metritis	0.010	0.666	<0.0001	0.0004	0.051	<0.0001
Milk fever	0.010	0.126	<0.0001	0.0005	0.103	0.0002
Depressed dehydrated fever	0.010	0.034	0.071	0.0005	0.0175	0.177
Digestive	0.010	0.050	<0.0001	0.0003	0.040	<0.0001
Foot problems	0.010	0.031	0.267	0.0005	0.0137	0.458
Ketosis	0.010	0.008	0.809	0.0005	0.0034	0.772

Table 11: Sensitivity and Specificity of lactose cow and mates alarms for different health disorders

	Cow alarm			Mates alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
Analysis on the day of diagnosis; ±0.10 cutoff									
Mastitis	34.8	95.0	0.65	34.8	95.6	0.65	34.8	94.7	0.65
Metritis	17.4	95	0.56	4.4	95.6	0.50	17.49	94.6	0.56
Milk fever	100	95	0.98	100	95.6	0.98	100	94.6	0.97
Dep. Dehy.[2]	13.1	95.0	0.54	4.4	95.6	0.50	13.0	94.6	0.54
Digestive	8	95.0	0.52	8	95.6	0.52	8	94.6	0.51
Lameness	5.0	100	0.53	4.5	100	0.52	5.4	100	0.53
Ketosis	5.4	95.0	0.50	5.4	95.6	0.51	5.4	95.6	0.50
Sick	12.5	94.7	0.54	10.1	95.6	0.53	12.5	94.7	0.54
Analysis on a day previous to diagnosis; ±0.10 cutoff									
Mastitis	13.0	95.8	0.54	13.1	96.1	0.55	13.0	95.2	0.54
Metritis	19.2	95.8	0.58	19.2	96.1	0.58	19.2	95.2	0.57
Milkfever	60	95.8	0.78	40	96.1	0.68	60	95.2	0.78
Dep.Dehy.[2]	8.3	95.8	0.52	8.3	96.1	0.52	8.3	95.2	0.52
Digestive	11.8	95.8	0.54	9.8	96.1	0.53	11.8	95.2	0.54
Lameness	4.2	100	0.52	3.9	100	0.52	4.8	100	0.52
Ketosis	13.9	95.8	0.55	11.1	96.1	0.54	13.9	95.2	0.55
Sick	11.2	95.9	0.54	9.4	96.2	0.53	11.2	95.3	0.53
Analysis on the day of diagnosis; ±0.15 cutoff									
Mastitis	26.1	97.6	0.62	26.1	98.0	0.62	26.1	97.5	0.62
Metritis	4.4	97.6	0.51	4.4	98.0	0.51	4.4	97.5	0.51
Milkfever	100	97.6	0.99	100	98.0	0.99	100	97.5	0.99
Dep.Dehy.[2]	4.4	97.6	0.51	4.4	98.06	0.51	4.4	97.5	0.51
Digestive	6	97.6	0.52	4	98.0	0.51	6	97.5	0.52
Lameness	2.5	100	0.51	2.1	100	0.51	2.5	100	0.51
Ketosis	5.4	97.6	0.52	5.4	98.0	0.52	5.4	97.5	0.52
Sick	8.3	97.6	0.53	7.7	98.0	0.53	8.3	97.6	0.53
Analysis on a day previous to diagnosis; ±0.15 cutoff									
Mastitis	4.4	98.2	0.51	4.4	98.2	0.51	4.4	97.9	0.51
Metritis	3.9	98.2	0.51	3.9	98.2	0.51	3.9	97.9	0.51
Milkfever	20	98.2	0.59	20	98.2	0.59	20	97.9	0.59
Dep.Dehy.[2]	8.3	98.2	0.53	8.3	98.2	0.53	8.3	97.9	0.53
Digestive	3.9	98.2	0.51	3.9	98.2	0.51	3.9	97.9	0.51
Lameness	1.8	100	0.51	1.8	100	0.51	2.1	100	0.51
Ketosis	5.6	98.2	0.52	5.6	98.2	0.52	5.6	97.9	0.52
Sick	**4.1**	**98.2**	**0.51**	**4.1**	**98.2**	**0.51**	**4.1**	**97.9**	**0.51**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 12: Average milk lactose indices

	Cow index			Mates index		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day of diagnosis						
Mastitis	-0.007	-0.079	<0.0001	-0.001	-0.070	<0.0001
Metritis	-0.007	-0.036	0.014	-0.001	-0.026	0.02
Milk fever	-0.007	-0.293	<0.0001	-0.001	-0.272	<0.0001
Depressed dehydrated fever	-0.007	-0.027	0.089	-0.001	-0.017	0.126
Digestive	-0.007	-0.033	0.001	-0.001	-0.023	0.003
Foot problems	-0.007	-0.017	0.546	-0.001	-0.016	0.330
Ketosis	-0.007	-0.018	0.243	-0.001	-0.014	0.126
Analysis on the day previous to diagnosis						
Mastitis	-0.005	-0.001	<0.0001	-0.0003	-0.049	<0.0001
Metritis	-0.005	-0.042	0.0001	-0.0004	-0.033	0.0003
Milk fever	-0.005	-0.114	<0.0001	-0.0004	-0.088	<0.0001
Depressed dehydrated fever	-0.005	-0.029	0.016	-0.0004	-0.024	0.014
Digestive	-0.005	-0.030	0.0003	-0.0003	-0.023	0.0005
Foot problems	-0.005	-0.005	0.979	-0.0004	-0.0006	0.992
Ketosis	-0.005	-0.029	0.004	-0.0004	-0.024	0.002

Table 13: Sensitivity and Specificity of fat by lactose cow and mates alarms for different health disorders

	Cow alarm			Mates alarm			Comb alarm		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
Analysis on the day of diagnosis: ±0.10 Cutoff									
Mastitis	77.8	58.8	0.68	80.3	60.3	0.72	83.3	53.2	0.68
Metritis	56.7	58.8	0.58	76.7	60.3	0.69	80	53.2	0.67
Milk fever	80	58.8	0.69	80	60.2	0.70	80	53.1	0.67
Dep. Dehy.[2]	51.7	58.8	0.55	48.3	60.2	0.54	55.2	53.1	0.54
Digestive	57.6	58.8	0.58	62.7	60.3	0.62	66.1	53.2	0.60
Foot problems	57.9	58.8	0.58	57.9	60.2	0.59	57.9	53.1	0.56
Ketosis	61.4	58.8	0.60	70.5	60.3	0.65	75	53.2	0.64
Sick	60.4	59.1	0.60	65.9	60.7	0.63	68.7	53.6	0.61
Analysis on day previous to diagnosis: ±0.10 cutoff									
Mastitis	69.4	61.8	0.66	72.2	62.8	0.68	72.2	57.0	0.65
Metritis	66.7	61.8	0.64	73.3	62.8	0.68	80	57.0	0.69
Milkfever	60	61.7	0.61	80	62.7	0.72	80	57.0	0.68
Dep.Dehy.[2]	55.2	61.8	0.59	55.2	62.8	0.59	62.1	57.0	0.60
Digestive	62.7	61.8	0.62	59.3	62.8	0.61	69.5	57.0	0.63
Footproblems	63.2	61.8	0.63	63.2	62.8	0.63	68.4	56.9	0.63
Ketosis	59.1	61.8	0.60	65.9	62.8	0.64	70.5	57.0	0.64
Sick	61.3	62.1	0.62	64.1	63.2	0.64	69.6	57.3	0.64
Analysis on the day of diagnosis: ±0.15 cutoff									
Mastitis	63.9	72.3	0.68	72.2	73.4	0.73	72.2	69.4	0.71
Metritis	46.7	72.2	0.60	56.7	73.4	0.65	60	69.4	0.65
Milkfever	80	72.2	0.76	80	73.3	0.77	80	69.3	0.75
Dep.Dehy.[2]	34.5	72.2	0.53	37.9	73.3	0.56	41.4	69.4	0.55
Digestive	44.1	72.3	0.58	47.5	73.4	0.60	50.9	69.4	0.60
Footproblems	52.6	72.2	0.62	52.6	73.3	0.63	52.6	69.4	0.61
Ketosis	43.2	72.2	0.58	56.8	73.4	0.65	56.8	69.4	0.63
Sick	46.5	72.5	0.60	52.1	73.8	0.63	53.5	69.7	0.62
Analysis on day previous to diagnosis: ±0.15 cutoff									
Mastitis	66.7	74.3	0.71	66.7	75.1	0.71	66.7	71.7	0.69
Metritis	53.3	74.2	0.64	60	75.0	0.68	63.3	71.7	0.68
Milkfever	40	74.2	0.57	60	75.0	0.68	60	71.6	0.66
Dep.Dehy.[2]	37.9	74.2	0.56	38.0	75.0	0.57	41.4	71.7	0.57
Digestive	47.5	74.3	0.61	44.1	75.0	0.60	47.5	71.7	0.60
Footproblems	47.4	74.2	0.61	52.6	75.0	0.64	52.6	71.6	0.62
Ketosis	54.5	74.3	0.64	54.6	75.1	0.65	54.6	71.7	0.63
Sick	**50.2**	**74.6**	**0.62**	**51.6**	**75.4**	**0.64**	**53.5**	**72.0**	**0.63**

[1]AUC =Area under the curve

[2] Dep. Dehy. = Depressed and/or dehydrated

Table 14: Average milk fat by lactose indices

	Cow index			Mates index		
	Healthy	**Sick**	**P value**	**Healthy**	**Sick**	**P value**
Analysis on the day of diagnosis						
Mastitis	0.025	0.162	<0.0001	0.002	0.158	<0.0001
Metritis	0.025	0.064	0.173	0.002	0.029	0.351
Milk fever	0.025	0.226	0.010	0.002	0.209	0.01
Depressed dehydrated fever	0.025	0.051	0.352	0.002	0.018	0.579
Digestive	0.025	0.135	<0.0001	0.0002	0.120	<0.0001
Foot problems	0.025	0.058	0.420	0.002	0.053	0.228
Ketosis	0.025	0.057	0.150	0.002	0.035	0.154
Analysis on day previous to diagnosis						
Mastitis	0.019	0.140	<0.0001	0.002	0.143	<0.0001
Metritis	0.019	0.085	0.006	0.002	0.066	0.011
Milk fever	0.019	0.158	0.012	0.002	0.160	0.006
Depressed dehydrated fever	0.019	0.054	0.171	0.002	0.046	0.093
Digestive	0.019	0.133	<0.0001	0.001	0.130	<0.0001
Foot problems	0.019	0.027	0.830	0.002	0.007	0.888
Ketosis	0.019	0.123	<0.0001	0.002	0.107	<0.0001

Table 15: Sensitivity and Specificity of serial and parallel combination of alarms for different health disorders

	RAM_SERIAL			RAM_PARALLEL		
	Sensitivity	**Specificity**	**AUC[1]**	**Sensitivity**	**Specificity**	**AUC[1]**
Analysis on the day of diagnosis; 0.10 cutoff						
Mastitis	21.1	98.3	0.60	81.6	49.2	0.65
Metritis	19.4	98.3	0.59	83.9	49.2	0.67
Milk fever	33.3	98.3	0.66	100	49.2	0.75
Dep. Dehy.[2]	6.9	98.3	0.53	79.3	49.2	0.64
Digestive	21.3	98.3	0.60	88.5	49.3	0.69
Foot problems	4.2	98.3	0.51	95.8	49.2	0.73
Ketosis	18.2	98.3	0.58	93.2	49.3	0.71
Sick	16.3	98.5	0.57	86.8	49.7	0.68
Analysis on a day previous to diagnosis; 0.10 cutoff						
Mastitis	13.2	98.5	0.56	76.3	52.3	0.64
Metritis	1.5	100	0.51	83.9	52.3	0.68
Milkfever	1.5	100	0.51	100	52.3	0.76
Dep.Dehy.[2]	1.5	100	0.51	78.3	52.3	0.66
Digestive	13.1	98.5	0.56	80.3	52.4	0.66
Footproblems	4.2	98.5	0.51	83.3	52.3	0.68
Ketosis	11.4	98.5	0.55	84.1	52.3	0.68
Sick	8.4	98.6	0.54	80.2	52.7	0.66
Analysis on the day of diagnosis; 0.15 cutoff						
Mastitis	21.1	99.2	0.60	68.4	65.4	0.67
Metritis	12.9	99.1	0.56	77.4	65.4	0.71
Milkfever	33.3	99.1	0.66	100	65.4	0.83
Dep.Dehy.[2]	6.9	99.1	0.53	62.1	65.4	0.64
Digestive	11.5	99.1	0.55	73.8	65.5	0.69
Footproblems	4.2	99.1	0.52	87.5	65.4	0.76
Ketosis	13.6	99.1	0.56	86.4	65.4	0.76
Sick	12.3	99.3	0.56	74.5	65.9	0.70
Analysis on a day previous to diagnosis; 0.15 cutoff						
Mastitis	5.3	99.3	0.52	68.4	68.8	0.69
Metritis	0.7	100	0.50	74.2	68.8	0.72
Milkfever	0.7	100	0.50	100	68.7	0.84
Dep.Dehy.[2]	0.7	100	0.50	68.9	68.8	0.69
Digestive	11.5	99.3	0.55	67.2	68.8	0.68
Footproblems	4.2	99.3	0.52	75	68.7	0.72
Ketosis	6.8	99.3	0.53	65.9	68.8	0.67
Sick	**5.3**	**99.3**	**0.52**	**67.4**	**69.2**	**0.68**

[1]AUC =Area under the curve
[2] Dep. Dehy. = Depressed and/or dehydrated

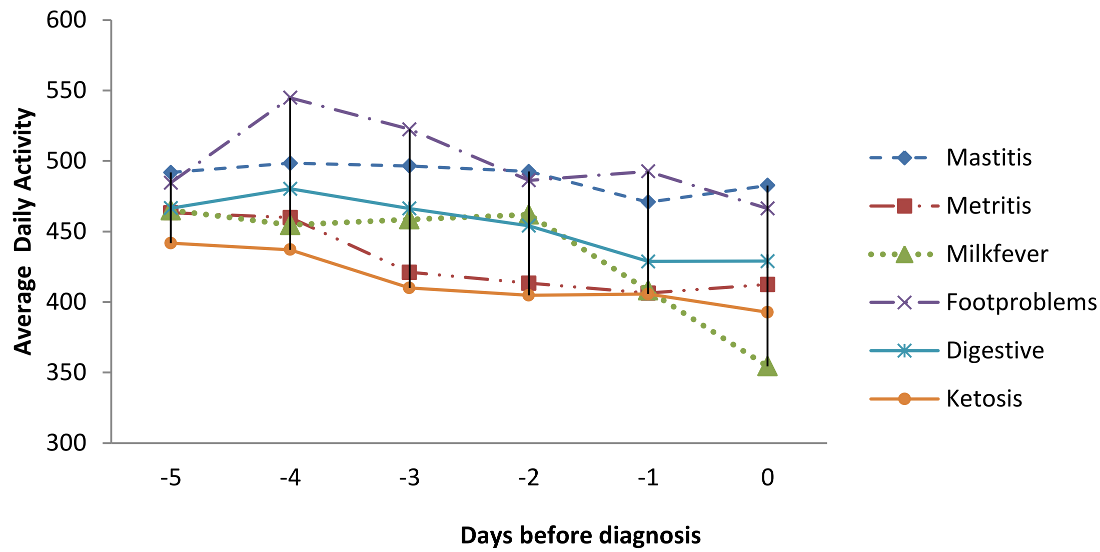

Figure 1: Average daily activity from d -5 to the day of disease diagnosis in affected cows

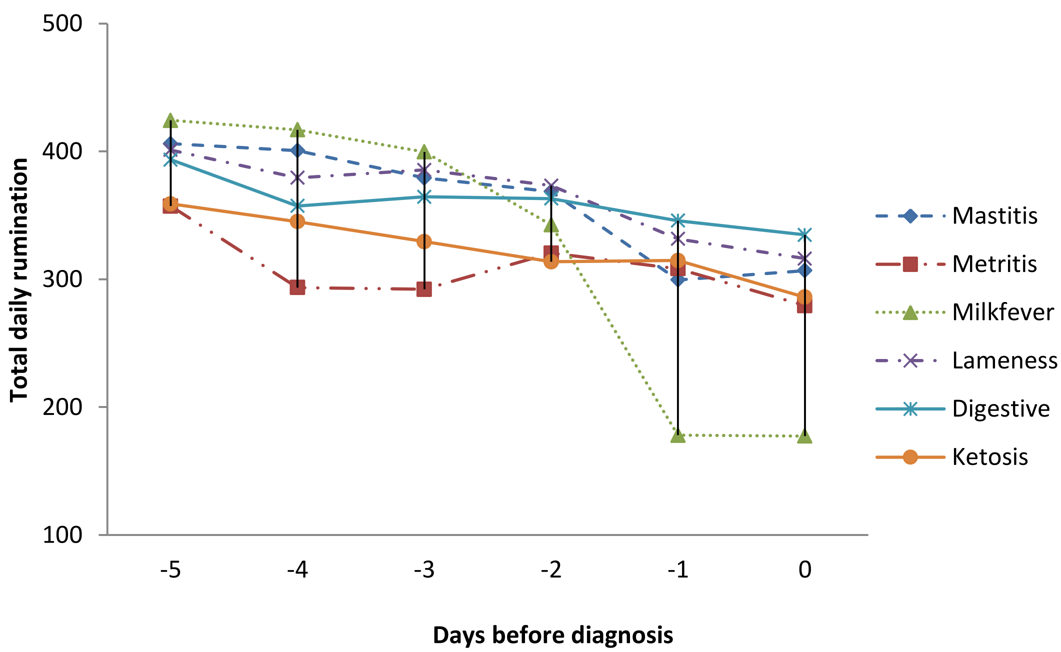

Figure 2: Average daily rumination time from d -5 to the day of disease diagnosis in affected cows

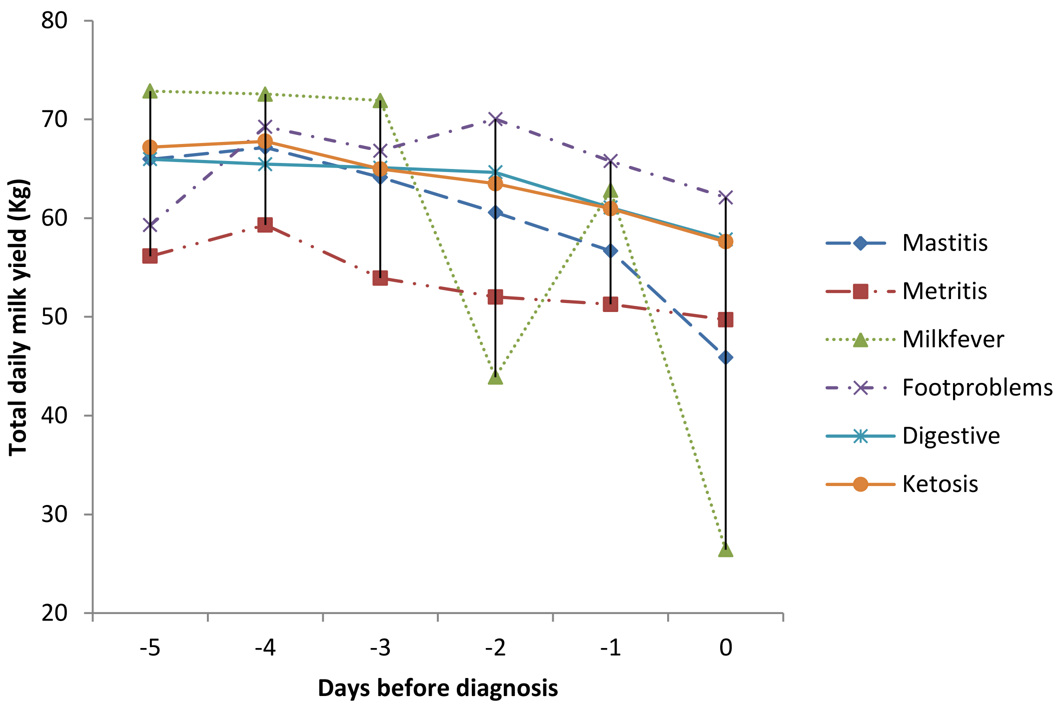

Figure 3: Total daily milk yield from d -5 to the day of disease diagnosis in affected cows

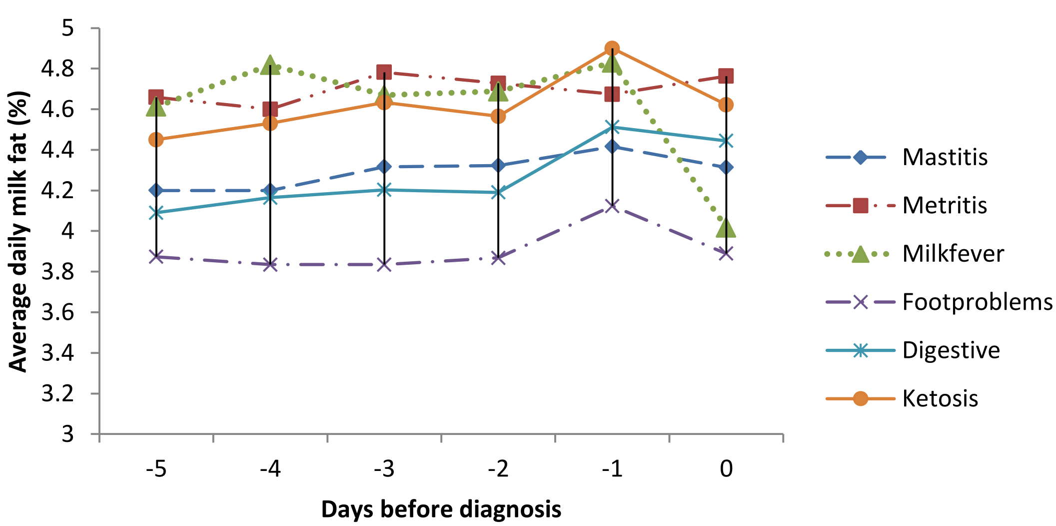

Figure 4: Average daily milk fat (%) from d -5 to the day of disease diagnosis in affected cows

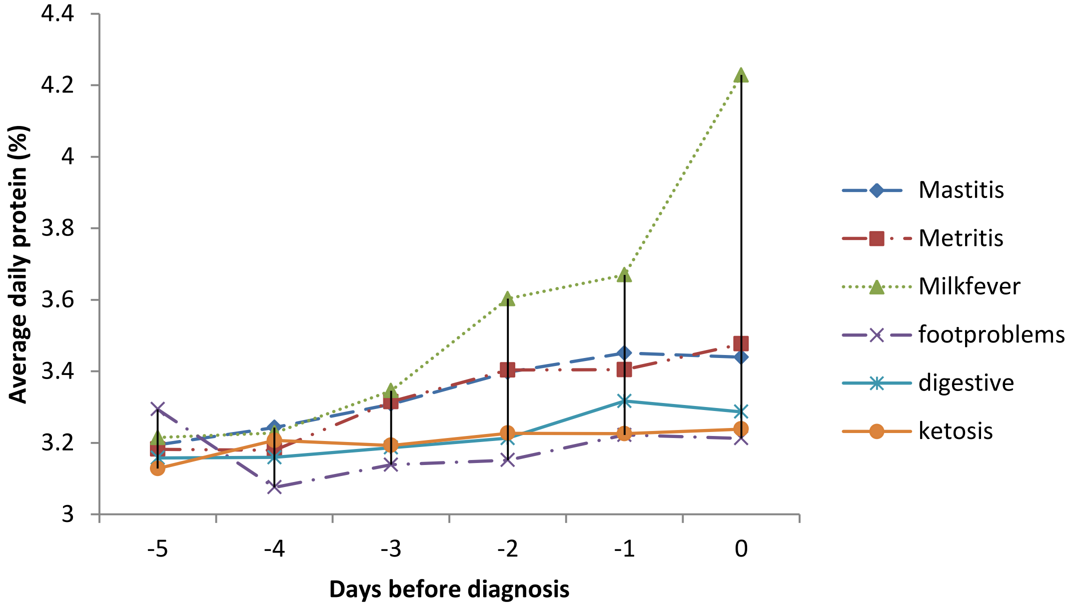

Figure 5: Average daily milk protein (%) from d -5 to the day of disease diagnosis in affected cows

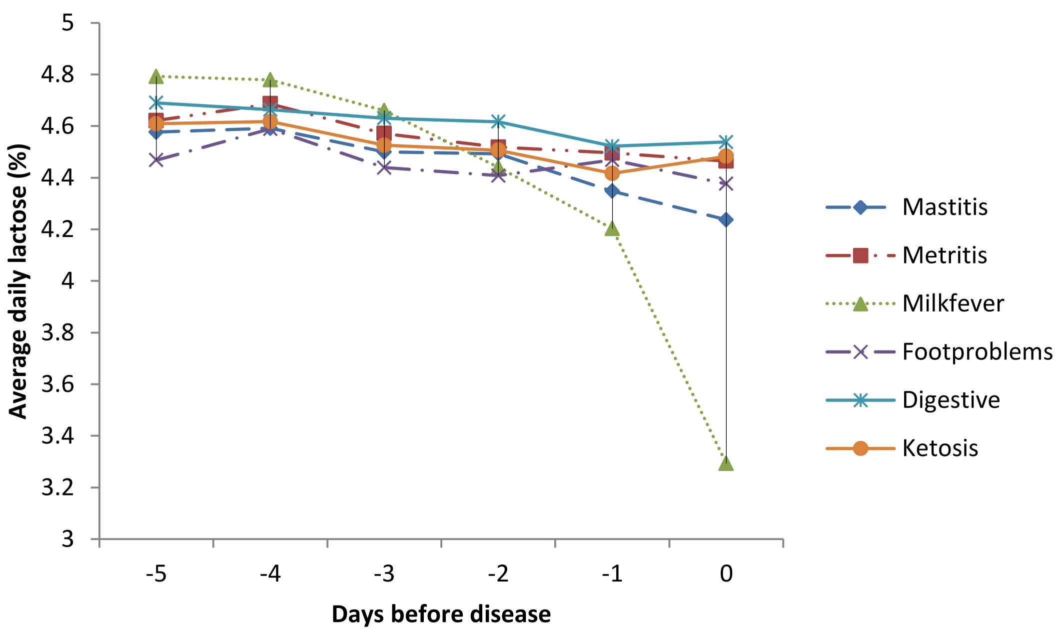

Figure 6: Average daily milk lactose (%) from d -5 to the day of disease diagnosis in affected cows

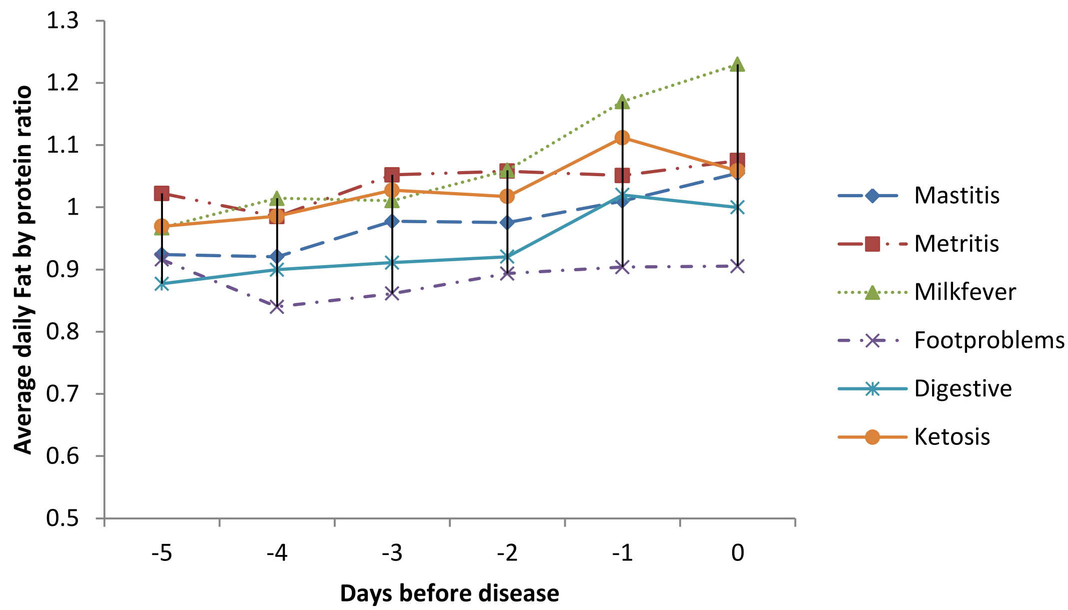

Figure 7: Average daily fat by lactose ratio from d -5 to the day of disease diagnosis in affected cows

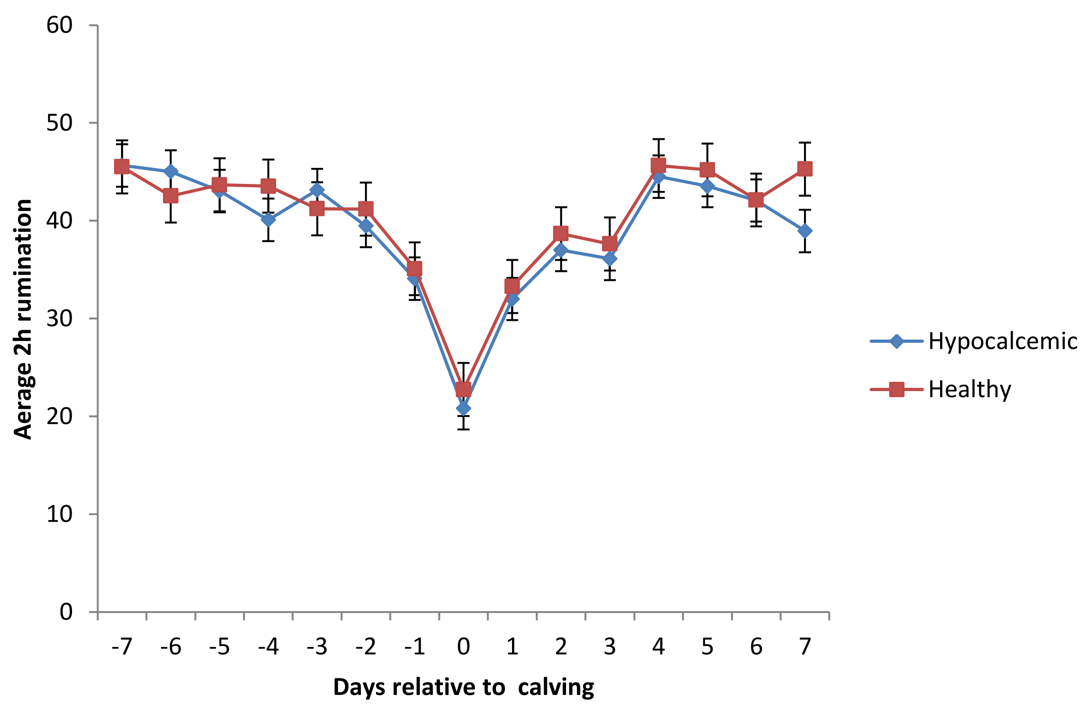

Figure 8: Variation of 2-hr rumination time from -7d to 7d after calving in healthy and hypocalcemic cows

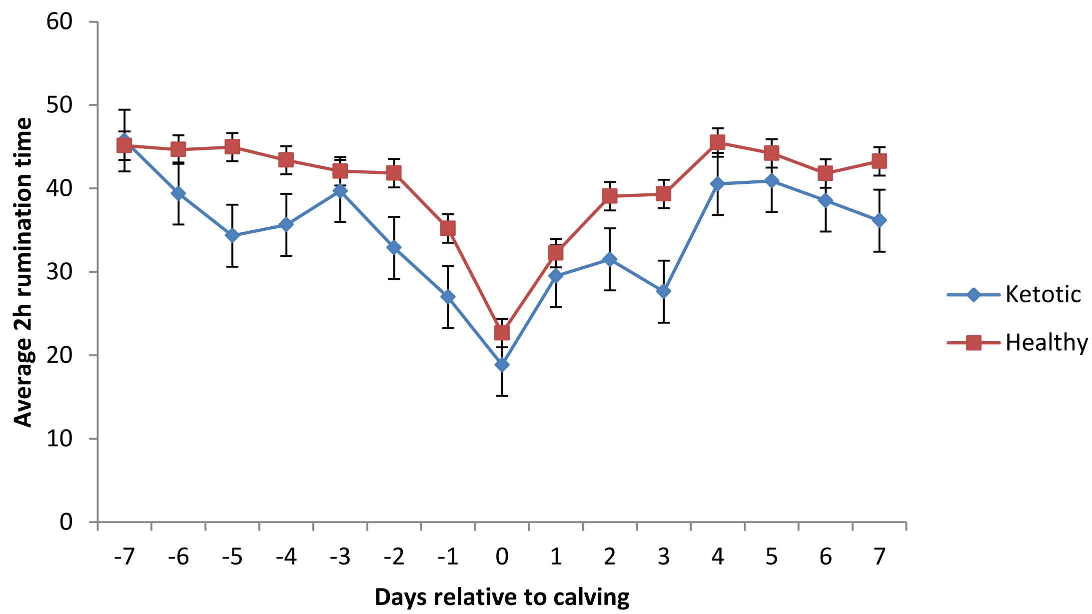

Figure 9: Variation of 2-hr rumination from -7d to 7d after calving in healthy and ketotic cows

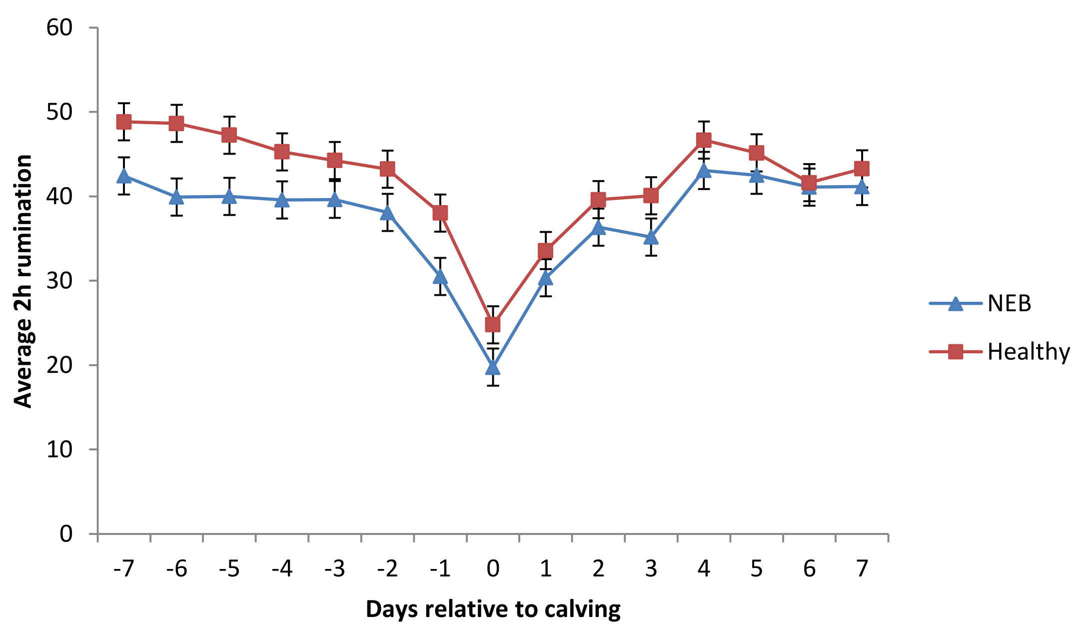

Figure 10: Variation of 2-hr rumination from -7d to 7d after calving in healthy and NEB cows