

MANUEL AGUSTIN SILVA

**Measuring rumination and physical activity as a tool
for fresh cows health monitoring**

Pirassununga-SP

2017

MANUEL AGUSTIN SILVA

Measuring rumination and physical activity as a tool for fresh cows health monitoring

Tese apresentada ao Programa de Pós-Graduação em Reprodução Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do Título de Doutor em Ciências

Departamento:

Reprodução Animal

Área de concentração:

Reprodução Animal

Orientador:

Prof. Dr. Ed Hoffmann Madureira

De acordo: _____

Orientador

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Pirassununga-SP

2017

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T.3462
FMVZ

Silva, Manuel Agustin
"Measuring rumination and physical activity as a tool for fresh cows health monitoring
/ Manuel Agustin Silva. -- 2017.
146 f.: il.

Título traduzido: Medição da ruminação e da atividade física como ferramenta no monitoramento de saúde de vacas recém paridas.

Tese (Doutorado) - Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Reprodução Animal, Pirassununga, 2017.

Programa de Pós-Graduação: Reprodução Animal.

Área de concentração: Reprodução Animal.

Orientador: Prof. Dr. Ed Hoffmann Madureira.

1. Rumination. 2. Activity. 3. Disease. 4. Dairy cow. I. Título.

**CERTIFICADO**

Certificamos que a proposta intitulada "Evaluacao de um sistema automatico de monitoramento para vacas leiteiras", protocolada sob o CEUA nº 9166221116, sob a responsabilidade de **Ed Hoffmann Madureira e equipe; Manuel Agustin Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 01/02/2017.

We certify that the proposal "Evaluation of an automated monitoring system for periparturient dairy cows", utilizing 610 Bovines (610 females), protocol number CEUA 9166221116, under the responsibility of **Ed Hoffmann Madureira and team; Manuel Agustin Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 02/01/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 08/2015 a 12/2015

Área: Reprodução Animal

Origem: Animais de proprietários

Espécie: Bovinos

sexo: Fêmeas

idade: 1 a 6 anos

N: 610

Linhagem: Holstein

Peso: 400 a 700 kg

Resumo: The objectives of the current experiment were to determine if the addition of rumination and activity monitoring to on-farm routine for fresh cow checking improves sick cows detection and diagnosis of disease during the transition period, and if it anticipates diagnosis of disease by farm personnel and milk production drops, and to compare economic profits between the addition of rumination and activity monitoring to on-farm routine diagnosis and on-farm routine diagnosis only. Holstein animals (nulliparous = 282, parous = 328) were enrolled in the experiment approximately 60 d before expected calving date, and were divided into two groups (Collar Monitoring-CM-, n=293 ; Control-C, n=317). Rumination and activity loggers (SCR Engineers Ltd., Netanya, Israel) were fitted on cows neck from enrollment until approximately 80 ± 3 d postpartum. Animal check and diagnose of disease were made by farm personnel following the routine of the farm, additionally daily rumination and activity screening was used to flag the cows from CM group to check them, then cows showing altered rumination pattern were checked. Serum calcium concentration was determined using blood a sample collected from 0 to 4 DIM. BHBA concentrations were determined twice using blood samples collected from 4 to 12 DIM and 7 to 20 DIM. Subclinical hypocalcemia was characterized by Ca <8.55 ng/dL and subclinical ketosis was characterized by BHBA >1,000 µmol/L in any sample. Daily rumination, daily activity, diagnose of disease data and daily milk production were collected using DataFlow II and PCdart Software for statistical purpose.

Local do experimento: O experimento foi realizado numa fazenda leiteira comercial nos Estados Unidos, com um sistema de confinamento.

São Paulo, 01 de fevereiro de 2017

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade
de São Paulo

Roseli da Costa Gomes
Secretaria Executiva da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade
de São Paulo

FOLHA DE AVALIAÇÃO

Nome: SILVA, Manuel Agustin

Título: Measuring rumination and physical activity as a tool for fresh cows health monitoring

Tese apresentada ao Programa de Pós-Graduação em Reprodução Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do Título de Doutor em Ciências

Data: ____/____/____

Banca Examinadora

Prof.Dr: _____

Instituição: _____ Julgamento: _____

AGRADECIMENTOS

Como reza a música da cantora Mercedes Sosa, “*gracias a la vida que me ha dado tanto*”. Eu me sinto favorecido pela sorte, sorte de ter tido a possibilidade de estudar em grandes instituições que me formaram como profissional e como pessoa. Por isso agradeço à vida, pelas oportunidades que a maioria das pessoas não tem, e me proponho devolver de alguma forma tudo o que recebi dela.

À Universidade de São Paulo e a comunidade uspiana por me aceitar e deixar ser parte delas.

Ao CNPq e a CAPES pelas bolsas e auxílios proporcionados para minha estadia no Brasil e nos Estados Unidos, e para a realização do experimento e auxílio a eventos.

À Universidade da Flórida por me aceitar como mais um de seus alunos durante minha estadia.

À minha família e amigos que ficaram na Argentina, sempre esperando minha volta, apoiando desde à distância, por esse amor que se sente a pesar dos quilômetros.

À minha companheira em todos os momentos, parceira, por seu amor e carinho, espero ter sido recíproco, obrigado por tanto. Minha negra chota.

Ao Departamento de Reprodução Animal e todo o pessoal que forma parte dele, por todo o conhecimento e experiência que me tornaram melhor profissional. Especialmente ao Clayton e à Harumi, pela paciência e boa disposição para me ajudar cada vez que precisei.

Ao professor Ed, por sua simplicidade, humildade, e carisma. Por mais professores como o ele, que tratam seus alunos como colegas e amigos, sem importar seus diplomas ou experiência, sempre com respeito e humildade.

A meus companheiros de equipe e amigos Thiago Santin, Milton Maturana Filho, Kleber Menegon Lemes, e Julianne Rezende, pelos conhecimentos compartilhados e os momentos vividos. Queria incluir aqui especialmente ao meu amigo Ismael “Arepa” Morales, e a todos os estagiários que participaram ativamente da equipe durante estes anos.

A meus companheiros e amigos Anderson Veronese e Anna Luiza Belli por sua grande e valiosa participação e ajuda no experimento. Por colocar colares no calor e no frio, pelo apoio moral e psicológico nos duros momentos, e pelos momentos de lazer vividos.

À fazenda North Florida Holstein, por me abrir as portas e permitir fazer o experimento na suas instalações e com seus animais.

Ao pessoal da fazenda, especialmente a meu amigo Angel Diaz. Muito obrigado Angel querido, pela disposição, ensinamentos, por me mostrar que um diploma não é sinônimo de conhecimento e capacidade sempre, e que com esforço e trabalho se pode chegar bem longe. Por esses dias em que acabava sem braço por palpar tantas vacas, com você do meu lado ajudando a melhorar minhas habilidades. A todos os funcionarios que me ajudaram, fazendo deste este experimento como seu também. Saudade desses dias eternos na fazenda!

Aos animais que fizeram parte do meu experimento, sem eles não existiria tese e nem conhecimento.

A todas a pessoas que fazem parte do Departamento de Ciencias Clinicas da Faculdade de Medicina Veterinaria da Universidade da Florida (FARMS), aonde fiz bons e importantes amigos, como Federico Cunha, uruguaio assador e “futebolero”. Especialmente ao professor Klibs Galvao, que anima o dia de todo mundo com seu sorriso permanente, mesmo em momentos duros.

Ao professor Ricardo Chebel, pela oportunidade de fazer o experimento nos Estados Unidos.

Aos funcionarios da USP, que trabalham com o gado do Campus, tanto de leite quanto corte, pela forca e paciencia durante esses anos. Especialmente ao Andre Valente, pela confianca em minhas capacidades.

Ao professor Paulo Mazza, por me introduzir no fascinante mundo da estatística e me induzir a gostar desta matéria. Por sua didática e paciência, as melhores aulas que já tive. E por sua disposição em cada uma das milhares de vezes fui na sua sala para fazer milhares de consultas.

A todas as pessoas da Faculdade de Zootecnia e Engenharia de Alimentos que me abriram as portas quando cheguei ao Brasil, e me ajudaram a transferir de unidade quando solicitei.

Ao Prof. Ivanor Prado da Universidade de Maringá, que lá por 2012 me convidou para vir para o Brasil, sem não teria achado ele, esta tese não existiria.

A todos os amigos que conheci no Brasil, que fizeram meus dias melhores, com quem compartilhei momentos que sempre ficarão na minha mente e meu coração. É muito difícil nomear a todos neste parágrafo mas queria reforçar este obrigado para o Fábio e sua família, que me abriram as portas de sua casa sempre. Aos amigos que a USP me deu, Luis, Julio,

Fabian, Ismael, Jose, Michael, Felipe, Romulo, e todos com os que vivi momentos que sempre lembrarei. Também meus amigos do futebol Guilherme Pereira, Juninho, Willinha, Jose, Belli e muitos mais que fizeram minhas tardes e finais de semana um “show de bola”. Aos amigos paraquedistas Igor Teofilo, Marcos Pettena, Antonio Raposo, Guinas, File, e toda a família da Azul do Vento paraquedismo com os que compartilhei o céu do Brasil! Aos amigos da vida, que alguma força maior colocou no meu caminho.

“Science is a delight”

-Carl Sagan-

“The known is finite, the unknown infinite”

-Thomas Henry Huxley-

***“Cada uno da lo que recibe,
y luego recibe lo que da...”***

-Jorge Drexler-

ABSTRACT

SILVA, M. A. **Measuring rumination and physical activity as a tool for fresh cows health monitoring.** [Medição da ruminação e da atividade física como ferramenta no monitoramento de saúde de vacas recém paridas]. 2017. 146f. Tese (Doutorado em Ciência) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

The objectives of the current experiment were to characterize patterns of daily rumination time, activity and milk production around the diagnosis of health disorders, and to determine if the addition of rumination and activity data to a commercial dairy farm fresh cow health monitoring program improves sick cow detection and diagnosis of disease during the first 30 DIM. Holstein animals (*primiparous* = 282, *parous* = 328) were enrolled in the experiment approximately 60 d before expected calving date, and were divided into two groups (Collar Monitoring-CM-, n=293 ; Control-C-, n=317). Electronic rumination and activity monitoring tags (SCR Engineers Ltd., Netanya, Israel) were fitted on cow's neck at enrollment and were kept until approximately 80 ± 3 DIM. Farm personnel checked the cows and performed the diagnosis of disease following the routine of the dairy. Cows from both of the groups were sent to check based on the parameters used by the farm. Additionally, cows from group CM were checked based on the data provided by the tags. Serum calcium concentration was determined using blood samples collected from 0 to 4 DIM. BHBA concentration was determined twice using blood samples collected from 4 to 12 DIM and 7 to 20 DIM. Subclinical hypocalcemia (SCHC) and subclinical ketosis (SCK) were characterized as Ca <8.55 ng/dL, and BHBA >1,000 $\mu\text{mol/L}$ in any blood sample, respectively. Daily rumination time (DRT), daily activity (ACT), and daily milk production patterns for cows with clinical disease showed differences with healthy cows around diagnosis ($P < 0.05$). Cows with subclinical disorders and calving problems had changes in DRT, ACT, and milk production patterns compared to healthy cows around calving ($P < 0.05$). DRT and ACT patterns of regrouped cows were characterized by differences with non-regrouped cows around regrouping ($P < 0.05$). No differences were found for DRT, ACT, and milk production between groups C and CM. The overall sensitivity (Se) of collars to identify health disorders was 56.4% (n = 402 cases), considering a positive outcome as at least 1 alert based on rumination and activity from -7 to +2 d relative to diagnosis. Se was higher for cows with more than one disorder (75.8%) than for cows with one disease only (45.5%) ($P < 0.001$). No differences between groups were found for overall Se, and Se for cows with one disease. However, for cows with more than one disorder, Se was higher in group CM than C ($P = 0.005$). Overall specificity, positive predicted values, and negative predicted values

were 74.5%, 46.4%, and 57.6%, respectively. The overall incidence of disease was 48%. No differences between groups were found for overall incidence of disease and each disease. Among *primiparous*, group CM (43.3%) had higher overall incidence of disease than group C (32.1%) ($P = 0.05$). Although there were no differences for *parous*, incidence of metritis tended to be greater in group C than CM ($P = 0.1$). Incidence of SCK and SCHC was not different between groups. A higher percentage of animals from group CM than C received treatment ($P = 0.04$), and these differences were seen in *primiparous* ($P = 0.03$), but not in *parous*. However, a higher percentage of *parous* not diagnosed as sick from group CM received support treatments (drenching and fluids) compared to C. No differences were shown for culling rate, service rate until 150 DIM, conception rate at first service, and percentage of cows marked as “do not breed” between groups. DRT and ACT patterns for sick cows showed differences around diagnosis compared to healthy cows. The use of DRT and ACT data was able to identify sick cows in a commercial dairy farm. Results suggest that it may be also useful to identify cows needing attention before clinical signs are visible, improving the prevention of health disorders. Its usefulness may vary according to parity, disease, severity of disease and health compromise, and the intensity of the farm system for checking cows. Future research should evaluate different parameters and parameters thresholds based on rumination and activity data for identifying sick cows, and their efficiency in dairies with different degrees of intensity for checking animal’s health.

Keywords: Rumination. Activity. Disease. Dairy cow.

RESUMO

SILVA, M. A. **Medição da ruminação e da atividade física como ferramenta no monitoramento de saúde de vacas recém paridas** [Use of rumination and physical activity as a tool for fresh cows health monitoring]. 2017. 146f. Tese (Doutorado em reprodução animal) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2017.

Os objetivos deste experimento foram caracterizar os padrões diários do tempo de ruminação, atividade, e produção de leite arredor do diagnostico de doenças, e determinar se a adição de dados de ruminação e atividade num programa de monitoramento de saúde de vacas de uma fazenda comercial melhora a detecção de vacas doentes e o diagnostico de doenças durante os primeiros 30 DEL. Animais Holstein (*primíparas* = 282, *multíparas* = 328) foram utilizados no experimento aproximadamente 60 ± 3 dias antes da data esperada de parto, e foram divididos em dois grupos (Collar Monitoring-CM-, n=293; Control-C-, n=317). Dispositivos eletrônicos para o monitoramento da ruminação e atividade acoplados a colares (SCR Engineers Ltd., Netanya, Israel) foram colocados nas vacas no enrolamento e mantidos ate aproximadamente 80 ± 3 DEL. O monitoramento de saúde das vacas e o diagnostico de doenças foram realizados pelos funcionários da fazenda seguindo a rotina do estabelecimento. Os animais dos dois grupos foram enviados para checagem de saúde baseados nos parâmetros utilizados pela fazenda. Adicionalmente, as vacas do grupo CM foram checadas baseadas na informação suprida pelos colares. A concentração de cálcio sérico foi determinada usando uma amostra de sangue coletada do dia 0 ao 4 em leite. A concentração de beta-hidroxibutirato (BHBA) foi determinada duas vezes usando amostras de sangue coletadas do dia 4 ao 12, e do 7 ao 20 do parto. Hipocalcemia subclínica (SCHC) e cetose subclínica (SCK) foram caracterizadas como $Ca < 8.55$ ng/dL, e $BHBA > 1000$ μ mol/L em qualquer amostra, respectivamente. Os padrões diários do tempo de ruminação (DRT), atividade (ACT), e produção de leite de vacas com doenças clinicas arredor do diagnostico mostraram diferencias comparados com vacas sadias ($P < 0.05$). Vacas com alterações subclínicas e problemas de parto tiveram alterações nos padrões de DRT, ACT, e produção de leite arredor do parto, quando comparadas a vacas controle ($P < 0.05$). Padrões de DRT e ACT de vacas reagrupadas se caracterizaram por diferencias com vacas não reagrupadas ($P < 0.05$). Não foram achadas diferencias em DRT, ACT, e produção de leite entre os grupos C e CM. A sensibilidade (Se) dos colares para identificar problemas de saúde foi de 56.4% (n = 402 casos), considerando como evento positivo a ocorrência de pelo menos uma alerta baseada em ruminação e atividade dentro dos 7 dias prévios ate 2 dias apos o diagnostico de doença. A Se foi maior para vacas com mais de

uma doença (75.8%) que em para vacas com uma doença somente (45.5%) ($P < 0.001$). Não se acharam diferenças na Se geral, nem Se para vacas com uma doença somente entre grupos. Porém, a Se foi maior no grupo CM que no grupo C ($P = 0.005$) em vacas com mais de uma doença. A especificidade (Sp), valores da predição positiva (PPV), e valores da predição negativa (NPV) foram 74.5%, 46.4%, e 57.6%, respectivamente. A incidência de doença foi de 48%. Não houve diferenças entre grupos na incidência de doença, nem na incidência de cada doença. Entre as *primíparas*, o grupo CM (43.3%) teve maior incidência de doença do que o grupo C (32.1%) ($P = 0.05$). Embora não teve diferença na incidência de doença entre grupos para *multíparas*, a incidência de metrite teve uma tendência a ser maior no grupo C do que no grupo CM ($P = 0.1$). A incidência de SCK e SCHC não foi diferente entre grupos. Maior percentagem de animais do grupo CM recebeu tratamento do que do grupo C ($P = 0.04$), e estas diferenças foram observadas em *primíparas* ($P = 0.03$), mas não em *multíparas*. Contudo, uma maior percentagem de animais não diagnosticados como doentes do grupo CM recebeu tratamentos de suporte, quando comparado ao grupo C. Não se acharam diferenças na taxa de descarte, taxa de serviço aos 150 DEL, taxa de concepção ao primeiro serviço, e percentagem de vacas de descarte reprodutivo entre grupos. Resumindo, os padrões de DRT e ACT de vacas doentes arredor do diagnóstico de doença mostraram diferenças comparados com os de vacas saudas. O uso da informação de DRT e ACT foi capaz de identificar vacas doentes numa fazenda comercial. Os resultados sugerem que a utilização dos colares pode ser útil para identificar vacas com necessidade de atenção antes da aparição de sinais clínicos visíveis, melhorando a prevenção de problemas de saúde. A utilidade da utilização do sistema pode variar de acordo a ordem de partos dos animais, doença em questão, severidade da doença e comprometimento de saúde do animal, e com a intensidade do sistema de monitoramento de saúde dos animais da fazenda. Próximas pesquisas deveriam avaliar diferentes parâmetros baseados na informação de ruminação e atividade para identificar vacas doentes, e a sua eficiência em fazendas com diferentes graus de intensidade para o monitoramento de saúde.

Palavras-chave: Ruminação. Atividade. Doença. Vaca leiteira.

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1. INTRODUCTION

Rumination is not just a digestive process by which the cow regurgitates previously consumed feed and masticates it a second time; it is also a natural behavioral necessity that ruminants have to satisfy and can be affected by many factors, such as feeding, stress (MOALLEM ET AL., 2010; SORIANI ET AL., 2013), and disease (LIBOREIRO et al., 2015), being considered as a sign of animal welfare (CALAMARI et al., 2014). It has been demonstrated that animals that suffer from metabolic and infectious diseases have disrupted rumination patterns when compared to healthy individuals (LIBOREIRO et al., 2015; SORIANI; TREVISI; CALAMARI, 2012). Based on this, some researchers have suggested that monitoring rumination time and activity could be useful for identifying cows suffering from health disorders during the postpartum period (STANGAFERRO et al., 2016a, 2016c). The early postpartum period is the most critical and vulnerable phase of life for dairy cows because 75% of diseases occur in the first month postpartum, affecting up to 50% of the cows (LEBLANC, 2010). Thus, the early detection of sick cows is important to ensure the productive, reproductive, and economic success of lactation. Dairy farms monitoring programs for fresh cows health are based on different parameters, such as days in milk (DIM), or milk production, and cows are checked following the schedule and frequency according to the necessity and problems of the farm (GUTERBOCK, 2004). The use of new technology is becoming more adopted by large dairy farms, and it can be useful for fresh cow health screening based on automatic devices that monitor milk characteristics, rumination and activity behavior, or body temperature (DE VRIES; RENEAU, 2010). The hypothesis of the current study is twofold: first, rumination and activity patterns are altered in sick cows around the diagnosis of disease during the first 30 DIM, and secondly, the addition of rumination and activity data provided by collars fixed to cow's necks can be used as a tool for identifying cows suffering from health disorders. Then, the objectives of this work were to characterize patterns of daily rumination time, daily activity, and daily milk production around the diagnosis of health disorders to determine if alterations in rumination and activity anticipate diagnosis of disease by farm personnel; and to analyze if the addition of rumination and activity monitoring to on-farm routine diagnosis improves sick cows detection and diagnosis of disease during the first 30 DIM.

2. LITERATURE REVIEW

2.1. Rumination behavior

Rumination is the process by which the ruminants regurgitate consumed feed and masticate it again in order to facilitate the digestive process as it is required to break down the size of feed particles so that they can pass through the reticulo-omasal orifice (WELCH, 1986). This process provides the rumen bacteria a greater access to feed particles to achieve a more efficient fermentation (RUSSELL, 2001) and increase saliva production, improving rumen health (BEAUCHEMIN, 1991). Cows ruminate between 400 and 500 min/d on average (CALAMARI et al., 2014; SCHIRMANN et al., 2013), and this may vary between pre-partum and post-partum periods and *primiparous* and *parous* animals (SORIANI; TREVISI; CALAMARI, 2012). Rumination is performed in 4 to 24 periods/d, each lasting 10 to 60 minutes (GÁSPÁRDY et al., 2014). During each period, approximately 30-60 boluses are produced. Each cycle lasts approximately 40 seconds and consists of 30 to 60 chewing movements. Between boluses, one bolus is swallowed and another one is regurgitated, occurring during the inter-cycle period. This lasts for approximately 4-8 seconds, and during this time, no chewing is performed (LINDGREN, 2009). Rumination occurs mostly at night but also to some extent during the afternoon resting period (SCHIRMANN et al., 2009).

The normal rumination behavior around calving would present a drop in rumination of up to 70% on the day of calving with a quick recovery during the first days in lactation (CALAMARI et al., 2014). However, Calamari et al. (2014) showed that the recovery rate may vary between cows according to their pre-partum rumination behavior. These authors divided the animals according to average pre-partum rumination time in “higher” and “lower” rumination, and their results showed that the first group reached post-partum levels faster than the second.

Breed, sex, body size, physiological state, age, parity, and level of production influences rumination (LINDGREN, 2009). Between animals, there are “better” or “more efficient” ruminators than others. The amount of time spent chewing per unit of intake has been implicated as a measure of mastication efficiency (DADO; ALLEN, 1994). It seems to be that these more efficient ruminators can eat more roughage, needing shorter time to eat and ruminate per unit of ingested feed. Higher rumination efficiency may be due to decreased time

between boluses, a larger number of chews per unit time, a lower proportion of pseudorumination (regurgitated material mainly consists of fluids only containing small quantities of solid particles), or more efficient regurgitation of large particles. Anatomical features like larger mouths, larger tooth surfaces, stronger jaw muscles, or enhanced articulation of the jaw may also explain higher rumination efficiencies (DADO; ALLEN, 1994).

It is expected that a strong relationship between rumination and dry matter intake (DMI), and that changes in rumination over time may reflect changes in DMI. However, Schirmann et al. (2012) indicated that the use of daily rumination times to estimate DMI is limited. These authors found negative correlation between daily rumination time (DRT) and DMI within 2-h periods. Moreover, they found a negative correlation between daily rumination time and feeding time, suggesting that the cows that ruminate more spent less time feeding. Moreover, Dado and Allen (1994) found that increases in chewing duration were not proportional to increases in DMI because ruminating and total chewing durations per unit of intake decreased as production and intake increased. During the first week of lactation, DRT quickly recovers levels observed before calving, reaching a maximum value between 7 and 15 days after calving and staying at an almost stable level over the following weeks. Conversely, DMI in general continues to increase until 6 weeks of lactation (CALAMARI et al., 2014).

According to Van Soest (1994), the time a cow spends ruminating is proportional to the dry matter (DM) content and the size of feed particles. Generally, cattle ruminate for 25-80 minutes per kg roughage consumed, but the time spent ruminating per kg roughage depends both on the chemical and physical nature of the ration (LINDGREN, 2009). As DM fiber content and particle size increase, the cow needs longer rumination times (Beauchemin, 1991; Krause et al., 2002). According to Mertens (1997), rumination is a response associated with physical effectiveness of the NDF (neutral detergent fiber) fraction. Unbalanced rations with excessive starch and easily fermented carbohydrates in relation to the content of effective fiber result in reduced rumination. However, Welch & Smith (1975) found that increased indigestible fiber loads were not effective in stimulating rumination beyond the normal 8 or 9 hours per day.

Rumination is a behavioral aspect that has to be satisfied by ruminants (LINDSTRÖM; REDBO, 2000), being considered an indicator of welfare, since it can be affected by several factors such as management, stress, and disease (BRISTOW; HOLMES, 2007; LIBOREIRO et al., 2015). Moreover, changes in rumination behavior may be the earliest warning signs noticing about potential health problems, allowing its possible utilization as a health indicator

(AMBRIZ-VILCHIS et al., 2015; GÁSPÁRDY et al., 2014; LIBOREIRO et al., 2015). Stressful situations such as high stocking density, group movements, weather, or calving can reduce rumination activity (BRISTOW; HOLMES, 2007; GREGORINI et al., 2012; SORIANI; PANELLA; CALAMARI, 2013; SORIANI; TREVISI; CALAMARI, 2012).

It has been demonstrated that sick animals ruminate less than healthy ones (LIBOREIRO et al., 2015; SORIANI; TREVISI; CALAMARI, 2012). When cows were divided according to their pre-partum rumination time in lower and high level, the first group had higher incidence of disease during the first 30 DIM (SORIANI; TREVISI; CALAMARI, 2012). Supporting this, Stangaferro et al., (2015) showed that pre-partum rumination patterns were altered in cows that suffered health disorders within 30 DIM. Liboreiro et al. (2015) found associations between daily rumination and disease and suggested that the changes of daily rumination time of a cow over time may be useful for the diagnosis of disease. Changes in rumination time may be used as a proxy measure of illness or changes in health status (i.e., if detected, subtle changes in rumination could help in the detection of subclinical diseases before they progress and become a clinically apparent concern).

2.1.1. Rumination measurement

Different methods to measure rumination activity have been implemented, ranging across pressure transducers, acoustic biotelemetry, to visual observation using a simple stopwatch, used often for validation of other methods nowadays.

During visual observation, the animal is observed by two or more previously trained observers, depending on the number of animals being studied (SCHIRMANN et al., 2009). The process can be improved using video recording, which allows a greater number of observations. Visual observation is labor intensive, and typically, only a few cows can be monitored simultaneously.

Pressure transducers can be used to measure rumination based on hydraulic or pneumatic processes. This method uses a plastic tube or ball that transduces the pressure generated by the animal's muzzle, which is converted into electrical signals that are processed and recorded (BALCH, 2007; NYDEGGER; GYGA; EGLI, 2010). This method provides useful information, however, it fell in disuse because of several disadvantages, such as

technical limitations and discomfort to the animals that may have affected their behavior. Among technical problems, restricting animals housed in metabolism crates or in tie-stalls, limited memory capacities for data storage, and the necessity of halter removal to download data were crucial disadvantages of this measurement method (KASKE et al., 2002; SCHIRMANN et al., 2009).

Acoustic biotelemetry constitutes a measurement method that is able to ascertain rumination by sound signals recorded using microphones (CLAPHAM et al., 2006). These microphones are attached to collars that are positioned on cow's neck, keeping the microphone on the left side of jaw (Figure 1). The characteristic sounds of rumination are recorded, digitally stored, processed, and then, data are presented as rumination time either min/2 h or min/d. The system consists of rumination loggers, stationary or mobile readers, and software for processing the electronic records (Data Flow software, SCR Engineers Ltd.). Data are recorded and stored in the memory of the logger for up to 22 h in eleven 2-h intervals (22 h), after which the first interval recorded is overwritten. Then, in situations in which the data have not been downloaded, results are lost data. Readers have to be installed in strategic points around the farm, as in the milking parlor or hospital barn where all collars can be read (the distance limit readers can receive data is up to 1000 feet). Readers send the data to a computer, and then, the software can read, process, and save them. Like any other automatic device system, this had to be tested and validated to ensure that the obtained data are reliable and accurate. Visual observation was used to validate collar performance and accuracy (SCHIRMANN et al., 2009).

2.2. Activity behavior

Cows need space to move and rest in a safe and comfortable manner (DAIRY NZ, 2015), and this is considered important for maximizing production as well as cow comfort and welfare (HALEY; RUSHEN; DE PASSILLÉ, 2000). Knowing the way cows walk and how they physically respond to situations are important. Cows normally walk with their heads down looking where they will place their front feet at an average speed of 45 meters/minute (2.7 km/h), and it is important to allow cows to walk and move at their own pace as pressuring them could result in injury or lameness issues (DAIRY NZ, 2015).

Many biological, environmental and management factors may influence the walking distance, speed, and movements of dairy cattle, such as breed, lactation period, estrus, phase of gestation, health status, hierarchy and dominance, milking and feeding routine, group and pen size, stocking density, floor and track condition and design, paths distance, climatic conditions, period of the year, lameness, and farm personnel labor (D'HOUR et al., 1994; DAIRY NZ, 2015; EDWARDS et al., 2004; GUSTAFSON, 1993; LIBOREIRO et al., 2015; PRATUMSUWAN, 1994; STANGAFERRO et al., 2016c; TALUKDER et al., 2015a; TELEZHENKO et al., 2012).

When in pasture, the distance cows walk varies according to several factors, such as the size of the grazing area, the amount of grass available, the proximity of drinking water, and management strategies (D'HOUR et al., 1994). In intensively housed animal dairy farms, the amount of space provided to the cows can be related to behavioral and health problems and is one of the most contentious issues for the public concerned about farm animal welfare (TELEZHENKO et al., 2012). Although most intensive production systems may appear to provide sufficient time for moving and resting, not all housing environments allow cows to move and rest properly. Cows in large pens moved greater distances than cows in small pens, and this is related to the stocking density (TELEZHENKO et al., 2012). The floor surface has an important effect on activity and hoof health. The flooring type and maintenance are important for bringing comfort as well as traction to ensure that it does not compromise cattle ease of movement or inflict hoof or limb issues (FRANCO-GENDRON et al., 2016). Hierarchy and dominance have impact on walking distance, time, and order. Dominant cows walk ahead of the herd guiding the group. It is important for cows to have space at all times to keep their distance and avoid forced contact with others around them of similar or higher dominance. The availability of space is important for maintaining adequate locomotor activity, and it has been shown that exercise promotes health in dairy cows (GUSTAFSON, 1993). In this regard, it has been demonstrated that cows suffering from disease are less active than healthy cows (LIBOREIRO et al., 2015; STANGAFERRO et al., 2016a). Edwards et al. (2004) found that sick cows made 8-15 steps/h less than healthy cows, and this difference was greater the day after the diagnosis of disease.

The walking distance may have an impact on milk yield and composition and feed intake, and this is related to the fact that it requires the expenditure of energy (D'HOUR et al., 1994; PRATUMSUWAN, 1994). As exercise is one of the many factors influencing the animal metabolic requirements for nutrients, feed intake by animals performing extra walking would be expected to increase. However, some authors found increased intake in exercised animals

others have found no increase, and sometimes even reduced feed intake, since the time spent walking may reduce the time available for eating (PRATUMSUWAN, 1994).

2.2.1. Activity measurement

Activity can be measured by different methods, such as visual observation, video recording, and accelerometers attached to a leg band or a collar. Activity and lying down behaviors are commonly used as indicators of animal comfort and welfare (MATTACHINI et al., 2013). The use of electronic data loggers to measure activity behavior has become increasingly common, as they record non-invasively and overcome the time-consuming limitations of video-based observations. Activity measurements from video recording and accelerometer devices are highly correlated (ELISCHER et al., 2013; MATTACHINI et al., 2013). Accelerometer devices can record number of steps, laying and standing bouts of time, and speed and angles of movements, depending on the design and brand. The ultimate technology in activity measurement is available in previously mentioned collars equipped with microphones for measuring rumination, also containing a 3-axis accelerometer system that records the speed and angle of head movements. Accelerometer devices use different scales for activity, such as steps and arbitrary units (unit of measurement to a predetermined reference measurement) per minutes or per hour. Based on that, there are differences in the activity of sick and healthy cows (EDWARDS et al., 2004; LIBOREIRO et al., 2015; STANGAFERRO et al., 2016c), and thus, the use of activity monitoring may be useful for improving the detection of animals suffering from health problems (EDWARDS et al., 2004; LUBABA et al., 2015).

2.3. Postpartum health problems

The transition period, which commences 3 weeks before calving and lasts until 3 weeks after calving (DRACKLEY, 2001), is the most critical and vulnerable phase of a cow's life. Approximately 75% of diseases in dairy cattle occur in the first month postpartum, and 50% of cows suffer from metabolic and infectious diseases in the transition period (LEBLANC, 2010).

After parturition, nutrient demand is not able to be met through feed intake alone because the rate of dry matter intake (DMI) increase is slower than the rate of milk energy output, and then, the total intake of energy by cows after calving usually is less than energy requirements (KOZLOSKI, 2016). In fact, requirements for net energy of lactation essentially double “overnight” as cows calve and commence lactation. As a consequence, all dairy cows experience a negative energy balance (NEB) in early lactation (KAUFMAN et al., 2016). This condition leads to lipomobilization from body reserves in order to meet the demands for energy, which in turn leads to high concentrations of non-esterified fatty acids (NEFA), which are used as fuel, reconverted to storage fat (triglycerides) by the liver, which in turn could lead to the development of fatty liver, or converted to ketone bodies in this organ (DRACKLEY, 2001). The ketones serve as alternate water-soluble fuels that can replace glucose in many tissues, thus conserving glucose for milk synthesis (KOZLOSKI, 2016). The increased production of ketones bodies by the liver can lead to high concentrations of them, which can result in ketosis (GOLDHAWK et al., 2009), that can be categorized as sub-clinical (SCK) or clinical (CK) depending on the presence of clinical signs. Some authors have determined that cows suffer from SCK when β -hydroxybutyrate (BHBA) concentration is over 1,000 $\mu\text{mol/L}$ (GOLDHAWK et al., 2009; LIBOREIRO et al., 2015; WALSH et al., 2007), however, others have used different thresholds (1,100, 1,200 or 1,400 $\mu\text{mol/L}$) (MCART et al., 2012; SUTHAR et al., 2012). The incidence of SCK was found to be up to 60% with a peak on the first week of postpartum (MCART et al., 2012; SUTHAR et al., 2012). Ketosis can manifest clinically as a decrease in appetite, weight loss, and a decrease in milk production, however, its incidence (2-15%) is much lower than SCK (MCART et al., 2012). Subclinical ketosis is a risk factor for subsequent diseases and has been associated with metritis, mastitis, CK, and DA (LEBLANC, 2010; OSPINA et al., 2010c; VANHOLDER et al., 2015). Additionally, cows suffering from SCK may increase their risk of removal from the herd during early lactation, decrease milk yield, and have poor reproductive performance (MCART et al., 2012; OSPINA et al., 2010a). The occurrence of displaced abomasum (DA) is directly associated with NEB, and cows with SCK are a higher risk of developing this metabolic disease (DRACKLEY, 2001). This is a highly disruptive disorder that affects fresh cows, typically around 10 days postpartum, and is preceded by great lipid mobilization and weight losses up to 3 weeks before its appearance (LEBLANC; LESLIE; DUFFIELD, 2005). The incidence of DA was around 2%; however, it has been reported that the occurrence of this disorder is increasing in the last years (LEBLANC; LESLIE; DUFFIELD, 2005). DA it has an strong economic impact because of the direct costs of surgery and treatment, milk production losses, and higher culling rate

(FOURICHON et al., 1999; LEBLANC; LESLIE; DUFFIELD, 2005; RAIZMAN; SANTOS, 2002).

Decreased DMI, NEB, and high concentrations of NEFA and BHBA are related with impaired immune function, in particular of neutrophils and lymphocytes, which predispose cows to suffer from infections such as metritis and mastitis during the early postpartum (HAMMON et al., 2006; LACETERA et al., 2005). All dairy cows experience reduced immune function for 1 to 2 weeks before and 2 to 3 weeks after calving, as well as bacterial contamination of the uterus for 2 to 3 weeks postpartum (LEBLANC, 2010; MALLARD et al., 1998). Uterine health problems affect up to half of dairy cows in the first 60 DIM (SHELDON et al., 2009). Retained placenta (RP) has reported incidence ranging from 3 to 40%, however, it is somewhat influenced by the definition of RP utilized, differing mostly in the time the placenta has to be retained for be considered as retained (LAVEN; PETERS, 1996). Impaired neutrophil function before calving has been suggested as a cause of RP, since these cells have a reduced chemotaxis, related to lower concentrations of interleukin-8 (IL-8), a chemoattractant in the cotyledons, in cows that develop RP compared to cows that expel it normally (KIMURA et al., 2002a). The economic losses of RP are related to the higher risk for developing metritis and infertility (GALVÃO et al., 2010) and also with decreased milk production (SHELDON et al., 2009). Metritis may affect up to 50% of dairy cows during the first 3 weeks of postpartum (SHELDON et al., 2009). Researchers defined metritis as clinical, puerperal, and endometritis, according to the presence or absence of systemic clinical signs of disease, and DIM when it is diagnosed (GIULIODORI et al., 2013). Basically, metritis can be defined as cows having watery, pink/brown, and fetid uterine discharge (LEBLANC, 2010; LIBOREIRO et al., 2015). Healthy cows clear the uterus of bacteria by approximately 3 weeks postpartum, and similar to RP, development of metritis depends largely on immune function in the early postpartum period (HAMMON et al., 2006). Retained placenta and other calving problems (such as twinning and stillbirth), and reduced DMI around calving are risk factors for the occurrence of metritis (GIULIODORI et al., 2013; HUZZEY et al., 2007; OSPINA et al., 2010b). The financial negative effect of metritis is derived from infertility, higher culling rate, reduced milk production, and the cost of treatment (FOURICHON et al., 1999; MELENDEZ et al., 2004; RAJALA; GRÖHN, 1998; SHELDON et al., 2009).

Clinical mastitis, which is defined and characterized by udder's inflammation and milk changes, can affect dairy cows throughout lactation (FUENZALIDA et al., 2015). Some authors related the presence of SCK with higher risk of mastitis (RABOISSON; MOUNIÉ; MAIGNÉ, 2014), while others found no relation (LEBLANC, 2010; SUTHAR et al., 2013).

However, Le Blanc (2010) reported that although SCK did not have association with the incidence of clinical mastitis, it increased severity and duration of this disorder. Even though the incidence of clinical mastitis is more influenced by environmental and sanitary conditions of the farm and may range from 5 to 22% (ALERI et al., 2016; FLEISCHER et al., 2001), it is well documented that the immunosuppression caused by NEB increases susceptibility to clinical mastitis (DRACKLEY, 2001). The severity of clinical mastitis is related to systemic signs of illness and ranges from light to severe cases (HARMON, 1994). Cows with mastitis have altered and reduced milk production, which leads to negative economic profits (FOURICHON et al., 1999; HARMON, 1994).

Lameness does not have a standard definition, and its diagnosis remains a subjective opinion (ARCHER; GREEN; HUXLEY, 2010). It is one of the most important welfare problems in dairy cattle (GALINDO; BROOM, 2002). The incidence of lameness can be as high as 30% with the highest rates observed in herds housed in free-stalls (ITO et al., 2010), and its risk period extends throughout lactation (FRANCO-GENDRON et al., 2016). The lack of comfort represents a risk for this health disorder, and this is related to floor and bed design, material, cleanness (FRANCO-GENDRON et al., 2016; OLECHNOWICZ; JASKOWSKI, 2011; RAJAPAKSHA et al., 2015). It has been reported that the risk of lameness was higher in cows with SCK (SUTHAR et al., 2012). This condition was related with high milk yields by some authors, but other did not find this relation (FLEISCHER et al., 2001). However, it was demonstrated that lame cows produced less milk than healthy ones (ARCHER; GREEN; HUXLEY, 2010; OLECHNOWICZ; JASKOWSKI, 2011). Fertility is also reduced in lame cows, which in conjunction with the reduced milk yield and premature culling have a strong negative economic impact on dairy farms (OLECHNOWICZ; JASKOWSKI, 2011).

Indigestion can be a problem during the transition period, because cows are under stressful events, including calving, physiological changes and social modifications, and dietary modification that require dairy cows to adjust to diets high in nonstructural carbohydrates (DEPETERS; GEORGE, 2014). According to Garry (2009), the term indigestion describes a group of problems that involve abnormal forestomach motility or abnormal fermentative activity, which in turn lead to abnormal reticulorumen contents. It can result from rapid change in feeding or introduction of substances that change the rumen fluid environment or alter the fermentation patterns, or when another disease reduces feed intake or specifically reduces rumen motility, such as DA, ketosis, or endotoxemia.

Colostrum and milk production are very demanding in calcium. In effect, 23 g of calcium is needed by the mammary gland to produce 10 kg of colostrum on the day of calving (GOFF et al., 1997). In consequence, metabolic adaptation mechanisms are activated in order to meet this demand, however, these are not rapid enough at the onset of lactation, taking 1 to 2 days to maximize calcium inflow from the gastrointestinal tract and from bone to the mammary gland (RAMBERG et al., 1970). As result, most cows experience some degree of hypocalcemia during the periparturient period (HORST et al., 1994). The incidence of subclinical hypocalcemia (SCHC), which was defined as calcium concentrations below 8.5 mg/dL by some authors (AMANLOU et al., 2016; LIBOREIRO et al., 2015; MARTINEZ et al., 2012), was reported by Reinhardt et al. (2011), as being of 25% and 47% for *primiparous* and *parous*, respectively. Hypocalcemia is a risk factor infectious and metabolic diseases, poor reproductive performance, and increased risk of culling. (ALERI et al., 2016; MARTINEZ et al., 2012). Calcium is required for the normal functioning of a wide variety of tissues and physiologic processes, such as muscle contraction and nerve transmission (HORST et al., 1994). Related to this fact, hypocalcemia causes loss of teat sphincter tone and can cause uterine inertia, which would lead to a failure to achieve tight closure of teat sphincters permitting entry of environmental pathogens into the teat canal predisposing animals to mastitis, and reduces the ability of the placenta to be detached and expelled from the uterus enlarging the risk of RP, respectively (ALERI et al., 2016; GOFF et al., 1997). In addition, calcium is essential for the immune system function in dairy cows, and the activity of neutrophils is decreased in cows with SCHC (KIMURA et al., 2006). Based on these findings, SCHC is associated with multiple postpartum disorders such as dystocia, uterine prolapse, retained fetal membranes (RFM), endometritis, poor fertility, mastitis, and reduced rumen and abomasum motility (ALERI et al., 2016; AMANLOU et al., 2016; MARTINEZ et al., 2012, 2016). The link between hypocalcemia and milk yield is less clear because many of the cows susceptible to hypocalcemia are also those that produce more milk. Cows with SCHC produced more milk during the first weeks of lactation than normocalcemic herd-mates (JAWOR et al., 2012), suggesting that affected cows might have a greater productive potential or that greater production increases the risk of low blood Ca because of increased losses in colostrum and milk.

Some cows that experience a breakdown in their ability to maintain plasma calcium suffer from severe hypocalcemia with the presence of clinical signs, a condition that is also known as milk fever (MLF) which usually occurs in cows in their third or greater lactation (HORST et al., 1994). The incidence of MLF has been reported to be around 5% in the United

States (AMANLOU et al., 2016; MARTINEZ et al., 2012). However, this value has decreased in the recent years, reaching an average of 2.4%, %, probably because of the increased adoption of feeding acidogenic salts in prepartum diets (MARTINEZ et al., 2012). Reports on the relationship between hypocalcemia and milk yield are inconsistent. The diagnosis of MLF is based on clinical signs, including dull appearance, lethargy, cold ears, or a down cow, while SCHC may be associated with more subtle changes in behavior, such as changes in intake or resting behavior, which make the diagnosis of this last condition difficult (JAWOR et al., 2012).

2.3.1. Postpartum health disorders and their relationship with rumination and activity

As it was previously mentioned, several studies have shown that sick animals ruminate less and are less active than their healthy herd-mates, suggesting that the use of rumination and activity data could be useful as a health indicator and a possible tool helping in the diagnosis of sick cows (LIBOREIRO et al., 2015; STANGAFERRO et al., 2016a, 2016b, 2016c). Changes in rumination and activity precede the signs of disease in many cases and could be used for the earlier detection of sick cows, as was shown in Stangaferro et al. (2016) studies. Moreover, Soriani et al. (2012) found that cows with lower prepartum rumination time had higher incidence of disease during postpartum, compared to cows with higher rumination time during prepartum.

It has been shown that cows with RP, metritis, and cows delivering twins and stillborn calves ruminate less during the first week of postpartum than healthy cows (LIBOREIRO et al., 2015). Stangaferro et al. (2016a, 2016b, 2016c) showed altered rumination and activity days before or around the diagnosis of DA, indigestion, clinical ketosis, metritis and mastitis. Moreover, it was shown that lame cows have altered rumination and are less active compared to non-lame cows (MIGUEL-PACHECO et al., 2014; THORUP et al., 2015). Besides, subclinical metabolic disorders have been related with lower rumination time by Hansen et al. (2003) and Kaufman et al. (2016), who showed that cows with SCK and SCHC, respectively, ruminated less than healthy ones. Supporting these findings, it has been seen that cows with RP, metritis, MLF, DA, mastitis, clinical ketosis, indigestion, fatty liver, lameness, respiratory problems, SCK, and SCHC have depressed feed intake and altered feeding behavior compared

to healthy cows (GOFF et al., 1997; GOLDHAWK et al., 2009; HANSEN et al., 2003; JACKSON et al., 2016; MIGUEL-PACHECO et al., 2014; SIIVONEN et al., 2011).

2.4. Regrouping cows during the postpartum period

Dairy cows are often grouped according to age, days in milk, feed requirements, reproductive and health status, which in turn allows for maintaining homogenous groups of cows in terms of these parameters. To create these groupings, cows are often moved to new groups several times per lactation. This management strategy permits proper ration formulation easier for meeting individual nutrient requirements in a more accurate way, decreases nutrient excretion, facilitates milking activities and routine, and permits an easier cows management (CLARK; RICKETTS; KRAUSE, 1977; GRANT; ALBRIGHT, 2001; GUTERBOCK, 2004). Grouping should minimize negative social interactions and encourage positive interactions (BASSO SILVA, 2012). However, changing animals from one group to another has negative influences in social hierarchy and behavior, forcing animals to reestablish social relationships through physical and nonphysical interactions (BASSO SILVA, 2012). In this regard, grouping is a component of the cow's feeding environment that can modulate intake as a result of its impact on cow comfort, competition for feed and other resources (GRANT; ALBRIGHT, 2001). In effect, cows that experience abrupt environmental and social changes during the periparturient period often exhibit aberrant feeding behavior and are more susceptible to metabolic disorders (BAZELEY; PINSENT, 1984). Cows recently introduced to a new group might be vulnerable to reductions in DMI because of the competition imposed by the group. Schirrmann et al. (2011) observed a reduction in rumination time after regrouping, possibly as a consequence of reductions in DMI and stress, reaching the lowest values on the day of regrouping for cows that stayed in the original pen and the day after regrouping for cows moved to another pen, usually experienced by heifers that were moved in the lactating group. Indeed, *primiparous* cows benefit from separate grouping from older animals by increased intake and productivity (ØSTERGAARD; THOMSEN; BUROW, 2010). When introduced to a new group of animals, cows modify their lying down behavior, increasing the number of lying bouts, possibly as a sign of restlessness (HASEGAWA et al., 1997; VON KEYSERLINGK; OLENICK; WEARY, 2008). Several researchers have noted a short-term and slight decrease in milk yield of cows that were mixed into a new social group (HASEGAWA et al., 1997;

VON KEYSERLINGK; OLENICK; WEARY, 2008), possibly as a result of increased competitive interactions at the feed bunk and DMI changes, but others have found no change in milk yield (CLARK; RICKETTS; KRAUSE, 1977; GRANT; ALBRIGHT, 2001).

2.5. Fresh cow health monitoring programs in dairy farms

Prediction or early detection of which cows have health problems is an important goal in any dairy farm (LEBLANC, 2010). Sick cows mean welfare, productive, and reproductive problems that translate to negative economic profits for dairy producers and the industry. Dairy producers, veterinarian practitioners, and researchers have developed and used different programs to monitor the health status of cows. It is true that there is not an ideal program for every herd; however, specific procedures should be developed for each farm based on the past history of fresh cow problems in the herd, the priorities of the management, the facilities, the skills and interests of the workers, and the objectives of the farm. Collaboration between the veterinarian, the nutritionist, the management, and the workers is needed in large farms to implement these programs. In a good and successful program for monitoring fresh cows, every farm needs a schedule, facilities, labor availability, and data collection. It is always better to prevent than to treat cows during the early postpartum. Prevention commences before calving, during the dry period, and should be based on nutrition, group management, and welfare because healthy cows resist the stresses around calving better than cows that are compromised by mismanagement or poor feeding (GUTERBOCK, 2004). Fat cows at calving, cows with long dry periods or health problems while they are dry, cows with dystocia, delivering twins or stillborn, or cows that aborted are in higher risk of getting into health problems during postpartum (BICALHO et al., 2007a; GIULIODORI et al., 2013; GOFF, 2008; GUTERBOCK, 2004; LEBLANC, 2010). Part of prevention is curing or removing sick dry cows before they calve.

The size of the farm and number of animals play an important role at the moment of designing a schedule and planning the activities and procedures to be done. Small farms permit a more individualized management of the animals, while large farms with hundreds or thousands animals need a group based management. Dividing the cows in groups facilitates monitoring them (BASSO SILVA, 2012; ESPADAMALA et al., 2013). In a 3000 cow herd where, on the average, 10 cows calve a day and labor is highly specialized, it is a much greater

challenge to pick out the cows that need attention from a fresh cow pen of 200 animals, examine them, and treat them appropriately. The maternity pen facilities and management are very important in the control of calving problems and the future health of the cows (PROUDFOOT; BAK JENSEN; VON KEYSERLINGK, 2014). Generally, large farms have an area destined for sick cows and cows under antibiotic treatment that can be called “hospital barn”. Usually fresh cows go into the hospital barn right after calving, in order to be checked for vaginal or uterine lesions, as well as to receive support treatment when needed. Then, cows are destined to the “fresh cow’s barn”, where they are kept during the first weeks of postpartum. As mentioned before, this phase is crucial for health, production, and reproduction success of the animal. Every cow should be checked during this period. The schedule and frequency for checking cows vary among farms. Some checked every cow, every day during the first weeks of postpartum, which it could be called an “intense” schedule; others do it based on milk yield, milk yield drops, DIM, a combination of these, or only when animals need attention.

The use of technology is becoming more adopted by large dairy farms, and it can be useful for fresh cow health screening based on automatic sensors that measure milk characteristics (yield, temperature, and electrical conductivity), animal behavior (feeding, rumination, activity), body weight, or body temperature. Using specific parameters and combining the above inputs farmers and veterinarians can guide the health check program for each individual animal or the group. The principle is to monitor deviations from expected performance, often based on the recent performance data of the animal (DE VRIES; RENEAU, 2010). For example, an increase in the conductivity of the milk is a good indicator of mastitis (NORBERG et al., 2004), and daily milk yield data have been shown to aid in early detection of ketosis, DA, and digestive disorders (EDWARDS et al., 2004). Although these technological devices have been tested and validated, some data misunderstanding could lead to mistakes in their use and interpretation. For example, healthy cows could show a drop in milk yield as a result of missing data during milking, or sick cows might not be identified if the software estimates yields of missing records (GUTERBOCK, 2004). Although technology is earning popularity among dairy producers, its use is still far from the expected in the screening of fresh cows health (ESPADAMALA et al., 2013). Only 7% of dairy farms in California were equipped with rumination sensors or electronic milk yield meters with most techniques used to screen for health disorders based on nonspecific and subjective observations, in a survey reported by Espadamala et al. (2013).

A screening method for determining which cow needs attention or is sick and needing treatment would ideally detect all these cows but not cows that do not need it. We could say that screening fresh cows is a matter of sensitivity and specificity (GUTERBOCK, 2004). If it examined and treated all cows, it will be 100% sensitive, however, it will not be specific because it will treat a large number, maybe the majority, of cows that do not need treatment, and this would be economical, labor, and time intensive for the farm.

On large dairy farms in the United States, there is no reason to think that only veterinarians will do the routine screening, examination, and treatment of cows if simple criteria can tell a trained worker what to do. The role of the veterinarian is to develop the program, train the dairy personnel, evaluate the results, help with unusual or difficult cases, and perform the surgeries. Workers screening, examining, and treating the fresh cows have to be given enough time and suitable facilities to do their jobs properly. In large farms, time can be scarce because a large number of cows have to be checked at a given time, and workers have to be well trained to do it. Cows are generally examined after milking. Some farms examine the cows in the freestall while others are examined when they leave the milking parlor. A convenient chute for examination is necessary in these cases. The veterinarian and the herd manager have to train the workers who will be doing the screening and examining the cows to provide them with practical protocols for diagnosis and treatment. Examination must be practical and follow an established pattern. Visual observation, udder and uterine palpation, milk yield and aspect, and body temperature measurement should be part of the routine (GUTERBOCK, 2004). Additional and increasingly objective methods, activities, and tools may be useful. Dipsticks and test strips for the screening of urine and milk ketone bodies are useful, quick, easy, and inexpensive tools for ketosis testing (OETZEL, 2004). Grading the locomotion using a standardized format for lameness diagnosis and several scoring systems are performed (ARCHER; GREEN; HUXLEY, 2010). Vaginal devices are often used for the diagnosis of metritis and endometritis, based on their ease of use and hygiene (PLETICHA; DRILLICH; HEUWIESER, 2009). Although these methods are being more adopted since farmers understand the benefits of them, Heuwieser et al. (2010) showed in a survey that most of dairy farms in Germany used subjective criteria to identify sick cows such as general appearance (97%) and appetite (69%) while less than half used more objective measurements such as temperature (33%), or ketone bodies (2.8%). Most of the surveyed dairy farms of this referenced work had less than 200 lactating cows. Data from Californian dairies indicate that some of these methods are more adopted in large farms, however, they should be more frequently incorporated into fresh cow evaluations (ESPADAMALA et al., 2013).

Fresh cow health monitoring programs could be categorized as low, moderate, and high with respect to intensity, based on schedule or frequency of cow checking methods and adoption of technology for deciding which cows need attention. A farm that checks cows based on subjective parameters only, such as appetite or appearance, and does it without a programmed schedule or frequency and without adopting additional methods for diagnosis can be defined as a low intensity program. An intense program (high intensity) could be defined as one that is based on technology for deciding which cows need attention, uses additional methods for diagnosis, and checks all cows during postpartum with high frequency. A moderate intensity program would be that one which adopts some technology with variation in schedule and frequency among low and high intensity programs.

3. OBJECTIVES

The objectives of this study were:

- To characterize patterns of daily rumination time, daily activity and daily milk production around the diagnosis of health disorders;
- To determine if alterations in rumination and activity anticipate diagnosis of disease by farm personnel;
- To determine if the addition of rumination and activity monitoring to on-farm routine diagnosis improves sick cows detection and diagnosis of disease during the first 30 DIM.

4. MATERIAL AND METHODS

4.1. Animals, housing, management and feeding

The experiment was conducted in North Central Florida, from August 2015 to December 2015, in one dairy farm milking approximately 4,800 Holstein cows thrice daily with a rolling herd average of approximately 11,500 kg/cow. Six hundred and ten ($n=610$) Holstein dairy animals (*primiparous* = 282, *parous* = 328) were enrolled in the experiment at 60 ± 3 days previous to expected parturition.

During the pre-partum period animals were housed in tunnel-ventilated freestall barns equipped with misters that were activated when environmental temperatures increased above 18°C . As animals showed signs of calving they were moved to a sand packed bedding pen for delivery of the newborn. After parturition, cows were moved to a fresh cow pen in a different freestall barn until completion of the transition period. After 17-24 DIM they were moved to groups depending on milk yield, parity (1st vs 2+) and genetic merit until the end of lactation. *Primiparous* and *parous* received the same pre-partum and post-partum total mixed ration (TMR). During the prepartum period, the TMR was offered three times a day, with the objective of keeping the feed bunk with food 24 hours a day. During the postpartum period, the TMR was delivered three times a day at 0700, 1500 and 2300 hours. The freestall beds and walking alleys were cleaned thrice daily. Twice weekly, clean and dry sand were added on the top of the freestall beds.

4.2. Rumination and activity measurement

Rumination and activity loggers (Hi-Tag, SCR Engineers Ltd., Netanya, Israel) (Figure 1) were positioned by a collar on the left side of the neck of each animal from -60 ± 3 to 80 ± 3 d relative to calving. Each logger contains a microphone that records and sends regurgitation and rumination sounds to stationary readers strategically situated in the farm. Information was processed, stored and collected using the software provided by the company (DataFlow II, SCR Engineers Ltd., Netanya, Israel). Rumination was recorded in minutes/2h intervals and

activity in arbitrary units/2h, finally total daily rumination (minutes per day) and daily activity (arbitrary units per day) were collected and used for statistical analysis.

Figure 1 - Rumination and activity logger



Legend: Rumination and activity loggers (Hi-Tag, SCR Engineers Ltd., Netanya, Israel) are positioned by a collar on the left side of the neck of each animal. Source: Silva, 2017.

4.3. Fresh cow monitoring, clinical examination and diagnosis of disease

Fresh cow health monitoring was performed following farm standard operating procedures, and following the information provided by the collars. Cows were divided into two groups (Collar based monitoring (CM), n=293 and Control (C), n=317) based on parity, age at conception, previous lactation disease diagnosis and BCS at enrollment (using a scale from 1 to 5 (1 = emaciated and 5 = obese; 0.25 unit increment as described by Ferguson et al. (1994)) in order to be checked for health disorders and compared the performance of checking cows based on farm routine and farm routine plus the addition of information provided by the collars for disease diagnosis. Animals from the group C were sent to be checked according to the routine of the farm, which consists of checking the animals after milking when they leave the milking parlor at 3, 5 and 9 DIM. Besides, milk production is followed during the first DIM; cows that do not match the desirable or expected milk production are sent to be checked. After

9 DIM, health screening is based on milk production drops, being the cow checked when a reduction of milk yield of 25% or more between milkings occurs.

Cows belonging to the CM group were sent to be checked following the routine of the farm, previously described, and the data supplied by the collars (rumination and activity). The daily selection of animals from the CM group for being checked based on the data provided by the collars was done according to the following parameters of DataFlow II software: daily rumination time (minutes per day the cow ruminates - DRT), 3 days total rumination variation (difference in minutes between today's DRT and the third previous day DRT - 3dRV) and Health Index (arbitrary units parameter calculated by an algorithm based on rumination and activity data that indicates cows may be sick - HI). Cows from the CM group showing any Rumination Alteration event (RA: DRT less than 200 minutes (DRT<200), negative 3dRV value (Neg3dRV), or HI less than 80 (HI<80)), were sent to be checked by the farm personnel. When a cow was flagged to be checked based on DIM, milk production, or collar's information (RA events), she was checked by the dairy personnel beside the milking parlor, where they looked for signs of retained placenta, metritis, mastitis, ketosis and/or any sign of disease the cow may show.

When the farm personnel assumed that a cow showed any sign of disease she was sent to the hospital barn in order to be checked in a deeper and accurate way by trained personnel. Diagnosis of retained placenta (RP), metritis (MET), milk fever (MLF), ketosis, displaced abomasum (DA) and other diseases (respiratory, digestive, lameness, septicemia, etc) were performed at the hospital barn based on the following parameters:

- Retained placenta: fetal membranes retained for at least 24 h after parturition.
- Metritis: watery, pink/brown, and fetid uterine discharge.
- Displaced abomasum: "ping" sound diagnosed by simultaneous auscultation and percussion on the right or left side between the 9th to 13th intercostal spaces.
- Clinical mastitis: abnormal characteristics of the milk or signs of inflamed mammary gland as pain, swelling, warmth, and redness of the quarter involved.
- Ketosis: anorexia, lethargy, low milk production accompanied by high ketones reading in urine dipsticks (KetoStix, Bayer Diagnostics, Tarrytown, NY).
- Milk Fever: low milk production, depression, ataxia, muscular alterations or/and decubitus.
- Indigestion: scant manure and lack of appetite with rumen and intestinal stasis.

- Other Diseases: signs related to diverse health disorders, such as pneumonia, udder sore, injuries, lameness, vaginitis and vaginal laceration, rupture of uterus, septicemia, abscesses, intestine torsion, kidney failure and fatty liver.

Treatments given to the cows and via of administration are described in Table 1. Cows received treatment at hospital barn when they were diagnosed as sick, or when the hospital barn personnel determined they needed some treatment. Treatments were chosen and administered following the farm criteria. Treatments were divided into three types: (1) antibiotics; (2) anti-inflammatories; (3) support treatments (minerals such as calcium, potassium, magnesium, and sodium, propylene glycol, amino acids, bismuth, vitamin B, dextrose, saline solution, mineral oil, rumen fluid, prostaglandins, and intrauterine bolus containing urea). Cows could receive more than one type of treatment, and more than one item of each type of treatment (i.e. one or more antibiotic drugs, such as penicillin and ampicillin, and, one or more support treatments, such as calcium and dextrose).

Table 1 - Treatments given to the animals

Type of treatment	Item	Via of administration
Antibiotics	Ampicillin	IM
	Ceftiofur	IM, intramammary, topic
	Penicillin	IM
Anti-inflammatories	Aspirin	Oral
	Dexamethasone	SC, IV
	Flunixin meglumine	IM
	Meloxicam	Oral
Support treatments	Amino Acids	Oral
	Bismuth	Oral
	Bolus containing urea	Intrauterine
	Dextrose	IV
	Mineral oil	Oral
	Minerals (Ca, K, Mg)	Oral, IV
	Prostaglandin (dinoprost)	IM
	Propylene glycol	Oral
	Rumen fluid	Oral
	Saline solution	IV
Vitamin B	Oral, IV	

SC, subcutaneous; IM, intramuscular; IV, intravenous

4.4. Blood sampling, BHBA and total calcium determination for the diagnosis of subclinical ketosis (SCK) and subclinical hypocalcemia (SCHC)

Three blood samples were collected from each cow between 0 and 20 DIM using empty evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) that were placed in ice after collection until centrifugation for serum separation (3,000 rpm for 15 min at 4 °C). Then, serum was aliquoted into microcentrifuge tubes and stored at -32 °C until analysis.

The first blood sample (0 to 4 DIM) was used to determine total serum calcium concentration using an enzymatic assay (Randox Calcium-CPC/AMP, Randox Laboratories, Antrim, UK). The second (4 to 12 DIM) and third (7 to 20 DIM) blood samples from each cow were used to determine β -hydroxybutyrate (BHBA) concentrations enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et. al., 2009). Diagnosis of subclinical hypocalcemia and ketosis were done based on calcium and BHBA concentrations, respectively.

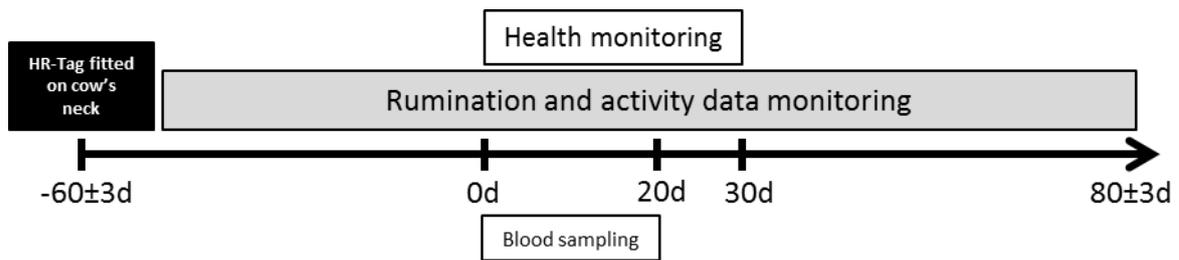
- Sub-clinical ketosis: BHBA concentration > 1,000 μ mol/L.
- Sub-clinical hypocalcemia: Ca concentration < 8.55 mg/dL.

It is important to note that the methodology and schedule for disease diagnosis adopted by the farm and exam and blood sampling schedule performed by our research group—despite being widely adopted by other research groups and the dairy industry—may result in some inaccuracies in the diagnosis of diseases and might not be sufficient to identify sudden changes in concentrations of metabolites. Figure 2 depicts the study design.

4.5. Milk production

Cows were milked thrice daily and individual milk yield was recorded at each milking using SmartDairy® Management Systems (Boumatic, Madison, WI, USA). For statistical analysis purposes, daily milk yield from calving to 80 DIM was collected.

Figure 2 - Graphical representation of study design



Legend: Cows were fitted with an electronic rumination and activity monitoring tag fixed to a collar (HR Tags; SCR Dairy, Netanya, Israel) approximately 60 d before calving to monitor rumination and activity until at least 80 days in milk approximately. Cows were divided in two groups (C = Control; CM = Collar Monitoring) based on parity, age at conception, previous lactation disease diagnosis and BCS at enrollment. Fresh cows health monitoring was performed by the farm personnel from 0 to 30 DIM following the routine of the farm. Additionally, cows from group CM were checked based on rumination and activity data. Three blood samples per cow were collected from 0 to 20 DIM for the diagnosis of subclinical ketosis and hypocalcemia based on BHBA and calcium concentrations, respectively. Source: Silva, 2017.

4.6. Data collection

Daily rumination time (**DRT**), daily activity (**ACT**) and source of cow's flagging ($DRT < 200$, $Neg3dRV$, $HI < 80$) were collected using DataFlow II software. Cows flagging will be mentioned as "system alert" or "rumination alteration" (**RA**), from now in the text. Disease diagnosis and production data were collected using PCdart software (DRMS-North Carolina, USA). All data were organized for statistical analysis using Microsoft Excel (Microsoft-Washington, USA) and statistical analyses were performed using the version 3.2.3 of R Studio (R Foundation for Statistical Computing, Vienna, Austria) and the version 9.4 of SAS (SAS Institute Inc., Cary, NC)

4.7. Statistical analysis

This was an observational cohort experiment. Animals were enrolled in weekly cohorts of 6 to 94 animals that were randomly divided into two groups according to parity, age at conception, previous lactation disease diagnosis and BCS at enrollment. The version 3.2.3 of R Studio and version 9.4 of SAS were used for statistical analyses. Correlations using the following variables were calculated: daily rumination time (DRT), daily activity (ACT), average DRT, average prepartum DRT, average postpartum DRT, average activity, average

prepartum ACT, average postpartum ACT, average milk yield during the first 30 DIM (30dMilk), average milk yield during the first 80 DIM (80dMilk), calcium concentration, BHBA concentration. All correlations were calculated using Pearson's correlation coefficient (r). T-test and Analysis of the Variance (ANOVA) were used to compare and analyze continuous variables. Binary variables were analyzed and compared using logistic regression and Wald test for logistic regression.

4.7.1. Rumination, activity and milk production patterns in cows with clinical disease, calving problems, subclinical disorders and regrouped animals

Daily rumination time (min/d) and daily activity (arbitrary units/d) were evaluated from 7 d preceding to 4 d after the diagnosis of retained placenta, metritis, ketosis, milk fever, displaced abomasum, mastitis, indigestion, lameness, and "other diseases". Daily milk production (Kg/d) was evaluated from 7 d preceding to 4 d after diagnosis of metritis, DA, mastitis, indigestion and other diseases, and the occurrence of regrouping; from the day of diagnosis to 4 d after diagnosis of RP; from 4 d preceding to 4 d after diagnosis of ketosis; from 2 d preceding to 4 d after diagnosis of milk fever; and from 3 d preceding to 4 d after diagnosis of lameness. This was done because data from all days from 7 d preceding to 4 d after diagnosis were not available in all cases (pre-partum days and data availability as main reasons). For SCK, SCHC, and calving problems, DRT and ACT were analyzed from 7 d before to 14 d after calving, and milk production was evaluated from the day of calving until 14 DIM. DRT, ACT, and milk production patterns were analyzed from 7 previous to 4 days after the occurrence of regrouping. Normality of the data was evaluated using Shapiro-Wilk and graphical methods, such as QQ-plot, histogram and box-plot. Data transformations were not necessary.

Cows were divided into three groups for the analysis of DRT, ACT and milk production around the diagnosis of clinical disease:

Healthy: cows not diagnosed with a health disorder during the period of study. The number of animals for the Healthy group varies for each disease depending of the number of sick animals. Cows that did not develop health disorders during the experiment were randomly selected to be part of group Healthy.

SickRAyes: cows that were diagnosed with the health disorder of interest, and showed at least one RA event within 7 d before, the day of, and 2 d after diagnosis.

SickRAno: cows that were diagnosed with the health disorder of interest and did not show any RA event within 7 d before, the day of, and 2 d after diagnosis.

For cows in groups *SickRAyes* and *SickRAno*, the day of diagnosis was considered as “Day 0”. For cows in the Healthy group, the average DIM at diagnosis for cows that suffered the disease in study was considered as “Day 0”.

For the analysis of DRT, ACT, and milk production around regrouping, cows were divided into two groups:

Control: Cows that were not moved to another pen.

Regrouped: Cows that were moved to another pen.

Cows were divided into two groups for the analysis of DRT, ACT, and milk production patterns for calving problems (dystocia, stillbirth, and twins):

Dystocia, Stillbirth, or Twins: Cows with dystocia, cows delivering stillborn calves, or cows delivering twins, respectively.

Control: Cows with normal calving, cows delivering live calves, or cows delivering singleton calves, respectively.

In the cases of dystocia and stillbirth, cows were also divided into three groups according to the presence of clinical disease during the first 14 DIM:

Dystociaonly or *Stillbronly*: Cows with dystocia or cows delivering stillborn calves, but not diagnosed with any clinical disease during the first 14 DIM.

Dystociadisease or *Stillbrondisease*: Cows with dystocia or cows delivering stillborn calves, diagnosed with clinical disease during the first 14 DIM.

For the analysis of the patterns in cows delivering twins, cows were not divided according to the occurrence of disease because only two cows having twins were not diagnosed as sick.

For the analysis of DRT, ACT, and milk production in cows with SCK and SCHC, cows were divided into two groups:

SCK or *SCHC*: Cows diagnosed with SCK and SCHC based on BHBA and calcium concentrations, respectively.

Control: Cows not diagnosed with SCK and SCHC.

Additionally, analysis of DRT, ACT, and milk production patterns in cows with SCK and SCHC were performed dividing the cows into three groups according to the presence of clinical disorders:

SCKonly or *SCHConly*: Cows diagnosed with SCK or SCHC, but not diagnosed with any clinical disorder during the first 14 DIM.

SCKdisease or *SCHCdisease*: Cows diagnosed with SCK or SCHC, and diagnosed with clinical disorders during the first 14 DIM.

Control: Cows not diagnosed with SCK and SCHC, neither clinical disorders.

In the case of SCHC, the analysis including the presence of clinical disorders was performed for *primiparous* and *parous* separately, because effect of parity was found ($P < 0.05$).

For the groups Healthy, Control, and Regrouped, only cows without any disease during the entire experimental period were included, in order to avoid any effect on rumination, activity, and milk production. When *primiparous* and *parous* cows suffered from disease, or were regrouped, the control groups (Healthy and Control) included a balanced number of heifers and cows.

Data were analyzed by ANOVA with repeated measures using PROC MIXED of the version 9.4 of SAS. Models for each variable of interest (DRT, ACT, and milk production) included group, time, and the interaction between group and time as explanatory variables. When *primiparous* and *parous* were present, parity and the interaction between group and parity were included also to model. The occurrence of any other disease from 7 d preceding, the day of, and 4 d after diagnosis was added to the model. Variations to the model were done according to the disorder of interest. The model for metritis, mastitis, lameness, “other diseases”, included as dependent variables all explanatory variables and all groups of the variable “group”. The model for RP included only 2 groups (Healthy and SickRAyes) because only two cows that developed RP did not show RA from 7 d preceding to 2 d after diagnosis and were not included for analysis. Displaced abomasum and indigestion models did not include the SickRAno group and parity, because all cows with DA showed RA, and cows with indigestion without RA had few data available for analysis, and only one and two *primiparous* suffered from DA and indigestion, respectively. The models for ketosis and milk fever included the three groups (Healthy, SickRAno and SickRAyes) but did not include parity because only one *primiparous* got sick. For lameness, the occurrence of other diseases during the period of study was not included into the model because only 2 animals suffered from other health disorder during the period of study. In the model for regrouping, parity was included, but the presence of disease was not, because only healthy cows were taken into account. Models for calving included parity and the occurrence of clinical disease during the first 14 DIM, except for twins because only three primiparous had twins, and two cows among cows

delivering twins, had clinical disease. For the model of SCK and SCHC, parity was included. Data were analyzed separately for *primiparous* and *parous* when the occurrence of clinical disease was included in the model of SCHC, because interaction between parity and group ($P < 0.05$) was found, and data showed different behavior among parities.

Cow within group was included as random effect in all models. The final model for each parameter of interest was selected by determination of the lowest value for the Akaike's Information Criterion (AIC). Cow was the subject of the repeated measurements. When the main effect or interaction between explanatory variables was significant, the LSD post-hoc mean separation test was used to determine specific differences between groups of means. For all tests a significance level of 5% was adopted.

4.7.2. Rumination and activity monitoring performance to detect sick cows and predict disease diagnosis

In order to analyze the performance of the collars system to detect sick cows, sensitivity (**Se**) was calculated using the clinical diagnosis performed by the farm personnel as reference. A positive outcome was defined as at least one RA event during the 7 d preceding, the day of, or 2 d after the diagnosis of disease. The calculation of Se of RA for any disease and for each disease of interest (RP, metritis, DA, ketosis, MLF, indigestion, mastitis and "other diseases") was done using R Studio, and was defined as the ability of RA to correctly identify cows with a positive disease diagnosis outcome. To evaluate the potential confounding effect of other health disorders (i.e., all disorders of interest) on the Se of RA, three separate analyses were conducted. The first analysis included all cows diagnosed with the disease of interest (regardless of the occurrence of another disorder during the 7 d preceding until 4 d after the disease diagnosis); a second analysis included cows diagnosed only with the disease of interest during the 7 d preceding until 4 d after the disease diagnosis; and a third analysis included cows that were diagnosed with the disorder of interest and at least another health disorder during the mentioned period around disease diagnosis. Differences in Se of RA between the subgroup of cows with the disorder of interest only and cows with the disorder of interest and at least another disorder were determined. Moreover, differences between groups (Control (C) and Collar based Monitoring (CM)) for overall Se, Se for cows with the disease of

interest only, and Se for cows with the disease of interest and at least another disorder were calculated. All differences for Se were performed by logistic regression using R Studio.

Besides, overall specificity (**Sp**), positive predicted values (**PPV**), and negative predicted values (**NPV**) were also accounted for system evaluation. Because RA is designed as an alert to identify cows for further clinical examination rather than to provide a definitive diagnosis of a health disorder, false-positive outcomes could not be assigned to a particular health disorder, because the reason for the alert was unknown. Therefore, Sp, PPV, and NPV were calculated as an overall test for all events recorded for the disorders of interest. Because each day was considered a new test, the total contribution of individual cows to the number of cow-days was determined from 0 to 30 DIM. A cow could contribute with a positive or negative outcome during specific periods of the study depending on her clinical status (without disease and with disease) as defined by the diagnosis performed by the farm personnel. In order to calculate the mentioned parameters (Se, Sp, PPV, and NPV), the following criteria were defined:

- True positive = positive RA event (DRT<200, Neg3dRV, and/or HI<80) within the 7 days before, the day of, and 2 d after the diagnostic of disease.
- False positive = positive RA event for a cow without a health disorder, or RA event outside the -7 to 2 days period relative to diagnosis for cows with health disorders.
- True negative = negative RA event (DRT>200, Positive 3dRV, and/or HI>80) for a cow without a health disorder, or negative RA event outside of the -7 to 2 days period relative to diagnosis for cows with health disorders.
- False negative = negative RA event during the -7 to 2 days period relative to diagnosis for a cow with a health disorder.

4.7.3. *Interval between the first RA event and disease diagnosis*

To determine if the collar system was capable of identifying cows with health disorders earlier than the farm personnel diagnosis, the interval (in days) between the first RA event (system alert) (during the 7 d preceding until 2 d after the disease diagnosis) and the day of disease diagnosis was evaluated for each disease of interest. As it was done for Se of RA, interval between the first RA event and disease diagnosis was calculated for all cows with the

disease of interest (regardless of the occurrence of another disorder during the 7 d preceding until 4 d after the disease diagnosis), cows with the disease of interest only, and cows with the disease of interest and at least one another disease during the period of interest around diagnosis. For this analysis, which only included cows flagged by the system during the 7 d preceding until 2 d after the disease diagnosis, the mean number of days from the first RA event and the day of diagnosis was compared with a paired t-test using R Studio. Moreover, differences between groups (C and CM) for the interval between the first RA event and the day of diagnosis for each disease were determined with an un-paired t-test using the same statistical software.

4.7.4. Farm diagnosis of disease, treatment of the animals, culling rate and reproductive performance

The overall incidence of disease and incidence of each disease, incidence of RA events, percentage of cows given treatments, percentage of cows sent to hospital barn and number of days staying at hospital, culling rate until 60 and 150 DIM, reproductive parameters, such as service rate until 150 DIM, conception rate and pregnancy loss at first service, and cows marked as “do not breed” (reproductive culling) were calculated for all cows, by parity and groups. Comparisons between parity and groups were calculated. Continuous and binary variables were compared by t-test, and logistic regression, respectively, using R Studio software. A significance level of 5% was chosen.

5. RESULTS

From a total of 683 cows enrolled in the study, 73 cows (10.6%) were removed from the data set for analysis due to tag malfunction, wrong collar placement or because of premature calving (calving occurred before the 7 d needed for the collar to draw a baseline). Thus, 610 cows were included in the final data set for analysis. The percentage of male calves, twin births and stillborn were 49.7% (301/610), 2.8% (17/610), and 4.4% (27/610), respectively. The overall incidence of disease during the first 30 DIM was 48% (293/610). Of the cows that got sick (293), 55.3% (162/293) suffered from one disease and 44.7% (131/293) presented more than one disease. Among parities, incidence of disease was higher for *parous* (57% (187/328)) than for *primiparous* animals (37.1% (106/282)) ($P < 0.001$). Table 2 shows the incidence for each disease. The incidence of stillbirth, dystocia, RP, metritis, milk fever, ketosis, DA, mastitis, indigestion, and “other diseases” according to farm personnel diagnosis was, 7.5% (45/598), 2.6% (16/610), 28.5% (174/610), 2.1% (13/610), 6.7% (41/610), 2.4% (15/610), 6.7% (41/610), 2.4% (15/610) and 16.7% (102/610) respectively.

Average milk yield during the first 30 DIM (30dMilk) was 39 ± 0.5 kg/d for *parous* and 31.8 ± 0.3 kg/d for *primiparous* animals. Average milk yield during the first 80 DIM (80dMilk) was 46.8 ± 0.06 and 37.2 ± 0.09 kg/d for *parous* and *primiparous* cows, respectively.

5.1. Subclinical metabolic disorders: SCK and SCHC

The incidence subclinical ketosis and subclinical hypocalcemia was 14.5% (86/593) and 77.6% (421/542), respectively. No differences were found for incidence of SCK (C = 14.5%, CM = 14.4%, $P = 0.96$) and SCHC (C = 77.45, CM = 77.9%, $P = 0.9$) between groups C and CM.

5.1.1. Subclinical Ketosis (SCK)

The incidence of SCK was higher for *parous* (23.3% (74/317)) than for *primiparous* (4.3% (12/276)) ($P < 0.001$). Average DRT ($P < 0.01$), ACT ($P < 0.001$), postpartum DRT ($P = 0.007$) and postpartum ACT ($P < 0.001$) were lower for cows diagnosed with SCK (497 min/d, 586 AU/d, and 464 min/d, 581 AU/d) than for healthy cows (515 min/d, 625 AU/d, and 498 min/d, 636 AU/d). Average Prepartum DRT ($P = 0.83$) and prepartum ACT ($P = 0.88$) were not different among cows with SCK (540 min/d and 573 AU/d) and healthy cows (541 min/d and 575 AU/d). However, average DRT ($P < 0.001$) and ACT ($P < 0.001$) during the last week of the prepartum period were higher for the healthy cows (497 min/d and 586 AU/d) compared to cows diagnosed with SCK (481 min/d and 568 AU/d) after calving. Average milk yield until 30 DIM ($P = 0.76$) and 80 DIM ($P = 0.3$) were not different among cows with (35.5 kg/d, 43.8 kg/d, respectively) and without SCK (35.9 kg/d, 42.3 kg/d, respectively). The incidence of disease was higher in cows with SCK (76.7% (66/86)) than in cows without SCK (46.6% (216/507)) ($P = 0.01$). Among cows with SCK, no differences between groups C and CM were found for any studied parameter.

5.1.2. Subclinical Hypocalcemia (SCHC)

The incidence of SCHC was not different among *primiparous* (76.8%) and *parous* (78.3%) cows ($P = 0.66$). Average DRT ($P = 0.37$), prepartum DRT ($P = 0.31$), postpartum DRT ($P = 0.55$) did not differ among cows with SCHC (512 min/d, 536 min/d, 489 min/d, respectively) and cows without SCHC (517 min/d, 541 min/d, 495 min/d, respectively). Average ACT ($P = 0.15$) and prepartum ACT ($P = 0.35$) were not different among cows with (615 AU/d, 574 AU/d, respectively) and without (628 AU/d, 565 AU/d, respectively) SCHC. Average postpartum ACT tended to be lower in cows diagnosed with SCHC (622 AU/d) than in healthy cows (639 AU/d) ($P = 0.09$). Average DRT ($P = 0.001$) and ACT ($P = 0.01$) during the first 7 DIM were higher in cows that did not developed SCHC (406 min/d and 657 AU/d) than in cows with SCHC (389 min/d, 632 AU/d). Average milk yield until 30 DIM ($P = 0.04$) and 80 DIM ($P = 0.006$) were higher in cows with SCHC (36 kg/d, 42.9 kg/d) than in cows without SCHC (34.2 kg/d, 40.1 kg/d) ($P = 0.04$). The incidence of disease was higher for cows

with SCHC (52% (219/421)) compared to cows without SCHC (36.3% (44/121)) ($P = 0.02$). Among cows with SCHC, no differences among groups were found for any studied parameter.

5.2. Daily rumination time and activity

Figure 3 and 4 show daily rumination time and daily activity of all cows, by parity, and by treatment. Average DRT, average prepartum DRT and average postpartum DRT were 515.14 ± 2.40 , 541.40 ± 2.66 and 487.47 ± 3.95 min/d, respectively. Average DRT, average prepartum DRT and average postpartum DRT for groups C and CM were 511.48 ± 3.29 and 514.19 ± 3.51 ($P = 0.57$), 537.35 ± 3.70 and 545.87 ± 3.84 ($P = 0.11$), and 489 ± 5.24 and 485.80 ± 5.98 min/d ($P = 0.68$) respectively, without differences between groups. Average ACT, average prepartum ACT and average postpartum ACT were 618.40 ± 3.75 , 573.40 ± 3.80 , 627.00 ± 4.12 arbitrary units/d respectively. Average ACT, average prepartum ACT and average postpartum ACT for groups C and CM were 615.03 ± 5.04 and 621.95 ± 5.58 ($P = 0.35$), 568.08 ± 5.24 and 579.08 ± 5.50 ($P = 0.14$), and 624.04 ± 5.51 and 630.05 ± 6.18 arbitrary units/d ($P = 0.45$), respectively, and no differences were found between groups.

5.2.1. Association between prepartum and postpartum DRT and activity

Average prepartum DRT was moderately correlated with average postpartum DRT ($r = 0.39$, 95% CI = 0.32, 0.45, $P = <0.01$). Average prepartum activity was highly correlated with average postpartum activity ($r = 0.62$, 95% CI = 0.57 – 0.66, $P = <0.01$).

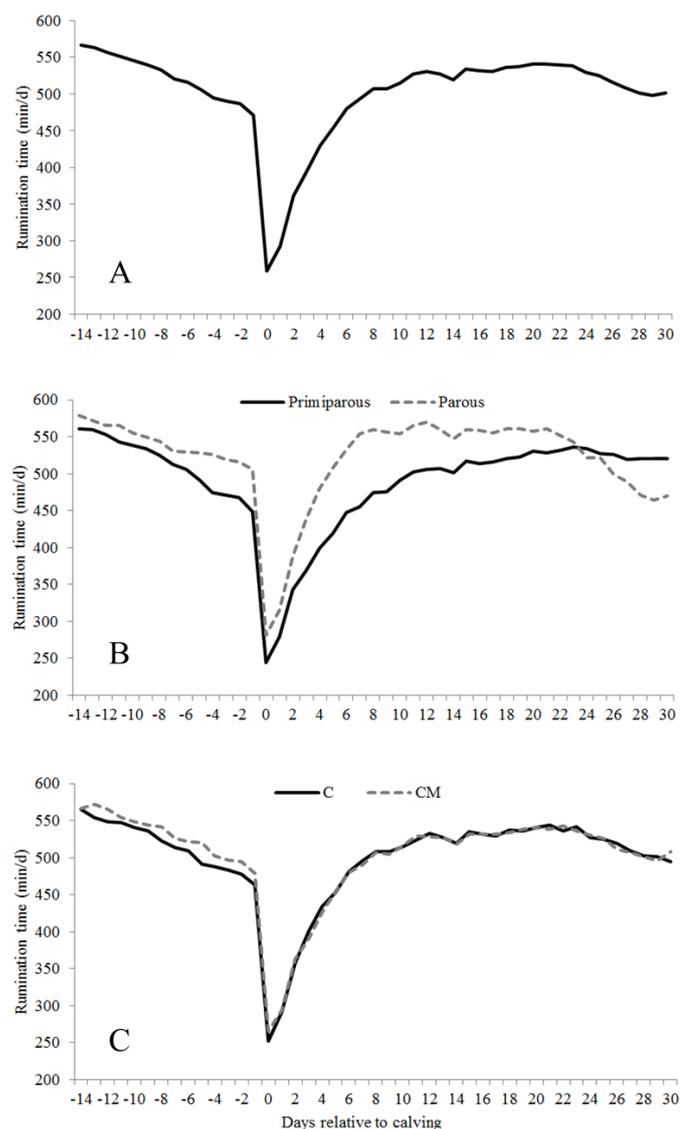
5.2.2. Association between milk yield and DRT and activity

Average milk yield during the first 30 DIM (30dMilk) was weakly correlated with average prepartum DRT ($r = 0.17$, 95% CI = 0.09, 0.25, $P = <0.01$). Correlation between 30dMilk and average postpartum DRT was moderate ($r = 0.34$, 95% CI = 0.27, 0.41, $P <0.01$).

30dMilk was weakly correlated with average prepartum activity and average postpartum activity ($r = 0.13$, 95% CI = 0.06, 0.21, $P = <0.01$ and $r = 0.10$, 95% CI = 0.02, 0.18, $P = <0.01$, respectively).

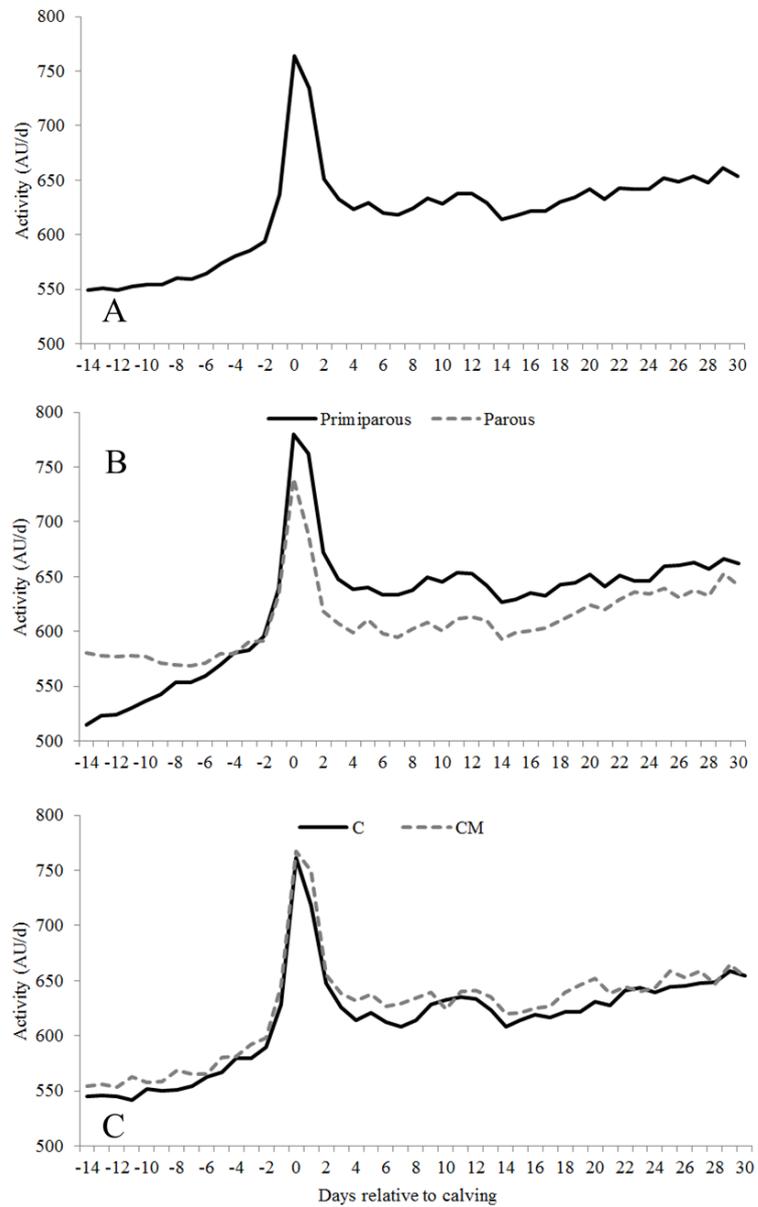
Average milk yield during the first 80 DIM (80dMilk) was weakly correlated with average prepartum DRT ($r = 0.20$, 95% CI = 0.11 – 0.26, $P = <0.01$). There was a moderate correlation between 80dMilk and postpartum DRT ($r = 0.36$, 95% CI = 0.29 – 0.43, $P = <0.01$). 80dMilk was weakly correlated with average prepartum activity ($r = 0.15$, 95% CI = 0.07, 0.23, $P = <0.01$) and average postpartum activity ($r = 0.10$, 95% CI = 0.025, 0.18, $P = <0.01$).

Figure 3 - Daily rumination time around calving



Legend: Daily rumination time (min/d) for all cows (A), by parity (B), and by treatment (C) from 14 d before to 30 days after calving; C, group Control; CM, group CM (collar monitoring). Values are presented as LSM \pm SEM. Source: Silva, 2017.

Figure 4 - Daily Activity around calving



Legend: Daily Activity (arbitrary units/d) for all cows (A), by parity (B), and by treatment (C) from 14 d before to 30 days after calving; C, group Control; CM, group CM (collar monitoring). Values are presented as LSM \pm SEM. Source: Silva, 2017.

5.3. Rumination alteration

The percentage of animals that had at least one RA event (RA) during the experimental period (first 30 DIM) was 54.9% (335/610), without differences between groups (C = 53.9% (171/317), CM = 55.9% (164/293), $P = 0.61$). Among the cows with altered rumination, 61.5% (206/335) suffered from some disease, but no differences were shown between groups (C = 63.1% (108/171), CM = 59.7% (98/164), $P = 0.52$). Thirty two percent (88/275) of the cows that never had altered rumination got sick (C = 28.7% (42/146), CM = 34.8% (45/129), $P = 0.22$). Of the cows that got sick, 70% (206/294) had altered rumination at some time, without differences between groups (C = 72% (108/150), CM = 68% (98/144), $P = 0.4$). Among the cows that never got sick during the experimental period (healthy cows), 40.8% had RA (C = 37.7% (63/167), CM = 44.2% (66/149), $P = 0.23$). Thus, cows that had altered rumination had higher risk of getting sick when compared to cows with unaltered rumination ($P < 0.01$; OR = 3.39).

The incidence of RA was not statistically different among *primiparous* (57.8% (163/282)) and *parous* (52.4% (172/328)) animals ($P = 0.18$). Nevertheless, the percentage of *primiparous* with RA tended to be higher for the CM (63.2% (86/136)) than for the C (52.7% (77/146)) group ($P = 0.07$). However, no differences between groups were found for the incidence of RA in *parous* (C = 54.9% (94/171), CM = 49.6% (86/157), $P = 0.33$).

RA occurred at 10.10 ± 6.89 DIM in average (C = 10.12 ± 6.80 , CM = 10.08 ± 6.98 , $P = 0.74$). Average DIM at first RA event was 7.41 ± 6.75 (C = 7.75 ± 6.65 , CM = 7.06 ± 6.86 , $P = 0.29$). *Primiparous* animals (8.9 ± 5.77 DIM in average) had RA earlier than *parous* animals (11.14 ± 7.58 DIM in average) ($P < 0.001$). First RA event occurred earlier for *primiparous* than *parous* (6.08 ± 4.83 DIM and 8.68 ± 7.98 DIM, respectively; $P < 0.001$).

5.4. Ruminantion, activity and milk production patterns in cows with clinical diseases, calving problems, regrouping, and subclinical metabolic disorders

5.4.1. Daily ruminantion time, daily activity, and daily milk production relative to the diagnosis of clinical diseases

5.4.1.1. Retained Placenta

For ruminantion, there was effect of interaction between group and day ($P < 0.001$) (Figure 5A). Animals that developed RP ($n = 14$) had lower ruminantion than healthy animals ($n = 71$) from “Day -4” throughout the period, with the greatest difference occurring on “Day -2” (~-1 DIM average) (211 min/d). The nadir for ruminantion for both groups was observed on “Day -1” (~0 DIM average), and it was lower for group SickRAYes than group Healthy (165 and 240 min/d, respectively). Thereafter, ruminantion increased for both groups but the increment was slower for cows in the SickRAYes group than for cows in the Healthy group. No effect of parity and interaction between parity and group were found ($P > 0.05$). No differences for DRT between cows that had other disease during the period of interest and cows that had no other disease were observed ($P > 0.05$).

For activity, there was effect of interaction between group and day ($P < 0.001$) (Figure 5B). Healthy animals had higher activity than SickRAYes group animals from “Day -1” throughout the period of study. The greatest difference occurred on “Day -1” (~0 DIM in average) (139 AU/d). No effect of parity and interaction between parity and group were found ($P > 0.05$). The occurrence of other disorders did not affect activity ($P > 0.05$).

For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 5C). Healthy animals produced more milk than animals that developed RP from “Day 1” to “Day 4”. Animals suffering from RP had a slower increment of milk production compared to healthy animals. No effect of parity and interaction between parity and group were found ($P > 0.05$). Milk production was not different between cows that had other disorder and those that had no other disorder during the period ($P > 0.05$).

5.4.1.2. Metritis

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 5D). Rumination was higher for Healthy ($n = 150$) and SickRAno ($n = 89$) groups when compared to the SickRAyes ($n = 85$) group during the entire period of study. No differences between Healthy and SickRAno groups were found. The nadir of DRT for Healthy and SickRAno group animals was observed on “Day -7” (461 and 483 min/d, respectively), since metritis was diagnosed on average at 11 DIM. Thereafter, an increment for rumination was observed as days go forward, slower for SickRAno than Healthy group. DRT decreased for SickRAyes from “Day -7”, reaching its nadir on “Day -4” (365 min/d), then a slow increment was observed. This increment was faster after the day of diagnosis (“Day 0”). The greatest difference between Healthy and SickRAyes and between SickRAno and SickRAyes was observed on “Day -3” (168 min/d) and “Day -2” (150 min/d), respectively. There was effect of parity ($P = 0.001$), with higher values for *parous* than *primiparous* animals. However, no effect of interaction between parity and group was found ($P > 0.05$). Rumination time was affected by the occurrence of other disease during the period of study ($P = 0.01$), cows that suffered from any other disease from 7 d preceding up to 4 d after diagnosis had lower DRT than cows that did not.

For activity, there was effect of group ($P = 0.02$) (Figure 5E). SickRAyes group animals showed the lowest values, with a nadir of 560 AU/d at the day of diagnosis (“Day 0”). However, no effect of day ($P = 0.07$) and interaction between group and day were found ($P = 0.13$). Regarding to parity, *parous* had lower values than *primiparous* animals ($P < 0.001$), nevertheless no effect of interaction between parity and group was found ($P > 0.05$). Finally, cows that developed other disease during the period of study had lower activity than those that did not ($P = 0.009$).

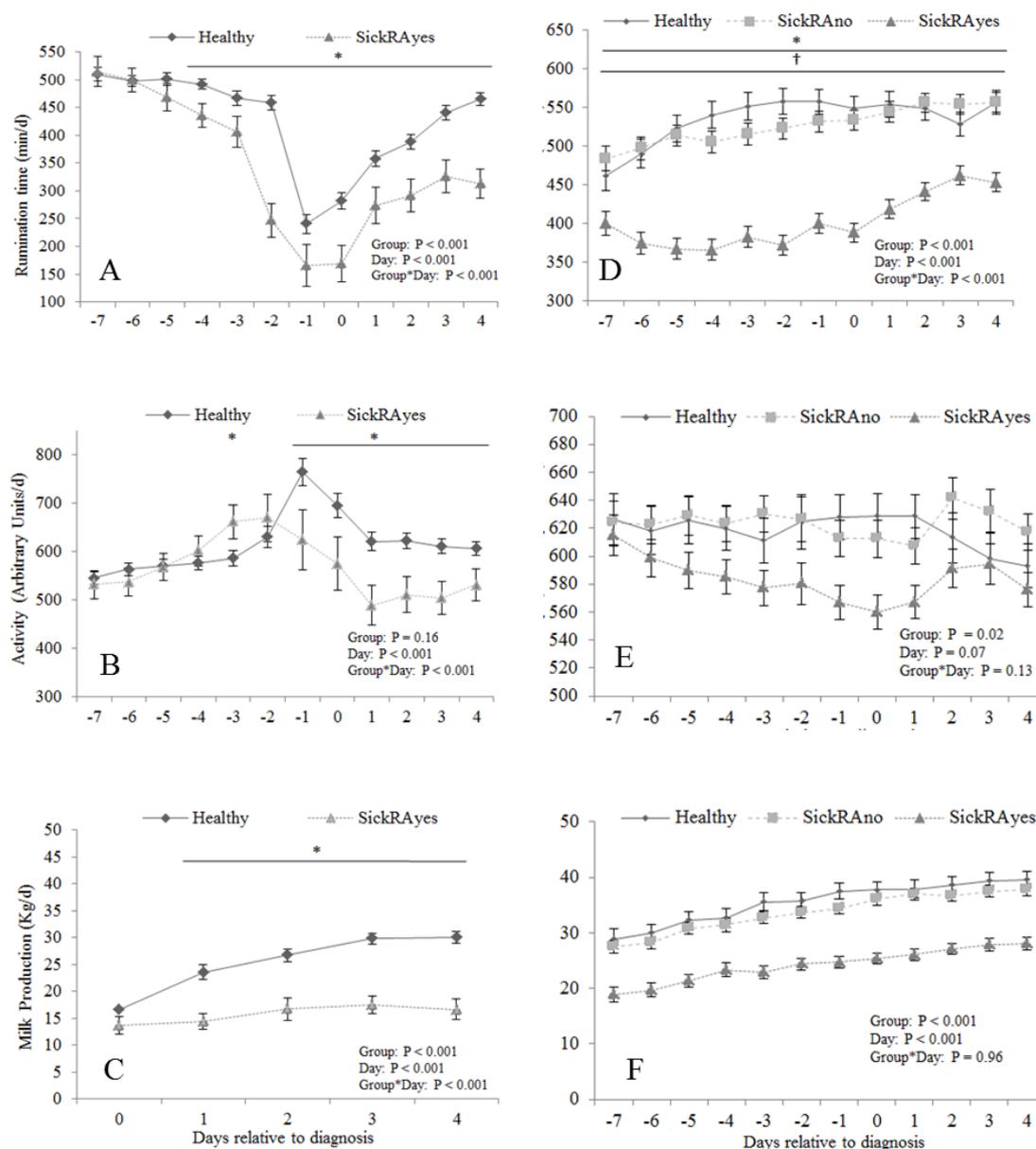
Animals belonging to SickRAyes group produced less milk than animals from Healthy and SickRAno groups ($P < 0.001$) (Figure 5F). However, no differences between Healthy and SickRAno groups were found. There was effect of day ($P < 0.001$), but no interaction between group and day was observed ($P = 0.96$). The nadir for milk production for all groups (28.8, 27.5 and 18.8 kg/d for Healthy, SickRAno and SickRAyes, respectively) was observed on “Day -7” (~4 DIM in average). Thereafter, milk production increased for all groups, but this increment was slower for cows in the SickRAyes group when compared to cows in the

SickRA_{no} and Healthy group. Milk production was affected by the occurrence of other diseases during the period of interest ($P < 0.001$), with lower milk production for cows that developed other disorder than cows that did not. There was effect of interaction between group and the occurrence of other disease ($P = 0.02$), because even if the cow had no other disease but had RA during the period, milk production was not different to cows with other disease.

5.4.1.3. Ketosis

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 6A). Because animals were diagnosed with ketosis on 6 DIM in average, “Day -6” corresponds to the day of calving for the Healthy group. For group SickRA_{no}, “Day -3” represents the day of calving, since animals were diagnosed with ketosis on ~3 DIM in average in this group. Abrupt decrements in rumination on these days are related to parturition. Differences for rumination between Healthy ($n = 48$) and SickRA_{yes} ($n = 28$) groups were found from “Day -4” throughout the period; differences between SickRA_{yes} and SickRA_{no} ($n = 11$) groups were found from “Day 0” on forward; and differences between Healthy and SickRA_{no} from “Day -3” to “Day 0”. Rumination for SickRA_{yes} began to decrease on “Day -6” until reaching its nadir (270 min/d) on the day of diagnosis (“Day 0”), followed by an increment during the following days. For the SickRA_{no} group, rumination increased after “Day -3” and no decrement was shown on “Day 0”. Maximum difference between Healthy and SickRA_{yes} occurred on “Day 0” (279 min/d). Rumination was not affected by the occurrence of other health disorders ($P > 0.05$) during the period of study around diagnosis of ketosis because rumination was not different between animals that had other disease and animals that had not.

Figure 5 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with retained placenta and metritis, and healthy cows



Legend: Patterns of daily rumination time (A) and daily activity (B), from -7 to 4 d relative to diagnosis, and daily milk production (C), from 0 to 4 d relative to diagnosis, for cows diagnosed with retained placenta (RP; group SickRAYes, $n = 14$) and healthy cows (group Healthy, $n = 71$). All cows with RP showed at least 1 rumination alteration (RA; daily rumination time <200 min/d, negative 3 days total rumination variation, and/or Health Index <80) event from -7 to 2 d relative to diagnosis. Patterns of daily rumination time (D), daily activity (E), and daily milk production (F) from -7 to 4 d relative to diagnosis of metritis (groups SickRAno and SickRAYes), and healthy cows (group Healthy, $n = 150$). Cows were assigned to the SickRAno ($n = 89$) and SickRAYes ($n = 85$) group if they showed or did not show at least 1 RA event from -7 to 2 d relative to diagnosis, respectively. For cows in groups SickRAno and SickRAYes, “day 0” belongs to the day of diagnosis. For cows in the Healthy group, the average DIM at diagnosis for cows with RP or metritis was considered as “day 0”. Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Healthy vs SickRAYes; † SickRAno vs SickRAYes. Source: Silva, 2017.

For activity, there was effect of interaction between group and day ($P < 0.001$) (Figure 6B). Activity for the Healthy group was higher than SickRAYes from “Day -2” throughout the period of study, showing a maximum difference (143 AU/d) on “Day -1”. For Healthy group, a peak in activity was observed on “Day -6”, belonging to day of calving. Thereafter, activity maintained similar values. Activity for SickRAYes showed a strong decrement on “Day -3” until its nadir (445 AU/d) on “Day -1”. A slightly recovery was observed during the following days. Activity was not different between animals with other health disorders and animals without other health disorders during the period of interest around diagnosis of ketosis ($P > 0.05$).

For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 6C). Healthy group animals produced more milk than SickRAno and SickRAYes group animals during the entire period. No differences were found between SickRAno and SickRAYes. The nadir for all groups was observed on “Day -4” (Healthy = 26 kg/d, SickRAno = 8.1 kg/d, SickRAYes = 15.9 kg/d), and it was followed by an increment (stronger and faster for Healthy group animals). Maximum differences between Healthy group and SickRAYes and SickRAno groups were observed on “Day 3” (17.9 kg/d) and “Day 4” (15.9 kg/d), respectively. Milk production was not affected by the occurrence of other health disorders during the period of study ($P > 0.05$), because no differences between cows diagnosed and cows non-diagnosed with other diseases were found.

5.4.1.4. Milk Fever

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 6D). Rumination for the Healthy group ($n = 40$) was higher on “Day -6”, “Day -5” and from “Day -3” to “Day 2” when compared to the SickRAYes group ($n = 8$); and higher on “Day -7” and from “Day -5” to “Day -3” when compared to the SickRAno group ($n = 5$). No differences for rumination were found between the SickRAno and SickRAYes groups. For the Healthy group, rumination went down abruptly on “Day -3”, reaching its nadir (261 min/d) on “Day -2” (day of calving), followed by a fast recovery, since this is normal around parturition. Rumination time began to decrease earlier in the SickRAYes and SickRAno groups when compared to the Healthy group, reaching rumination’s nadir on the day of diagnosis (“Day 0”)

(114 and 178 min/d, respectively). After diagnosis, rumination showed a fast increase. DRT was affected by the occurrence of other diseases during the period of study ($P = 0.01$), because cows that developed other disorders had lower rumination than cows that had not.

For activity, there was no effect of group ($P = 0.25$), neither interaction between group and day ($P = 0.26$) (Figure 6E). However, the effect of day was observed ($P = 0.005$). Activity increased from “Day -7”, reaching its maximum (751 AU/d) on “Day -2” (~0 DIM), followed by a fast decrease to previous values in the Healthy group. The increase was slower and smaller in the SickRAno group, with a maximum on “Day -3” (~2 d before calving in average), followed by a strong decrement, reaching the nadir (464 AU/d) on “Day 1” (~2 DIM in average). No increment was observed for the SickRAyes group, with a slow decrement beginning on “Day -2” (~DIM 0 in average), reaching the nadir (467 AU/d) on “Day 1”, however, no recovery of activity was seen after this point until “Day 4”. The occurrence of other diseases did not affect activity ($P > 0.05$), no differences in activity were found between cows with other disease and cows without other disease.

For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 6F). Milk production increased from “Day -2” (~0 DIM average), however, the increment was slower and lower for sick animals when compared to healthy animals. Milk production for the Healthy group was higher than for groups SickRAyes and SickRAno on “Day 0”, and higher than SickRAyes on “Day 2”. Besides, SickRAno animals produced more milk than SickRAyes animals on “Day 2”. The nadir for milk production was observed on the day of diagnosis (“Day 0”) for SickRAyes and SickRAno (13.7 and 13.9 kg/d, respectively). Cows that suffered from other diseases during the period of study produced less milk than cows that suffer from MLF only ($P = 0.02$).

5.4.1.5. Displaced Abomasum

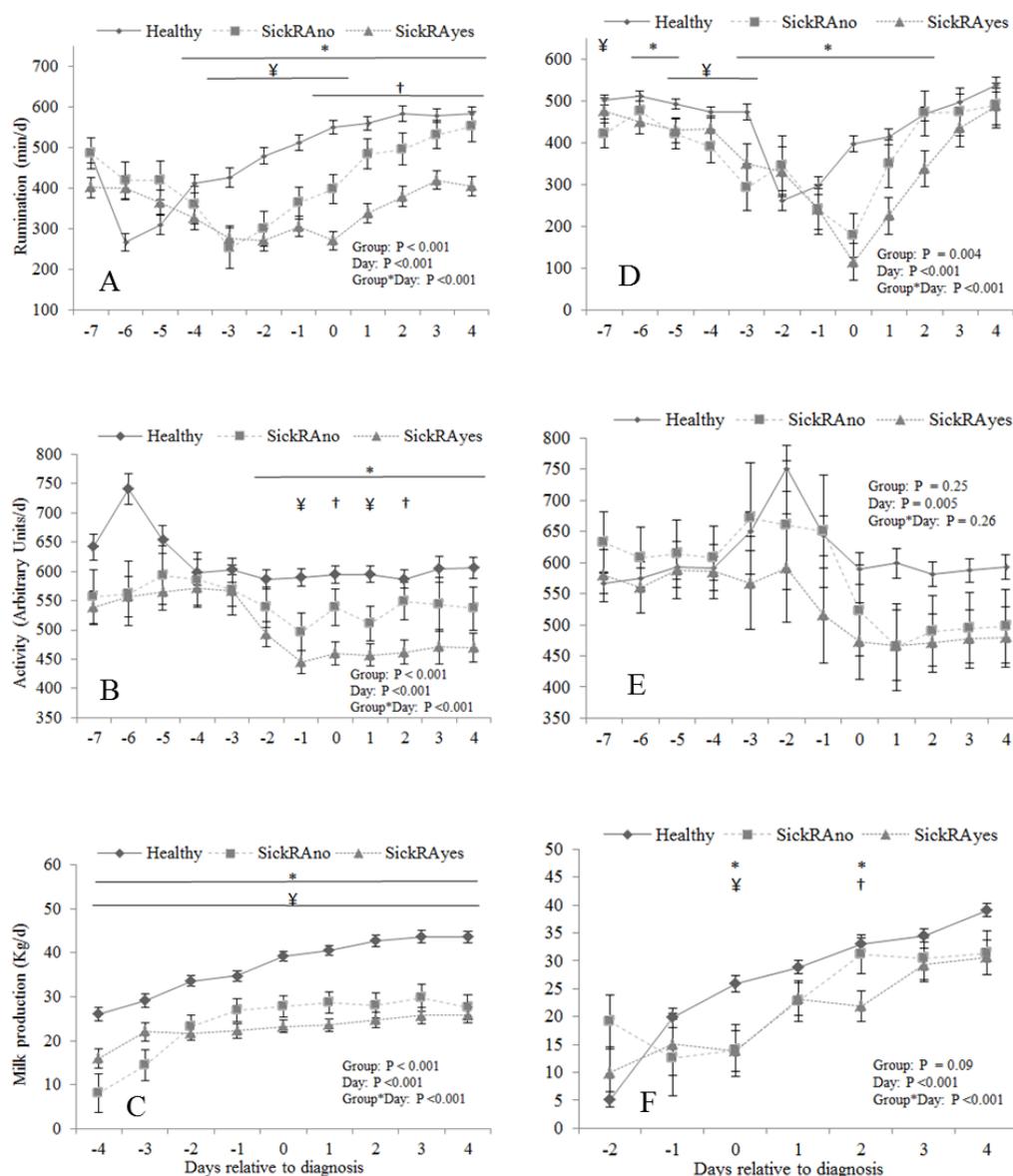
For rumination, there was effect of interaction between group and day ($P = 0.03$) (Figure 7A). All cows diagnosed with DA had RA events around diagnosis. Animals from the Healthy group ($n = 64$) ruminated more than animals that developed DA during the entire period of study ($P < 0.001$). The rumination’s nadir for the SickRAyes group ($n = 14$) was observed on “Day 0”, in coincidence with the maximum difference between groups (228

min/d). Thereafter, rumination increased quickly on “Day 2” for this group. There were no differences between cows that developed any other disease and those that did not during the period ($P = 0.52$).

For activity, animals that suffered from DA were less active than healthy animals ($P < 0.001$) (Figure 7B). There was a tendency for the effect of interaction between group and day ($P = 0.08$). The lowest activity for sick animals was observed on “Day 1” (407 AU/d). The greatest difference between groups was observed on “Day -1” (172 AU/d). Activity was affected by the occurrence of other diseases during the period ($P = 0.01$), cows with other disease were less active than cows without other disease during 7 d preceding up to 4 d after the diagnosis of DA.

For milk production, effect of interaction between group and day was observed ($P < 0.001$) (Figure 7C). Milk production for healthy cows was higher during the entire period than for cows that suffered from DA ($P < 0.001$). The nadir for milk production occurred on “Day -7” (37 kg/d) and on “Day 0” (18kg/d) for Healthy and SickRAYes group, respectively. Thereafter, milk production increased over time for both groups. The maximum difference between groups occurred on “Day 0” (24.6 kg/d). Milk production was not affected by the occurrence of other disease during the period ($P = 0.49$).

Figure 6 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with clinical ketosis and milk fever, and healthy cows



Legend: Patterns of daily rumination time (A) and daily activity (B), from -7 to 4 d relative to diagnosis, and daily milk production (C), from -4 to 4 d relative to diagnosis, for cows diagnosed with clinical ketosis (groups SickRAno and SickRAYes) and healthy cows (group Healthy, $n = 48$). Cows were assigned to the SickRAno ($n = 11$) and SickRAYes ($n = 28$) group if they showed or did not show at least 1 rumination alteration event (RA; daily rumination time <200 min/d, negative 3 days total rumination variation, and/or Health Index <80) from -7 to 2 d relative to diagnosis, respectively. Patterns of daily rumination time (D) and daily activity (E), from -7 to 4 d relative to diagnosis, and daily milk production (F), from -2 to 4 d relative to diagnosis of milk fever (groups SickRAno and SickRAYes), and healthy cows (group Healthy, $n = 40$). Cows were assigned to the SickRAno ($n = 5$) and SickRAYes ($n = 8$) group if they showed or not showed at least 1 RA event from -7 to 2 d relative to diagnosis, respectively. For cows in groups SickRAno and SickRAYes, “day 0” belongs to the day of diagnosis. For cows in the Healthy group, the average DIM at diagnosis for cows with clinical ketosis or milk fever was considered as “day 0”. Values are presented as LSM \pm SEM. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Healthy vs SickRAYes; † SickRAno vs SickRAYes; ¥ Healthy vs SickRAno. Source: Silva, 2017.

5.4.1.6. Indigestion

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 7D). Only cows that had RA events around diagnosis of indigestion were used for analysis. Rumination was higher for animals belonging to the Healthy group ($n = 55$) from “Day -5” to “Day 4” when compared with animals belonging to the SickRAyes group ($n = 8$). For the Healthy group, the nadir for rumination (413 min/d) was observed on “Day -7”, and it was followed by an increment on the following days. A gradual decrement of rumination for the group SickRAyes began on “Day -6”, reaching the nadir (213 min/d) on the day of diagnosis (“Day 0”), thereafter, rumination time increased quickly. The occurrence of other diseases during the period of study did not affect rumination time ($P = 0.9$).

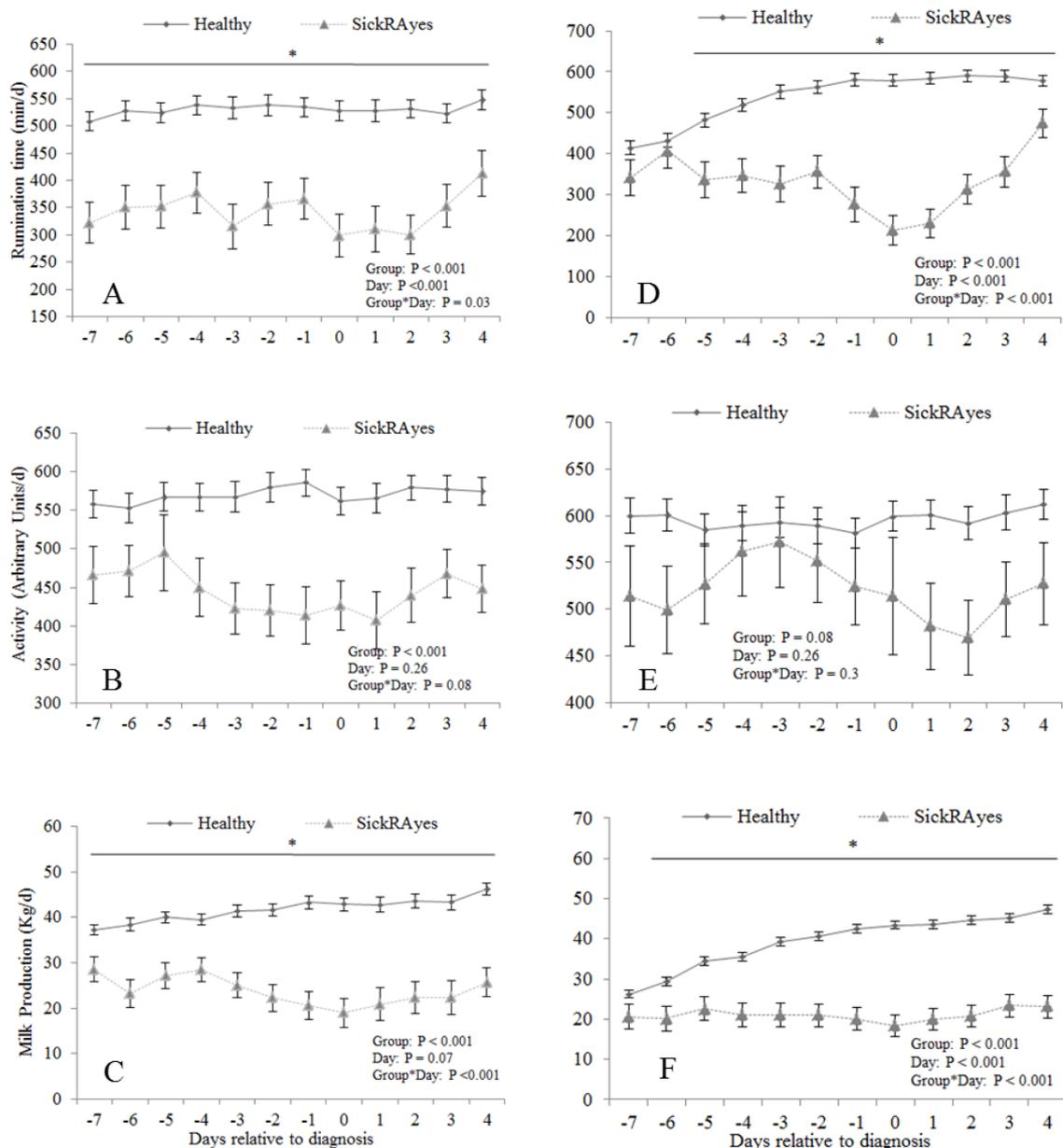
For activity, there was no effect of interaction between group and day ($P = 0.3$) (Figure 7E). A tendency between groups ($P = 0.08$) and no effect of day ($P = 0.26$) were observed. For the Healthy group, activity maintained similar values all over the period. Activity for the SickRAyes group showed a more variable behavior, with a decrement beginning on “Day -3” reaching a nadir on “Day 2” (469 AU/d), followed by a fast increase. Activity was not affected by the occurrence of other diseases from 7 d preceding to 4 d after diagnosis of indigestion ($P = 0.4$).

For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 7F). Milk production was higher for the Healthy group than for the SickRAyes group ($P < 0.001$). Differences were first observed on “Day -6” and continued throughout the period of study, with the greatest difference observed on “Day 0” (25 kg/d). The nadir for milk production for healthy animals (26.2 kg/d) occurred on “Day -7”, followed by an increment during the entire period. Milk production values for SickRAyes were maintained from “Day -6” until “Day -2”, when they showed a slightly decrement until the nadir (18.3 kg/d) on the day of diagnosis (“Day 0”), showing a gradual increment thereafter. The occurrence of other health disorders did not affect milk production ($P = 0.6$).

5.4.1.7. Mastitis

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 8A). Animals from the Healthy group ($n = 101$) ruminated more than animals from the SickRAyes group ($n = 26$), from the “Day -5” throughout the period. Moreover, rumination for cows belonging to the Healthy group was higher than for cows from the SickRAno ($n = 10$) group from “Day -4” to “Day 3”. Differences between SickRAyes and SickRAno were found on only two days (“Day -6” and “Day 0”), with higher values for SickRAno. The nadir of rumination in the Healthy group (367 min/d) occurred on “Day -7”, followed by an increment during the following days. Rumination for group SickRAno showed a decrement from “Day -7” until “Day 0”, when the nadir was reached (384 min/d). After “Day 0”, rumination increased. For the group SickRAyes, rumination showed a decrement on “Day -7” but maintained similar values until “Day -2”, when a strong decrement was observed, reaching the nadir (302 min/d) on the day of diagnosis (“Day 0”). The nadir was followed by a fast increment in the following days. The maximum difference for rumination between the Healthy and the SickRAyes groups (233 min/d) was observed on the day of diagnosis (“Day 0”). Moreover, there was effect of parity ($P = 0.02$), with *parous* ruminating more than *primiparous*, but effect of interaction between group and parity was not shown ($P = 0.26$). Therefore, no differences for rumination were found between cows that suffered from other disorders during the period and cows that did not ($P = 0.37$).

Figure 7 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with displaced abomasum and indigestion, and healthy cows



Legend: Patterns of daily rumination time (A), daily activity (B), and daily milk production (C) from -7 to 4 d relative to diagnosis for cows diagnosed with displaced abomasum (DA; group SickRAyes, $n = 14$) and healthy cows (group Healthy, $n = 64$). All cows with DA showed at least 1 rumination alteration (RA; daily rumination time < 200 min/d, negative 3 days total rumination variation, and/or Health Index < 80) event from -7 to 2 d relative to diagnosis. Patterns of daily rumination time (D), daily activity (E), and daily milk production (F) from -7 to 4 d relative to diagnosis for cows diagnosed with indigestion (group SickRAyes, $n = 8$), and healthy cows (group Healthy, $n = 55$). All cows with indigestion included for analysis showed at least 1 RA event from -7 to 2 d relative to diagnosis. For cows in group SickRAyes, "day 0" belongs to the day of diagnosis. For cows in the Healthy group, the average DIM at diagnosis for cows with DA or indigestion was considered as "day 0". Values are presented as LSM \pm SEM. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Healthy vs SickRAyes. Source: Silva, 2017.

For activity, there was tendency for the effect of interaction between group and day ($P = 0.09$) (Figure 8B). Activity was not different between groups ($P = 0.43$), but it was between days ($P = 0.05$). An increment for the SickRAno group was observed on “Day 0”. Healthy and SickRAyes groups maintained similar values all over the period of study. There was effect of parity ($P = 0.01$), *parous* animals were more active than *primiparous* animals, but no effect of interaction between group and parity was seen ($P = 0.51$). Activity was not affected by the occurrence of other diseases from 7 d preceding to 4 days after diagnosis of mastitis ($P = 0.38$).

Milk production data from “Day -7” to “Day -3” for the SickRAno group were not included because of missing data. For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 8C). Healthy group animals produced more milk than SickRAyes and SickRAno group animals from “Day -4” to “Day 4” and from “Day -2” to “Day 4”, respectively. No differences between SickRAyes and SickRAno were observed. An increment for milk production was observed during the entire period for healthy animals, from its nadir (23.9 kg/d) on “Day -7” (2 DIM in average). From “Day -7” to “Day -5”, milk production for SickRAyes increased, however a decrement beginning on “Day -5” until the nadir (19 kg/d) for milk production on “Day 0” was observed. After diagnosis, milk production increased. SickRAno had a similar behavior to SickRAyes, decreasing milk production from “Day -1” to “Day 0” (nadir’s day, 16.3 kg/d), showing an increment on “Day 1”. There was effect of parity ($P = 0.001$) and interaction between group and parity ($P = 0.01$). *Parous* animals produced more milk than *primiparous* animals. Regarding to groups, the decrement of milk production on “Day 0” for SickRAno was stronger for *parous* than *primiparous* animals. Therefore, the occurrence of other diseases during the period between 7 d preceding to 4 d after diagnosis of mastitis did not affect milk production ($P = 0.23$).

5.4.1.8. Lameness

For rumination, there was effect of interaction between group and day ($P < 0.001$) (Figure 8D). Differences between the groups Healthy ($n = 101$) and SickRAyes ($n = 8$) were observed during almost the entire period, being not different only on “Day -6”. Rumination for the SickRAno group ($n = 4$) was higher than for the SickRAyes group from “Day -3” until “Day 3”. For the Healthy group animals, rumination began with its nadir (367 min/d) on “Day

-7", and showed an increment during the following days. The SickRAno group maintained homogenous values during the studied period. Behavior of rumination data for SickRAyes was different, showing a strong decrement beginning on "Day -7" reaching the nadir (257 min/d) on "Day -3", maintaining similar values until "Day 1" when rumination started to increase. *Primiparous* animals tended to ruminate more than *parous* ($P = 0.08$). Moreover, the effect of interaction between parity and group was observed ($P < 0.001$), because the decrement of rumination was greater and the nadir was lower for *parous* than *primiparous* animals belonging to the SickRAyes group. It is important to note that the sample size for this observation was very small, with only 2 animals from the SickRAno group and 4 animals from the SickRAyes group, for *primiparous* and *parous*.

For activity, there was effect of interaction between group and day ($P = 0.03$) (Figure 8E). However, no differences were found when analysis was performed looking for differences for each day between groups. This could have happen due the great data dispersion in SickRAno and SickRAyes groups. Besides, no effects of group ($P = 0.75$) and day were found ($P = 0.75$). A decrement in SickRAyes was found on "Day -2". The lowest values were observed for this last group around "Day -1" to "Day 3".

For milk production, no effect of interaction between group and day was found ($P = 0.49$), but effects of group and day were observed ($P < 0.001$) (Figure 8F). Milk production was lower for the SickRAyes group animals when compared to Healthy and SickRAno group animals, but no differences between Healthy and SicRAno were found. For all groups, milk production had a similar behavior, increasing slowly over time from "Day -4" to "Day 4". However, a decrement on "Day 2" was observed for SickRAyes, followed by a quick recovery one day after. The presence of other diseases during the period of study around the diagnosis of lameness was not included because only two cows suffered from other disorders.

5.4.1.9. Other diseases

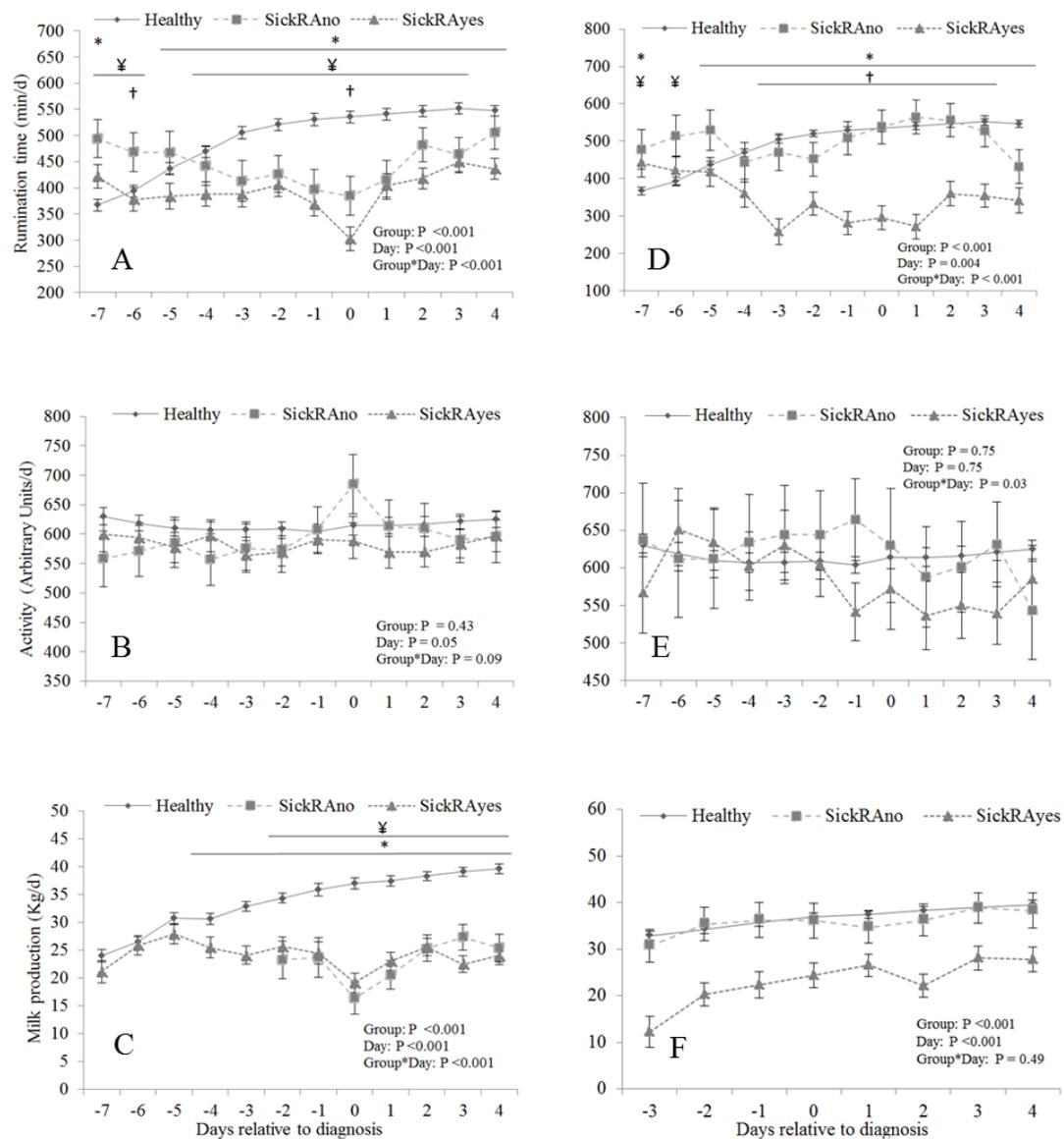
For rumination time, there was effect of interaction between group and day ($P < 0.001$) (Figure 9A). Rumination for the Healthy group ($n = 150$) was higher than for the SickRAyes group ($n = 50$) during almost the entire period, being not different only on "Day -6". SickRAno ($n = 49$) and SickRAyes groups showed differences from "Day -7" to "Day 4", with higher values for

SickRAno. The Healthy and SickRAno groups showed differences from “Day -3” to “Day 2”, with lower values for SickRAno. The nadir for rumination (419 min/d) for healthy animals was observed on “Day -7” (~3 DIM in average), showing an increment until reach the maximum value on “Day -2”, maintaining similar values throughout the period of study. The SickRAno group showed a slow and small decrement for rumination beginning on “Day -5” until the nadir on “Day 0” (461 min/d), followed by a gradual increment during the following days. Rumination for the SickRAyes group decreased from “Day -7” until reach its nadir (286 min/d) on “Day 0”, followed by a fast and strong increment. There was effect of parity ($P < 0.001$); *parous* animals had higher rumination than *primiparous* animals. No effect of interaction between parity and group was observed ($P = 0.12$). The occurrence of other health disorders during the period of interest affected rumination ($P = 0.02$), because cows that suffered from other diseases had lower rumination than cows that did not suffered from other diseases.

For activity, there was effect of interaction between group and day ($P < 0.001$) (Figure 9B). Activity was lower for SickRAyes when compared to SickRAno and Healthy groups from “Day -5” to “Day 4”, and from “Day -3” to “Day 4”, respectively. Activity data behavior was similar for the Healthy and SickRAno groups, with similar values during the entire period of study. SickRAyes showed a strong decrement for activity on “Day -4”. The nadir (511 AU/d) of activity for the SickRAyes group occurred on “Day 1”; followed by an increment during the next days. There was effect of parity ($P = 0.001$); *primiparous* were more active than *parous* animals. However, no interaction between parity and group was observed ($P = 0.33$). The occurrence of other diseases affected activity ($P = 0.008$), because animals that suffered from other health disorders had higher activity than animals that did not suffer from other health disorder.

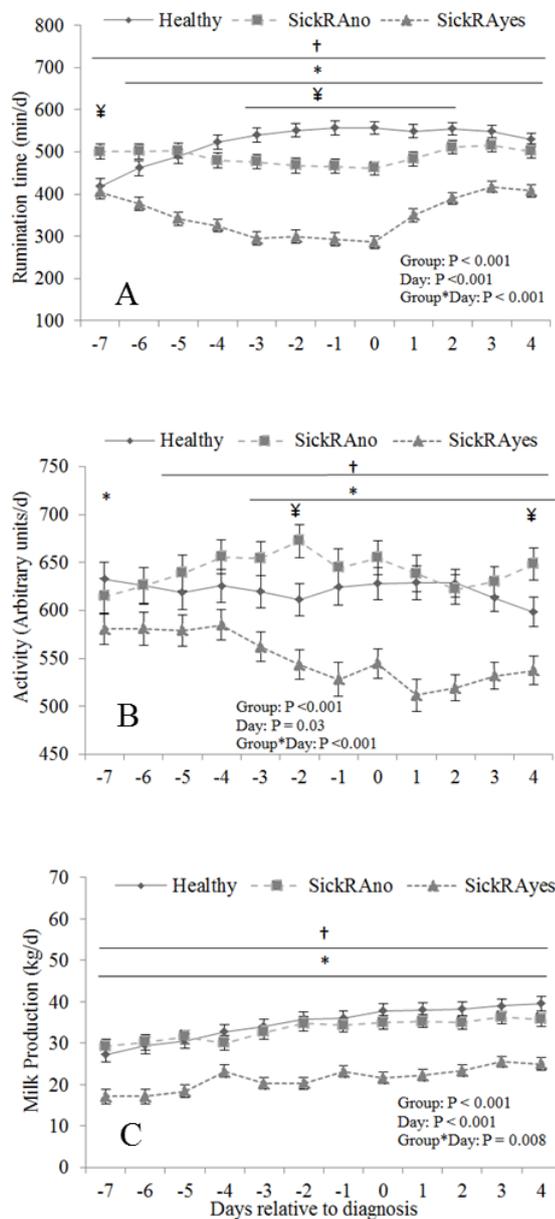
For milk production, there was effect of interaction between group and day ($P = 0.008$) (Figure 9C). Milk production for SickRAyes was lower when compared to the Healthy and SickRAno groups ($P < 0.001$) from “Day -7” to “Day 4”, but not differences were found between the SickRAno and the Healthy group. Milk production showed a similar behavior for all groups, with an increment from “Day -7” throughout the period. There was effect of parity ($P < 0.001$) because *parous* animals produced more milk than *primiparous* animals. No effect of interaction between parity and group was observed ($P = 0.16$). The occurrence of other health disorders affected milk production ($P < 0.001$), because animals with other diseases produced less than animals without other diseases.

Figure 8 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with mastitis and lameness, and healthy cows



Legend: Patterns of daily rumination time (A), daily activity (B), and daily milk production (C) from -7 to 4 d relative to diagnosis for cows diagnosed with mastitis (groups SickRAYes and SickRAno) and healthy cows (group Healthy, n = 101). Cows were assigned to the SickRAno (n = 10) and SickRAYes (n = 26) group if they showed or did not show at least 1 rumination alteration event (RA; daily rumination time <200 min/d, negative 3 days total rumination variation, and/or Health Index <80 AU/d) from -7 to 2 d relative to diagnosis, respectively. Patterns of daily rumination time (D), daily activity (E) from -7 to 4 d relative to diagnosis, and daily milk production (F) from -3 to 4 d relative to diagnosis for cows diagnosed with lameness (groups SickRAYes and SickRAno), and healthy cows (group Healthy, n = 101). Cows were assigned to the SickRAno (n = 4) and SickRAYes (n = 8) group if they showed or did not show at least 1 RA event from -7 to 2 d relative to diagnosis, respectively. For cows in groups SickRAno and SickRAYes, “day 0” belongs to the day of diagnosis. For cows in the Healthy group, the average DIM at diagnosis for cows with DA or indigestion was considered as “day 0”. Values are presented as LSM ± SEM. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Healthy vs SickRAYes; † SickRAno vs SickRAYes; ¥ Healthy vs SickRAno. Source: Silva, 2017.

Figure 9 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with “other diseases”, and healthy cows



Legend: Patterns of daily rumination time (A), daily activity (B), and daily milk production (C) from -7 to 4 d relative to diagnosis for cows diagnosed with “other diseases” (groups SickRAYes and SickRAno) and healthy cows (group Healthy, $n = 150$). Cows were assigned to the SickRAno ($n = 49$) and SickRAYes ($n = 50$) group if they showed or did not show at least 1 rumination alteration event (RA; daily rumination time < 200 min/d, negative 3 days total rumination variation, and/or Health Index < 80 AU/d) from -7 to 2 d relative to diagnosis, respectively. For cows in groups SickRAno and SickRAYes, “day 0” belongs to the day of diagnosis. For cows in the Healthy group, the average DIM at diagnosis for cows with “other diseases” was considered as “day 0”. Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Healthy vs SickRAYes; † SickRAno vs SickRAYes; ¥ Healthy vs SickRAno. Source: Silva, 2017.

5.4.2. DRT, Daily ACT, and Daily Milk Production around calving for cows with calving problems

5.4.2.1. Dystocia

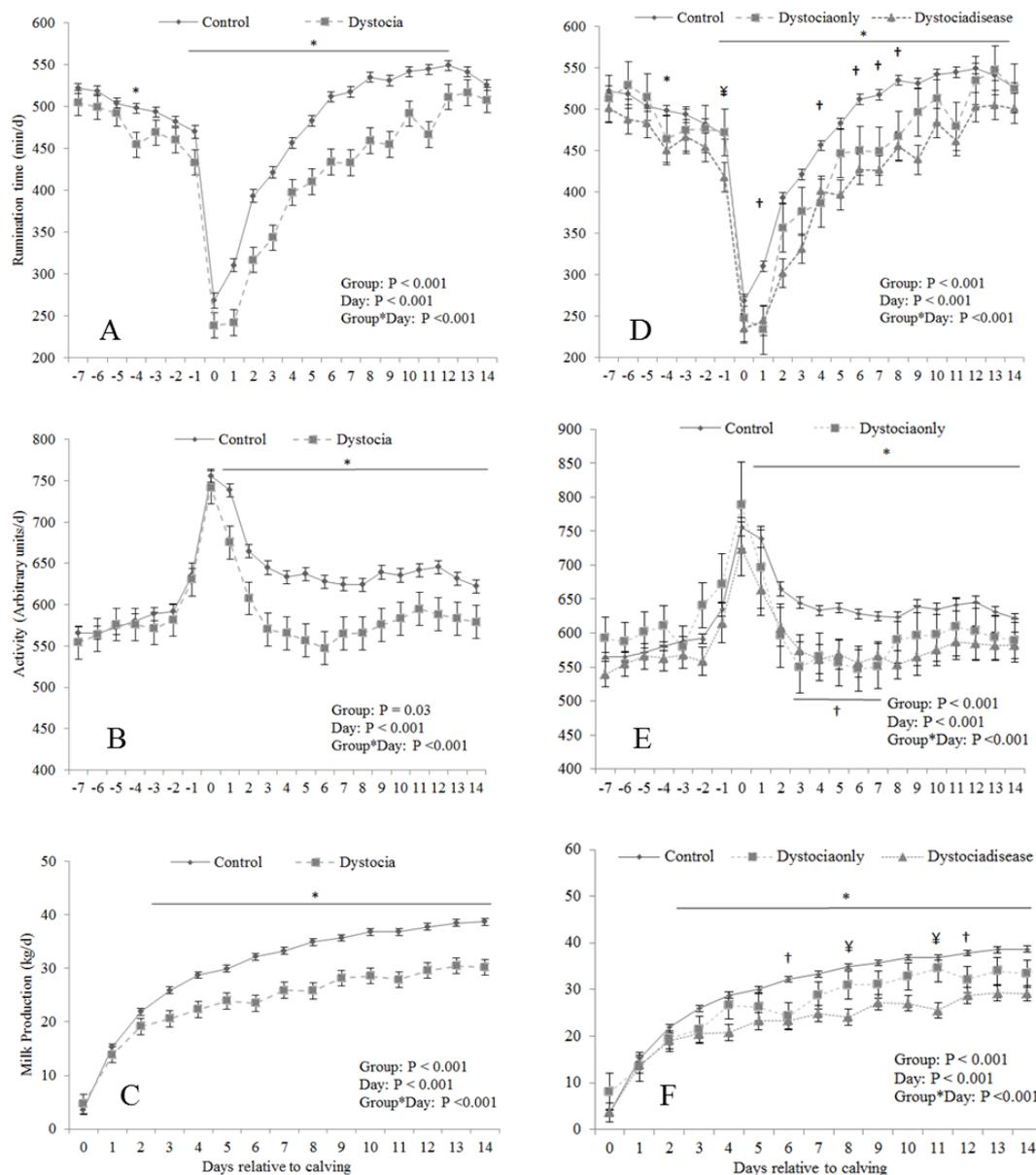
For rumination time, there was effect of interaction between group (Control and Dystocia) and day ($P < 0.001$) (Figure 10A). Rumination was higher for control cows ($n = 253$) than for cows with dystocia ($n = 43$) on “Day -4”, and from “Day -1” to “Day 14”. For the analysis including the occurrence of clinical events during the first 14 DIM, there was effect of interaction between group (Control, Dystociaonly and Dystociadisease) and day ($P < 0.001$) (Figure 10D). Cows that had dystocia but without any clinical disease (Dystociaonly, $n = 12$) ruminated less than control cows ($n = 253$) on “Day 1”, “Day 4”, and from “Day 6” to “Day 8”. Dystocic cows with clinical problems (Dystociadisease, $n = 31$) had lower rumination from “Day -1” to “Day 13” compared to control cows. Rumination from the Dystociadisease was lower than from the Dystociaonly group on “Day -1”. The greatest difference between the Dystociaonly and Control groups and between the Dystociadisease and the Control groups was observed at “Day 1” (76 min/d) and “Day 9” (92 min/d), respectively.

For activity, there was effect of interaction between group and day ($P < 0.001$) (Figure 10B). Differences among dystocic and control cows were observed from “Day 1” to “Day 14”. Effect of interaction between group and day was also observed in the analysis including the presence of clinical disorders ($P < 0.001$) (Figure 10E). Activity from control cows was higher than that from cows that had dystocia and clinical problems from “Day 1” to “Day 14”. Besides, control cows were more active than cows from the Dystocionly group from “Day 3” to “Day 7”. No differences between the Dystociaonly and Dystociadisease groups were found. The greatest difference between the Dystociaonly and Control groups was observed on “Day 3” (95 AU/d). Maximum difference between control cows and cows from the Dystociadisease group was found on “Day 9” (74 AU/d).

For milk production, the effect of interaction between group and day was found ($P < 0.001$) (Figure 7C), with higher milk production for cows without dystocia from “Day 3” to “Day 14” relative to calving. Regarding to the presence of clinical disease, effect of interaction

was observed between group and day ($P < 0.001$) (Figure 10F). Milk yield from control cows was higher than from cows from the group Dystociaonly on “Day 6” and “Day 12”. Cows from

Figure 10 - Daily rumination time, daily activity, and daily milk production patterns for cows having dystocia, and cows having normal calving



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for cows having dystocia (group Dystocia = 43) and cows having normal calving (group Control, $n = 253$). Patterns of daily rumination time (D) and daily activity (E) from -7 to 14 d relative to calving, and daily milk production (F) from 0 to 14 d relative to calving for cows having dystocia (groups Dystociaonly and Dystociadisease), and cows having normal calving (group Control, $n = 253$). Cows were assigned to the Dystociaonly ($n = 12$) and Dystociadisease ($n = 31$) groups if they were not or were diagnosed with any disease from 0 to 14 d relative to calving, respectively. The day of calving was considered as “day 0”. Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs Dystocia; * Control vs Dystociadisease; † Control vs Dystociaonly; ¥ Dystociaonly vs Dystociadisease. Source: Silva, 2017.

the Control group produced more milk than cows from the Dystociadisease group from “Day 3” to “Day 14”. On “Day 8” and “Day 11”, milk production was higher in cows from group Dystociaonly compared to cows from group Dystociadisease. The greatest difference among group Control and Dystociaonly, Control and Dystociadisease, and Dystociaonly and Dystociadisease was observed on “Day 6” (7.9 kg/d), “Day 11” (11.2 kg/d) and “Day 11” (8.9 kg/d), respectively. For the three studied parameters, there was effect of parity ($P < 0.05$), however, analysis were not run separately because the reason for this effect was that rumination, activity and milk yield were lower in *primiparous* than in *parous*, but the data behavior showed no important differences and the effect of interaction between group and parity was not found ($P > 0.05$).

5.4.2.2. Twins

For rumination time, there was effect of interaction between group (Twins and Singleton) and day ($P < 0.001$) (Figure 11A). Cows delivering singletons ($n = 254$) ruminated more than cows delivering twins ($n = 17$) from “Day 2” to “Day 13”, with the maximum difference occurring on “Day 6” (149 min/d).

For activity, the interaction between group and day was significant ($P < 0.001$) (Figure 11B). Cows that delivered twins were less active than cows that delivered singletons from “Day 1” to “Day 14”, and the greatest difference was observed on “Day 2” (183 AU/d).

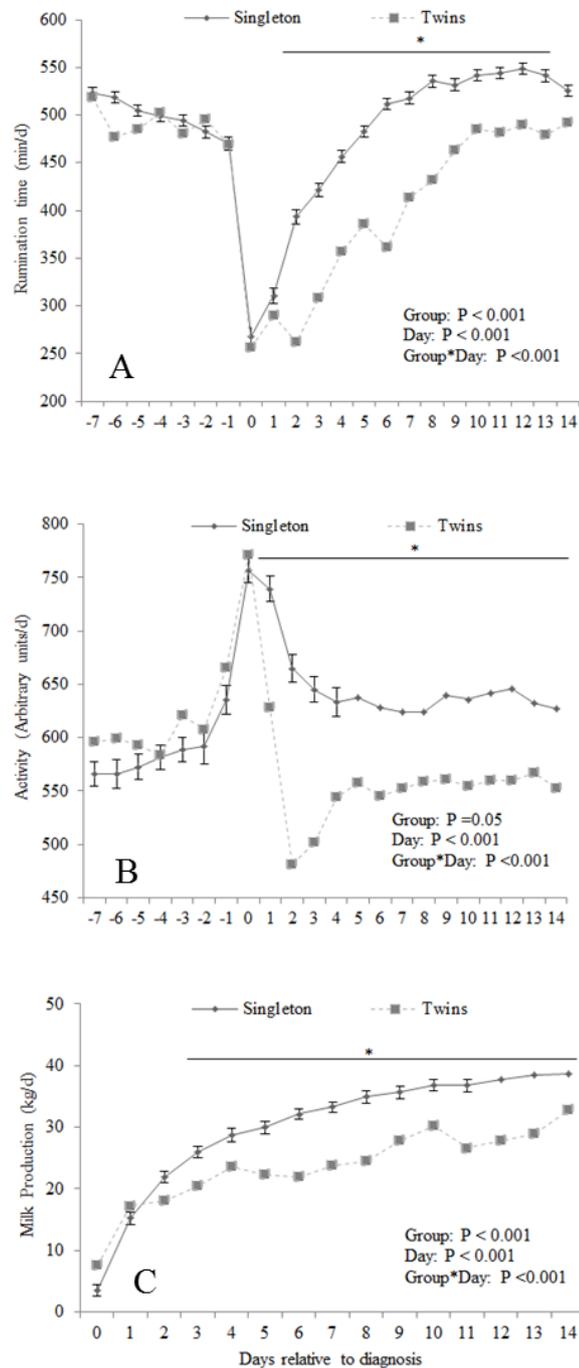
For milk production, there was effect of interaction between group and day ($P < 0.001$) (Figure 11C). Cows delivering singletons produced more milk than cows delivering twins from “Day 3”, throughout the period. The maximum difference occurred on “Day 8” (10.4 kg/d). Parity and the occurrence of clinical disorders were not included into the model because the samples size was too small, only 3 *primiparous* had twins, and among cows delivering twins only 2 of them developed clinical disease.

5.4.2.3. Stillbirth

For rumination time, there was effect of interaction between group (Stillborn and Alive) and day ($P < 0.001$) (Figure 12A). Cows delivering stillborn calves ($n = 27$) ruminated less than cows delivering live calves ($n = 308$) from the day before calving (“Day -1”), throughout the period of study. Regarding to the presence of clinical disease during the first 14 DIM, there was effect of interaction between group (Control, Stillbornonly, and Stillborndisease) and day ($P < 0.001$) (Figure 12D). Cows that delivered stillborn but had no disease (Stillbornonly) ruminated less than control cows on “Day -1”, “Day 0”, “Day 3”, “Day 6” and “Day 7” relative to calving. Rumination was lower for cows that delivered stillborn calf and had disease (Stillborndisease) than control cows on “Day -7”, and from “Day -1” to “Day 14”. Differences among the Stillbornonly and Stillborndisease groups were observed on “Day -7”, “Day 2”, “Day 5” and “Day 11”, with lower values for the last group. The greatest differences between groups Stillbornonly and Control, Stillborndisease and Control, and Stillbornonly and Stillborndisease were observed on the day of calving (“Day 0”) (105 min/d), “Day 3” (156 min/d), and “Day 5” (100 min/d), respectively.

For activity, there was effect of interaction between group and day ($P < 0.001$) (Figure 12B). Activity was lower in cows delivering stillborn calves than live calves. The interaction between group and day was also significant when the presence of clinical disorders was included ($P < 0.001$) (Figure 12E). Differences among groups Stillbornonly and Control were observed between “Day 0” and “Day 6”, being cows from the first group more active than from the second. Cows from the Stillborndisease group were more active than control cows from “Day 0” to “Day 14”. The only difference between groups Stillbornonly and Stillborndisease was found on “Day 12”. The greatest differences between group Control and Stillbornonly, and Control and Stillborndisease were observed on “Day 2” (124 AU/d), and “Day 1” (181 AU/d), respectively.

Figure 11 - Daily rumination time, daily activity, and daily milk production patterns for cows delivering singleton and twin calves



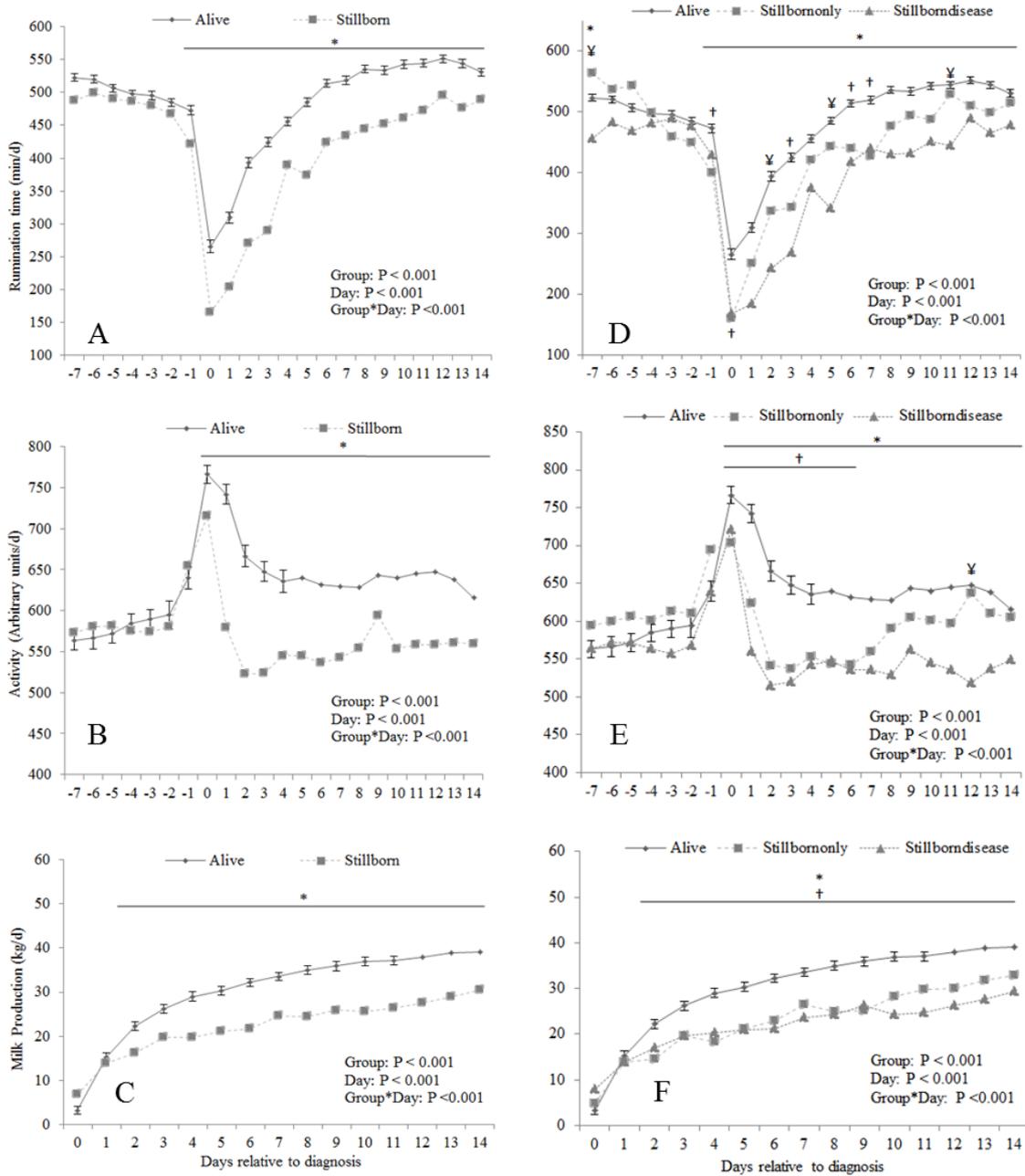
Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for cows delivering a singleton calf (group Singleton, $n = 254$) and cows delivering twin calves (group Twins, $n = 17$). The day of calving was considered as “day 0”. Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Singleton vs Twins. Source: Silva, 2017.

For milk production, the effect of interaction between group and day was significant ($P < 0.001$) (Figure 12C). Cows delivering stillborn calves produced less milk than cows delivering live calves. The effect of interaction between group and day was also significant when the presence of clinical disease during the first 14 DIM was included ($P < 0.001$) (Figure 12F). Cows from groups Stillbornonly and Stillborndisease produced more milk than control cows from “Day 2” to “Day 14”. No differences for milk production were found between the Stillbornonly and Stillborndisease were found. For the analyses including the occurrence of stillbirth only, there was effect of parity for the three parameters in study ($P < 0.005$). DRT and milk yield were lower in *primiparous* than *parous*, occurring the opposite with activity ($P = 0.01$). Parity was not included into the model containing the presence of clinical disorders, because the small number of *primiparous* cows in each group. Effect of parity ($P < 0.05$) was observed for rumination, activity, and milk production, however, effect of interaction among group and parity was not found ($P > 0.05$), and the data showed no important differences in behavior among *primiparous* and *parous*.

5.4.3. DRT, Daily ACT and Daily Milk Production relative to Regrouping

For rumination, there was tendency for the effect of parity ($P = 0.08$). However, the effect of triple interaction between group, parity and day was significant ($P = 0.001$). Thus, rumination for animals moved to another group (Regrouped) behaved different among *primiparous* and *parous*. Thus, *primiparous* and *parous* were analyzed and plotted separately. For *primiparous* animals, there was effect of interaction between group and day ($P = 0.03$) (Figure 13A). Rumination for the animals moved to another group (Regrouped, $n = 32$) was lower than for the control animals ($n = 55$) only one day (“Day 1”), and three days after (“Day 3”) the day of regrouping, and the differences were 32 min/d and 34 min/d respectively. For *parous* animals, there was effect of interaction between group and day ($P < 0.001$) (Figure 13D).

Figure 12 - Daily rumination time, daily activity, and daily milk production patterns for cows delivering stillborn, and cows delivering live calves



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for cows delivering stillborn calves (group Stillborn = 27) and cows delivering live calves (group Alive, $n = 108$). Patterns of daily rumination time (D) and daily activity (E) from -7 to 14 d relative to calving, and daily milk production (F) from 0 to 14 d relative to calving for cows delivering stillborn calves (groups Stillbornonly and Stillborndisease), and cows delivering live calves (group Alive, $n = 108$). Cows were assigned to the Stillbornonly ($n = 8$) and Stillborndisease ($n = 19$) groups if they were or were not diagnosed with any disease from 0 to 14 d relative to calving, respectively. The day of calving was considered as "day 0". Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs Stillborn; * Control vs Stillborndisease; † Control vs Stillbornonly; ¥ Stillbornonly vs Stillborndisease. Source: Silva, 2017.

Differences between the Regrouped group ($n = 70$) and the Control group ($n = 80$) occurred from the day of regrouping (“Day 0”) throughout the period of study. A strong and fast decrement for rumination was observed for the group Regrouped on “Day 0”, reaching its nadir on “Day 2” (465 min/d), the same day the maximum difference (96 min/d) between groups were found.

Activity was also analyzed and plotted separately for *primiparous* and *parous*. For *primiparous* animals, there was effect of interaction between group and day ($P = 0.02$) (Figure 13B). However, when analyses were performed to compare each day, no differences were found. This could have happened because there was no effect of group ($P = 0.35$) but effect of day was observed ($P < 0.001$). For the Regrouped group, activity showed an increase on “Day 0” that was followed by a recovery to previous values on the next day (“Day 1”). However, activity remained with a slight increment during the following days. For *parous* animals, there was effect of interaction between group and day ($P = 0.007$) (Figure 13E). Activity was higher for cows that were moved than control cows on “Day 0”. For the Regrouped group, activity showed a strong increase on “Day 0”, but this was followed by a fast decrement on the next day (“Day 1”).

For milk production, effect of parity was found ($P < 0.001$) because *parous* produced more milk than *primiparous* animals, but no interaction between parity and group, or the triple interaction found for rumination and activity, were found for milk yield. Then, milk production was analyzed for all cows together. No effect of interaction between group and day ($P = 0.29$) was found (Figure 13C). There were no differences between cows that were moved to another group and control cows ($P = 0.15$). Milk production data showed the same behavior for both group of animals, increasing from “Day -7” throughout the period.

5.4.4. DRT, Daily ACT and Daily Milk Production around calving for cows with subclinical metabolic disorders

5.4.4.1. Subclinical Ketosis (SCK)

For rumination, there was effect of interaction between group (SCK vs Control) and day ($P = 0.02$) (Figure 14A). Cows with SCK ($n = 86$) ruminated less than control cows ($n =$

297) on “Day -6”, and from “Day 0” to “Day 10”, and tended to ruminate less on “Days -4”, “Day -3” and “Day -2” relative to calving. The nadir of rumination for both groups occurred on the day of calving (“Day 0”), and the maximum difference between groups on “Day 1” (62 min/d).

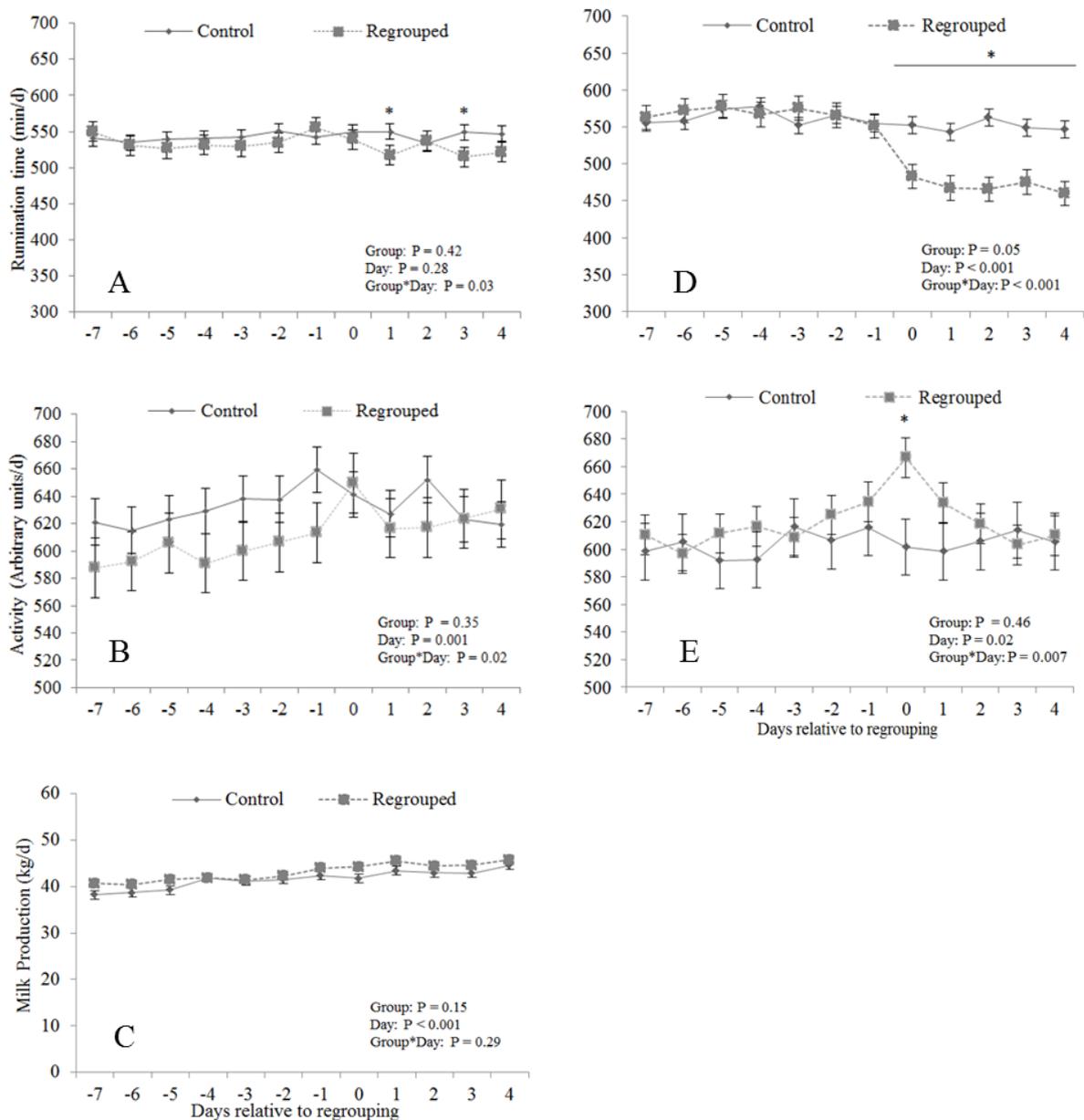
Regarding to the analysis performed dividing the cows in three groups according to the presence of SCK and clinical disease during the first 14 DIM, there was effect of interaction between group (*Control, SCKonly and SCKdisease*) and day ($P < 0.001$) (Figure 14D).

Although there was effect of parity ($P = 0.01$), data were not analyzed separately for *primiparous* and *parous*, because no effect of interaction between parity and group was observed ($P = 0.59$). Data from cows with SCK did not behave different among parities. The effect of parity was related to the higher rumination and milk production found in *parous* compared to *primiparous*.

Rumination of cows with SCK and clinical disorders during the first 14 DIM (SCKdisease, $n = 55$) was lower than control cows ($n = 297$) during the entire period of study (-7 to 14 d relative to calving). Cows with SCK only (SCKonly, $n = 31$) ruminated more than control cows on “Day -6”, “Day 4”, “Day 5”, and from “Day 8” to “Day 14”; and ruminated less on “Day 1”. Differences between the SCKonly and SCKdisease groups were observed on “Day -6” and from “Day 2” to “Day 14”, with lower values for the SCKdisease group. The nadir of rumination time was observed on the day of calving for the three groups (with the lowest value in the SCKdisease group), therefore rumination recovered prepartum levels around “day 5” and “Day 4” in groups Control and SCKonly, respectively. However, the recovery was slower for the SCKdisease group, because levels observed before calving were reached around “Day 7”.

For activity, there was effect of interaction between group (Control and SCK) and day ($P < 0.001$) (Figure 14B). Cows with SCK were less active than control cows from “Day -4” to “Day -2” and from “Day 1” to “Day 14” relative to calving. The nadir of activity was observed on “Day -7” and “Day 4” for the Control (564 AU/d) and SCK (519 AU/d) group, respectively. The maximum difference between groups was observed on “Day 1” (145 AU/d). There was effect of interaction between group (Control, SCKonly and SCKdisease) and day ($P < 0.001$) in the analysis performed with cows with SCK and clinical disorders (Figure 14E). Differences between the Control group and the SCKonly group were observed on “Day -3” and between “Day 1” and “Day 14”, with lower values for the last group.

Figure 13 - Daily rumination time, daily activity, and daily milk production patterns for regrouped and control cows



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 4 d relative to regrouping for regrouped (group Regrouped, $n = 32$) and control (group Control, $n = 55$) *primiparous* animals. Patterns of daily rumination time (D) and daily activity (E) from -7 to 4 d relative to regrouping for regrouped (group Regrouped, $n = 70$) and control (group Control, $n = 80$) *parous* animals. Daily milk production (C) from -7 to 4 d relative to regrouping for regrouped (Group Regrouped, $n = 102$) and control (Group Control, $n = 100$) cows. Cows from both groups were not diagnosed with any disease during the period of study. The day of regrouping was considered as “day 0”. For cows in the Control group, the average DIM at regrouping for regrouped cows was considered as “day 0”. Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs Regrouped. Source: Silva, 2017.

Cows from the SCKdisease group were less active than control cows and cows from the SCKonly group from “Day 1” to “Day 14”, and from “Day 2” to “Day 13”, respectively. The maximum difference between the SCKonly and Control and between SCKdisease and Control group was observed on “Day 2” (102 AU/d and 171 AU/d, respectively). The greatest difference between groups SCKonly and SCKdisease occurred on “Day 6” (80 AU/d).

For milk production, there was effect of interaction between group (Control and SCK) and day ($P = 0.02$) (Figure 14C). Cows with SCK produced more milk than control cows on “Day 1”, but less on days 7, 8, 10, 12, 13 and 14 relative to calving. Regarding to cows with SCK and clinical disorders, there was effect of interaction between group (Control, SCKonly and SCKdisease) and day ($P < 0.001$) (Figure 14F). Cows from the SCKdisease group produced less milk than cows from the Control and SCKonly groups from “Day 5” to “Day 14” and from “Day 4” to Day 14”, respectively. Differences between the SCKonly and SCKdisease group were observed on “Day 1”, “Day 2”, from “Day 5” to “Day 10” and from “Day 11” to “Day 14”, with lower values for the first group. The greatest difference between Control group and SCKonly (6.7 kg/d) and SCKdisease (7.2 kg/d) group was observed on “Day 11” and “Day 10”, respectively. The maximum difference between the SCKonly and SCKdisease occurred on “Day 11” (12.5 kg/d).

5.4.4.2. Subclinical Hypocalcemia (SCHC)

For rumination, there was effect of interaction between group (Control and SCHC) and day ($P < 0.001$) (Figure 15A). Differences among cows with SCHC ($n = 421$) and controls ($n = 77$) were observed from “Day -1” to “Day 6” relative to calving, with the higher values belonging to the Control group. For activity, effect of interaction between group and day was observed ($P < 0.001$) (Figure 12B), with differences among groups from “Day 1” to “Day 6”, being the control cows more active than cows with SCHC. For milk production, there was no effect of interaction ($P = 0.91$), nor effect of group ($P = 0.69$), because no differences between cows with and without SCHC were observed (Figure 15C).

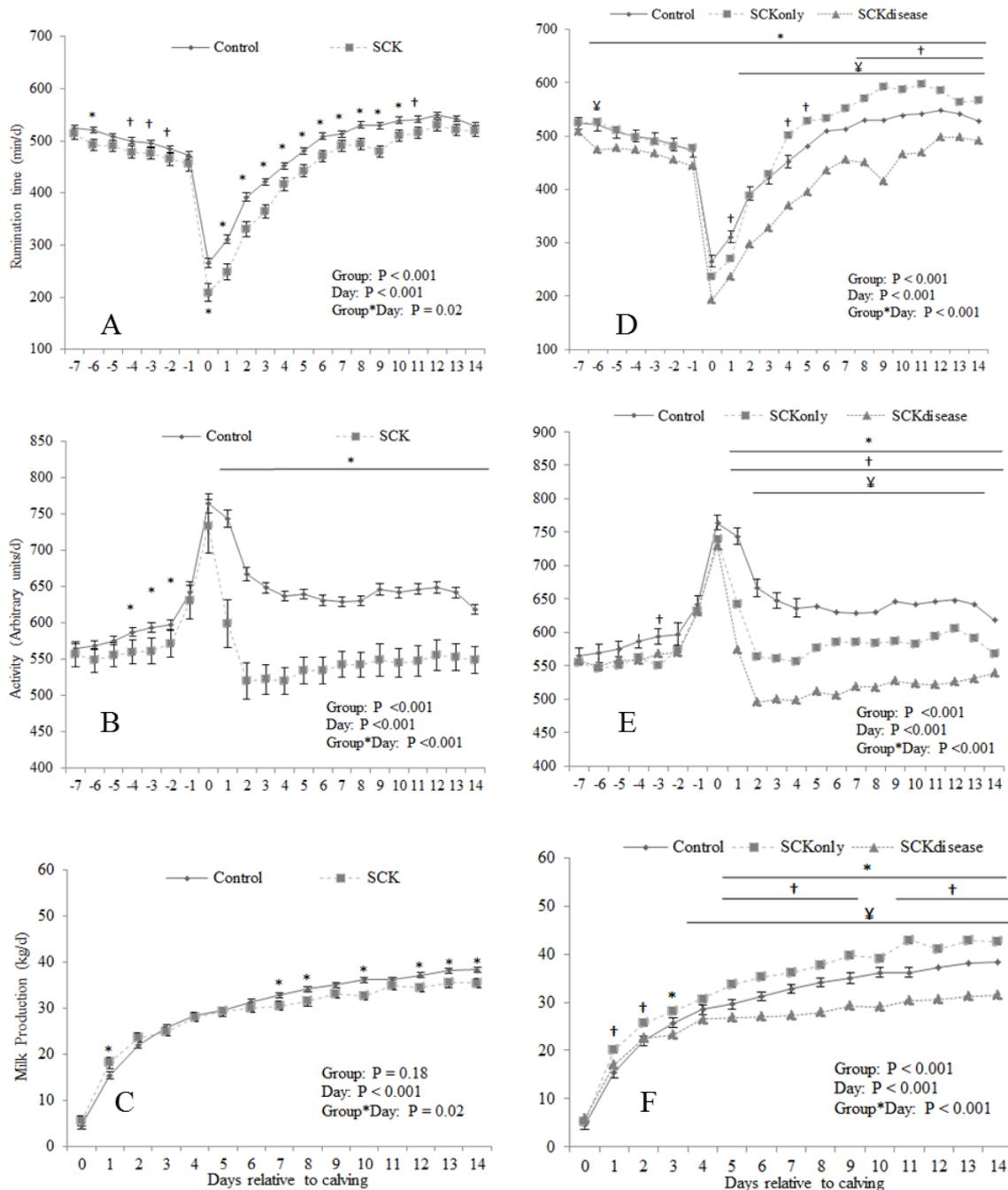
For the analysis of cows with SCHC and clinical disorders during the first 14 DIM, animals were divided according to parity because effect of parity was found ($P < 0.05$). The behavior of the studied parameters showed differences among *primiparous* and *parous*. For rumination, there was effect of interaction between group (Control, SCHConly, SCHCdisease)

and day ($P < 0.001$), for *primiparous* (Figure 16A) and *parous* (Figure 15D). In the case of *primiparous*, cows with SCHC but without clinical disorders (SCHConly, $n = 122$) ruminated less than control cows ($n = 47$) on “Day 6”; and cows with SCHC and clinical disorders (SCHCdisease, $n = 67$) ruminated less than control cows from “Day 1” to “Day 13”. For *parous*, cows from the SCHConly group ($n = 124$) had lower rumination than control cows ($n = 30$) on “Day 1” and “Day 2”; and cows from the SCHCdisease group ($n = 108$) ruminated less than control cows from “Day -6” to “Day 9”. Rumination time was lower for the SCHCdisease group than for the SCHConly group from “Day 1” to “Day 13” and from “Day -7” to “Day 14”, for *primiparous* and *parous*, respectively. The greatest difference among the SCHCdisease and Control groups was observed on “Day 3” and “Day 1”, for *primiparous* (89 min/d) and *parous* (128 min/d), respectively. The maximum difference among groups SCHCdisease and SCHConly occurred on “Day 9” for *primiparous* (66 min/d) and *parous* (97 min/d).

For activity, there was effect of interaction among group and day ($P < 0.001$), for *primiparous* (Figure 16B) and *parous* (Figure 16E). *Primiparous* cows from group SCHConly were more active than control cows on “Day -1”, while *parous* with SCHC only were less active than *parous* from group Control on “Day 2”. *Primiparous* and *parous* cows with SCHC and clinical disorders had lower activity than control animals from “Day 1” to “Day 9”, and from “Day 1” to “Day 14”, respectively. Cows from the SCHConly group showed higher activity than cows from the SCHCdisease group on “Day 1”, “Day 2”, “Day 4” and from “Day 6” to “Day 13” in *primiparous*, and from “Day 0” to “Day 14” in *parous*. The greatest difference among groups SCHCdisease and Control was observed on “Day 1” and “Day 3”, in *primiparous* (68 AU/d) and *parous* (143 AU/d), respectively. “Day 1” and “Day 3” showed the maximum difference between the SCHConly and the SCHCdisease groups, in *primiparous* (47 AU/d) and *parous* (92 AU/d), respectively.

For milk production, there was effect of interaction between group and day ($P < 0.001$), for *primiparous* (Figure 16C) and *parous* (Figure 16F). *Primiparous* cows from the SCHConly group had lower milk production than control cows on “Day 0”. However, *parous* from the SCHConly group produced more milk than control cows from “Day 6” to “Day 11”. Control cows had higher milk production than cows from the SCHCdisease group on “Day 0” and from “Day 4” to “Day 14”, in *primiparous*, and from “Day 8” to “Day 14”, in *parous*. Milk production was higher for cows from the SCHConly than for cows from the

Figure 14 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with subclinical ketosis and control cows



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for cows diagnosed with subclinical ketosis (SCK, group SCK, $n = 86$) and control cows (group Control, $n = 297$). Patterns of daily rumination time (D) and daily activity (E) from -7 to 14 d relative to calving, and daily milk production (F) from 0 to 14 d relative to calving for cows diagnosed with SCK (groups SCKOnly and SCKdisease), and control cows (group Control, $n = 297$). Cows were assigned to the SCKbornonly ($n = 31$) and SCKdisease ($n = 55$) groups if they were not or were diagnosed with any clinical disease from 0 to 14 d relative to calving, respectively. The day of calving was considered as "day 0". Values are presented as $LSM \pm SEM$. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs SCK; * Control vs SCKdisease; † Control vs SCKOnly; ¥ SCKOnly vs SCKdisease. Source: Silva, 2017.

SCHCdisease group from “Day 4” to “Day 14”, in *primiparous* and *parous*. The greatest difference between the SCHCdisease and Control groups was observed on “Day 6” and “Day 12”, for *primiparous* (6.8 kg/d) and *parous* (5.7 kg/d), respectively. “Day 10” and “Day 11” showed the maximum difference between the groups SCHConly and SCHCdisease, for *primiparous* (7.4 kg/d) and *parous* (8.8 kg/d), respectively.

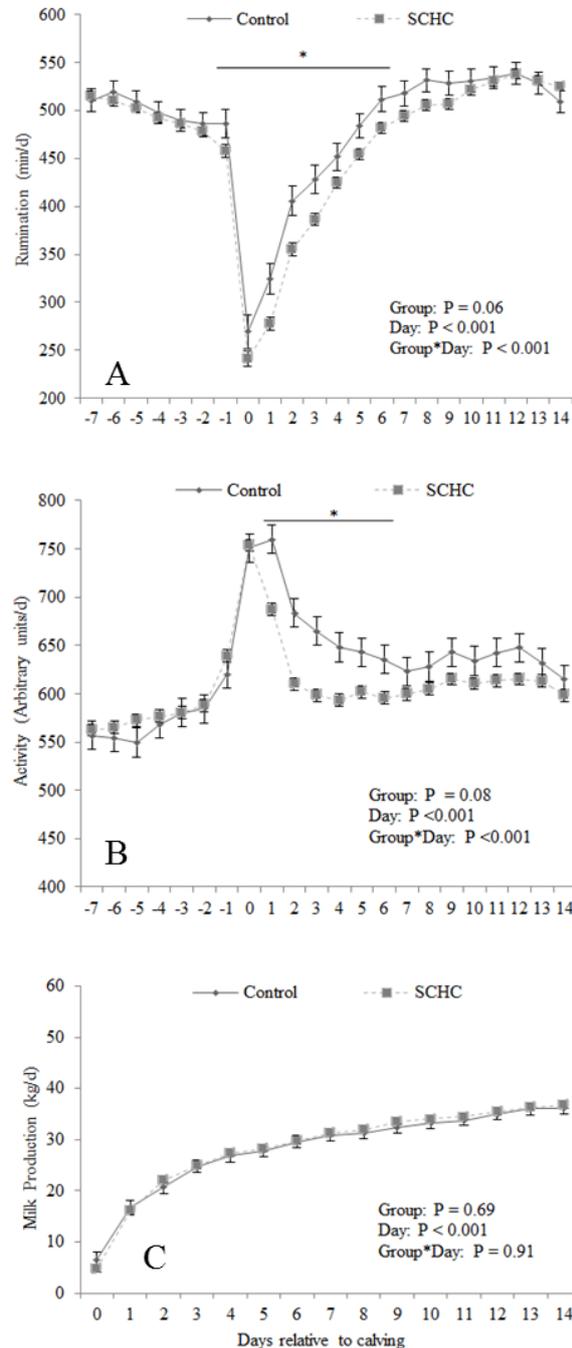
5.5. Use of rumination and activity monitoring to identify sick cows

5.5.1. Incidence of disease and DIM at diagnosis among groups

Table 2 shows the incidence of disease and average DIM at diagnosis for each disease by groups. No differences were found for overall incidence of disease (C = 47.3% (150/317), CM = 49.1% (144/293), $P = 0.59$) and for incidence of each disease between groups. Table 3 summarizes the incidence for each disease by parity. *Primiparous* animals from the CM group (43.3% (59/136)) had higher incidence of disease than *primiparous* (32.1% (47/146)) from the C group ($P = 0.05$). No differences were found for *parous* animals between groups ($P = 0.26$), however, incidence of metritis for group CM tended to be lower than for group C ($P = 0.1$).

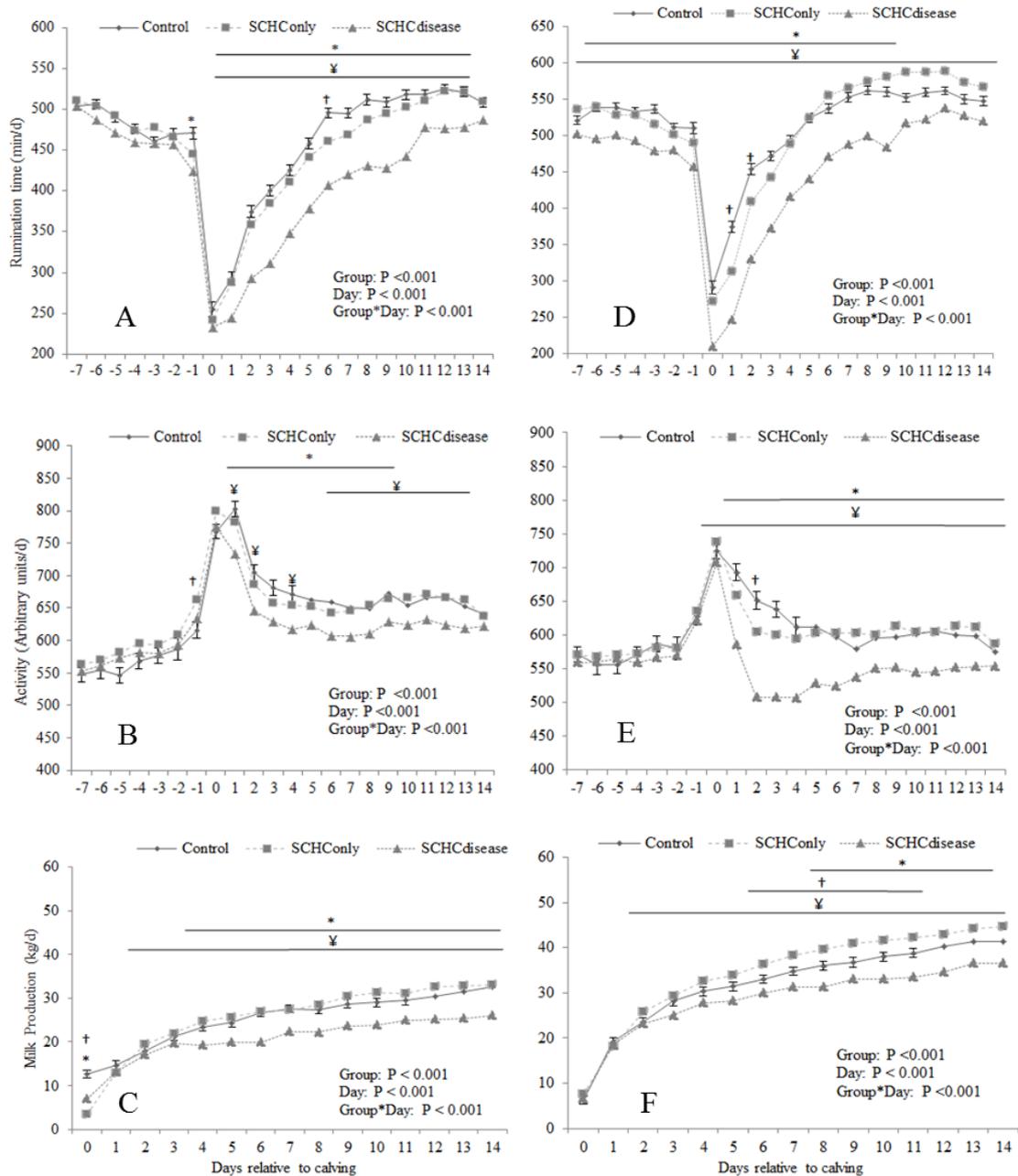
For average DIM at diagnosis, differences were found only for ketosis ($P = 0.03$) and mastitis ($P = 0.02$). Diagnosis of ketosis for cows from the CM group that had other disorders during the period of interest around diagnosis occurred earlier than for cows from the C group (4.2 DIM and 7.8 DIM, respectively) ($P = 0.03$). For mastitis, differences between C and CM groups were found for cows diagnosed only with this disease during the period of interest, diagnosis occurred earlier for the C (4.8 DIM) than for the CM group (13 DIM) ($P = 0.02$).

Figure 15 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with subclinical hypocalcemia and control cows



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for cows diagnosed with subclinical hypocalcemia (SCHC, group SCHC, $n = 421$) and control cows (group Control, $n = 77$). The day of calving was considered as “day 0”. Values are presented as LSM \pm SEM. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs SCHC. Source: Silva, 2017.

Figure 16 - Daily rumination time, daily activity, and daily milk production patterns for cows diagnosed with subclinical hypocalcemia and control cows (bis)



Legend: Patterns of daily rumination time (A) and daily activity (B) from -7 to 14 d relative to calving, and daily milk production (C) from 0 to 14 d relative to calving for *primiparous* cows diagnosed with subclinical hypocalcemia (SCHC; groups SCHCOnly, $n = 122$; SCHCdisease, $n = 67$) and *primiparous* control cows (group Control, $n = 47$). Patterns of daily rumination time (D) and daily activity (E) from -7 to 14 d relative to calving, and daily milk production (F) from 0 to 14 d relative to calving for *parous* cows diagnosed with SCHC (groups SCHCOnly, $n = 124$; SCHCdisease, $n = 108$), and *parous* control cows (group Control, $n = 30$). Cows were assigned to the SCHCOnly and SCHCdisease groups if they were not or were diagnosed with any clinical disease from 0 to 14 d relative to calving, respectively. The day of calving was considered as “day 0”. Values are presented as LSM \pm SEM. Within a day, significantly different pairwise comparisons ($P \leq 0.05$) based on LSD are represented as follows: * Control vs SCHC; * Control vs SCHCdisease; † Control vs SCHCOnly; ‡ SCHCOnly vs SCHCdisease. Source: Silva, 2017.

5.5.2. Rumination alteration performance to detect sick cows and predict disease diagnosis

Table 4 summarizes the sensitivity (Se) of RA to identify sick cows (from 7 d preceding until 4 d after disease diagnosis) and the mean interval between the first positive RA event and the day of disease diagnosis by groups (C and CM). The overall sensitivity of RA to detect the occurrence any health disorder, from -7 to 2 days relative to diagnosis, was 56.4% (227/402), and it was not different between groups (C = 53.4% (109/204), CM = 59.5% (118/198), $P = 0.21$). Sensitivity was higher for cows with more than one disease from -7 d to 4 d relative to the diagnosis of the disease of interest (75.8% (110/145)) than for cows with the disease of interest only (45.5% (117/257)) ($P < 0.001$). No differences between groups were found for Se for cows that had the disease of interest only (C = 45.6% (58/127), CM = 45.3% (59/130), $P = 0.96$). However, for cows that had more than one disorder during the period from -7 d to 4 d relative to diagnosis of the disease of interest, Se was higher for the CM group than for the C group (C = 66.2% (51/77), CM = 86.7% (59/68), $P = 0.005$).

For each disease, sensitivity of RA ranged from 40% to 100%. Sensitivity was not different between groups for: RP, metritis, ketosis, DA, and mastitis. For “other diseases”, the overall Se tended ($P = 0.07$) to be higher for the CM than for the C group. Se for cows that had other disorders from -7 d to 4 d relative to the diagnosis of “other diseases” was higher for the CM group than for the C group ($P = 0.04$).

Sensitivity of RA was higher for cows that had more than one disorder than for cows with the disorder of interest only around diagnosis for: metritis ($P = 0.001$), ketosis ($P = 0.04$), and “other diseases” ($P = 0.001$). However, sensitivity was not statistically different between cows with one and cows with more than one health disorder during the period of interest around the diagnosis for: RP ($P = 0.99$), DA ($P = 1$), MLF ($P = 0.92$), and mastitis ($P = 0.42$). For the CM group, Se for cows with more than one health disorder during the period of interest around diagnosis was higher for metritis ($P = 0.004$), and “other diseases” ($P = 0.004$). However, no differences for Se between cows with only one disease and cows with more than one disease in the period of interest were found for C group.

Table 2 - Incidence of disease and DIM at diagnosis

	Incidence (%)				DIM at diagnosis (mean)			
	Overall	Groups			Overall	Groups		
		C	CM	P-value		C	CM	P-value
RP	2.6 (16/610)	2.8 (9/317)	2.4 (7/293)	0.72	2	1.6	2.4	0.4
Only	0.8 (5/610)	1.2 (4/317)	0.3 (1/317)	0.24	1.4	1.5	1	0.49
With other	1.8 (11/610)	1.5 (5/317)	2 (6/293)	0.76	2.27	1.8	2.6	0.3
Metritis	28.5 (174/610)	29 (92/317)	27.9 (82/293)	0.77	11.4	11.9	10.8	0.27
Only	21.8 (133/610)	21.4 (68/317)	22.1 (65/293)	0.82	12.4	13.1	11.7	0.22
With other	6.7 (29/41)	7.5 (24/293)	5.8 (17/293)	0.38	8	8.6	7.2	0.28
Ketosis	6.7 (41/610)	6.3 (20/317)	7.1 (21/293)	0.67	6.2	7.7	4.8	0.03
Only	2.6 (16/610)	1.9 (6/317)	3.4 (10/293)	0.24	6.18	7.3	5.5	0.34
With other	4 (25/610)	4.4 (14/317)	3.7 (11/293)	0.68	6.28	7.8	4.2	0.03
DA	2.4 (15/610)	1.9 (6/317)	3 (9/293)	0.35	14.6	15.3	14.1	0.69
Only	0.5 (3/610)	0.3 (1/317)	0.6 (2/293)	0.52	18	12	21	*
With other	1.9 (12/610)	1.5 (5/317)	2.3 (7/293)	0.47	13.7	16	12.1	0.36
MLF	2.1 (13/610)	3.1 (10/317)	1 (3/293)	0.06	2	1.8	2.6	0.72
Only	1.3 (8/610)	2.2 (7/317)	0.3 (1/293)	0.07	2.3	2.4	2	*
With other	0.8 (5/610)	0.9 (3/317)	0.6 (2/293)	0.71	1.4	0.3	3	*
Mastitis	6.7 (41/610)	6.3 (20/317)	7.1 (21/293)	0.67	9.7	6.7	12.7	0.02
Only	3.9 (24/10)	3.1 (10/317)	4.7 (14/293)	0.3	9.6	4.8	13	0.02
With other	2.7 (17/610)	3.1 (10/317)	2.4 (7/293)	0.56	10	8.6	12	0.34
Indigestion	2.4 (15/610)	2.2 (7/317)	2.7 (8/293)	0.67	10.6	8.4	12.5	*
Only	1.6 (10/610)	0.9 (3/317)	2.3 (7/293)	0.17	10.8	5.6	13	*
With other	0.8 (5/610)	1.2 (4/317)	0.3 (1/293)	0.24	10.2	10.5	9	*
Others	16.7 (102/610)	14.8 (47/317)	18.7 (55/293)	0.17	10.4	11.2	9.6	0.18
Only	11.1 (68/610)	9.7 (31/317)	12.6 (37/293)	0.24	12.1	13	11.3	0.27
With other	5.5 (34/610)	5 (16/317)	6.1 (18/293)	0.53	7	8.1	5.8	0.24

RP = retained placenta; DA = displaced abomasum; MLF = milk fever; Others = "other diseases"; Only = cows diagnosed with the disease of interest only from -7 d to 4 d relative to diagnosis; With Other = cows diagnosed with the disease of interest and at least another health disorder from -7 d to 4 d relative to diagnosis; C = control group; CM = collar monitor group

Table 3- Incidence (%) of disease among parities

Disease	Overall			Groups					
	Nulliparous	Parous	P-value	Nulliparous			Parous		
				C	CM	P-value	C	CM	P-value
RP	2.4 (7/282)	2.4 (8/328)	0.97	2.7 (4/146)	2.2 (3/136)	0.77	2.3 (4/171)	2.5 (4/157)	0.9
Metritis	24.8 (70/282)	31.7 (104/328)	0.06	21.2 (31/146)	28.6 (39/136)	0.14	35.6 (61/171)	27.4 (43/157)	0.1
Ketosis	0.3 (1/282)	12.2 (40/328)	<0.001	0.6 (1/146)	0 (0/136)	0.98	11.1 (19/171)	13.3 (21/157)	0.53
MLF	0.3 (1/282)	3.6 (12/328)	0.02	0 (0/146)	0.7 (1/136)	0.99	5.8 (10/171)	1.2 (2/157)	0.04
DA	0.3 (1/282)	4.2 (14/328)	0.01	0.6 (1/146)	0 (0/136)	0.99	2.9 (5/171)	5.7 (9/157)	0.21
Mastitis	7 (20/282)	6.4 (21/328)	0.73	5.4 (8/146)	8.8 (12/136)	0.27	7 (12/171)	5.7 (9/157)	0.63
Indigestion	1 (3/282)	3.6 (12/328)	0.05	1.3 (2/146)	0.7 (1/136)	0.6	2.9 (5/171)	4.4 (7/157)	0.46
Others	15.2 (43/282)	18 (59/328)	0.36	13.6 (20/146)	16.9 (23/136)	0.45	15.7 (27/171)	20.3 (32/157)	0.28
Any disease	37.1 (106/282)	57 (187/328)	<0.001	32.1 (47/146)	43.3 (59/136)	0.05	59.6 (102/171)	54.1 (85/157)	0.26

Incidence of disease (%), percentage of cows diagnosed as sick during first 30 DIM; RP, Retained placenta; MLF, Milk Fever; DA, Displaced Abomasum; Others, “other diseases”; C, Control group. CM, Collar monitoring group

RA was capable to anticipate the diagnosis of almost all health disorders ($P \leq 0.05$), except for RP and MLF. The interval in days between the first RA event and the day of diagnosis was not different between groups for almost all health disorders, except for cows that had mastitis only during the period of interest around diagnosis, shorter for the C group (-0.03 d) than for the CM group (-3.2 d) ($P = 0.01$).

Summarizing, the overall sensitivity to detect any health disorders from 7 d preceding to 2 d after diagnosis was 54.8% (214/390 cow-days), and specificity (Sp) was 74.5% (13438/18028 cow-days). Moreover, positive predicted values (PPV) and negative predicted values (NPV) were 46.4% (511/1100) and 57.6% (9691/16812), respectively.

Table 4 - Sensitivity of RA to detect cows with health disorders (from -7 d to +2 d relative to diagnosis) and interval between first RA and diagnosis

		Sensitivity (%)				First RA to diagnosis (mean, d)				
		Overall	Groups			Overall		Groups		
			C	CM	P-value	Mean	P-value	C	CM	p-value
RP		87.5 (14/16)	77.7 (7/9)	100 (7/7)	0.9	0.5	0.89	1	0	0.2
	Only	100 (5/5)	100 (4/4)	100 (1/1)	1	1.4	0.99	1.2	*	*
	With other	81.8 (9/11)	60 (3/5)	100 (6/6)	0.9	0	0.5	0.6	-0.3	0.3
Metritis		48.8 (85/174)	48.9 (45/92)	48.7 (40/82)	0.98	-3.2	<0.001	-3.3	-3	0.67
	Only	42.1 (56/133)	44.1 (30/68)	40 (26/65)	0.63	-3.4	<0.001	-3.7	-3	0.73
	With other	70.7 (29/41)	62.5 (15/24)	82.3 (14/17)	0.17	-2.8	<0.001	-2.7	-3	0.7
Ketosis		68.2 (28/41)	70 (14/20)	66.6 (14/21)	0.81	-1.7	<0.001	-1.8	-1.5	0.76
	Only	50 (8/16)	50 (3/6)	50 (5/10)	1	-1.5	<0.001	-1.3	-1.6	0.9
	With other	80 (20/25)	78.5 (11/14)	81.8 (9/11)	0.84	-1.8	<0.001	-2	-1.5	0.7
DA		100 (15/15)	100 (6/6)	100 (9/9)	1	-4.5	<0.001	-5	-4.2	0.56
	Only	100 (3/3)	100 (1/1)	100 (2/2)	1	-4	<0.001	*	*	*
	With other	100 (12/12)	100 (5/5)	100 (7/7)	1	-4.6	<0.001	-4.6	-4.7	0.93
MLF		61.5 (8/13)	50 (5/10)	100 (3/3)	*	-0.1	0.51	0	-0.3	*
	Only	62.5 (5/8)	57.1 (4/7)	100 (1/1)	*	-0.4	0.54	-0.5	*	*
	With other	60 (3/5)	33.3 (1/3)	100 (2/2)	*	0.3	*	*	*	*
Mastitis		63.4 (26/41)	65 (13/20)	61.9 (13/21)	0.83	-2.2	0.008	-1.2	-3.3	0.05
	Only	58.3 (14/24)	60 (6/10)	57.1 (8/14)	0.88	-2	0.008	-0.3	-3.2	0.01
	With other	70 (12/17)	70 (7/10)	71.4 (5/7)	0.94	-2.5	0.01	-2	-3.4	0.48
Indigestion		64.2 (9/14)	85.7 (6/7)	42.8 (3/7)	*	-1.75	*	-1.8	-0.2	*
	Only	44.4 (4/9)	66.6 (2/3)	33.3 (2/6)	*	0	*	-1	0.5	*
	With other	100 (5/5)	100 (4/4)	100 (1/1)	*	-2.8	*	-2	-6	*
Others		50 (51/102)	40.4 (19/47)	58.1 (32/55)	0.07	-2.7	<0.001	-3.2	-2.3	0.23
	Only	38.2 (26/68)	32.2 (10/31)	42.2 (16/37)	0.35	-2.4	<0.001	-2.7	-2.2	0.71
	With other	73.4 (25/34)	56.2 (9/16)	88.8 (16/18)	0.04	-3	<0.001	-3.8	-2.5	0.24

RP = retained placenta; DA = displaced abomasum; MLF = milk fever; Others = "other diseases"; Only = cows diagnosed with the disease of interest only from -7 d to 4 d relative to diagnosis; With Other = cows diagnosed with the disease of interest and at least another health disorder from -7 d to 4 d relative to diagnosis; C = control group; CM = collar monitor group; Sensitivity = percentage of events detected by RA alteration from -7 d to 2 d relative to diagnosis; First RA to diagnosis = interval in days between first RA event and diagnosis of disease; 1 = to determine if RA was capable to identify sick cows earlier than diagnosis, the interval between first RA and diagnosis was evaluated by paired t-test, comparing the mean number of days from the first RA and the day of diagnosis.

5.5.3. Milk production

Daily milk production during the first 30 DIM (30dMilk) was not different between groups (C = 36 ± 0.4 kg/d, CM = 35.3 ± 0.5 kg/d, $P = 0.31$) (Table 6). Likewise, milk production until 80 DIM (80dMilk) showed no differences ($P = 0.3$) between the C (42.7 ± 0.5 kg/d) and the CM group (41.8 ± 0.6 kg/d). *Parous* animals had higher daily milk production

during the first 30 DIM ($P < 0.001$) and 80 DIM ($P < 0.001$) than *primiparous* animals. Daily milk production during the first 30 DIM ($P < 0.001$) and 80 DIM ($P < 0.001$) was higher for cows that never had RA events (38.2 ± 0.4 kg/d and 44.5 ± 0.5 kg/d, respectively) than for cows that had at least one RA event (33.6 ± 0.4 kg/d and 40.4 ± 0.5 kg/d, respectively). Cows diagnosed as sick during the experimental period had lower daily milk production during the first 30 DIM (Sick = 33.3 ± 0.4 kg/d, Healthy = 37.8 ± 0.5 kg/d, $P < 0.001$) and 80 DIM (Sick = 40.7 ± 0.4 , Healthy = 43.7 ± 0.6 kg/d, $P < 0.001$) when compared to healthy cows.

5.5.4. Hospital Barn

One hundred and fifty eight cows (25.9%) were sent to a “hospital barn” during the first 30 DIM. This percentage was not statistically different between groups (C = 24.6% (78/294), CM = 27.3% (80/317), $P = 0.44$). Cows stayed at hospital 12.12 ± 6.99 days in average, and no differences were found between groups (C = 12.74 ± 6.29 , CM = 13.48 ± 7.65 , $P = 0.47$). The percentage of cows that stayed at hospital barn ($P = 0.57$) and the number of days they stayed at hospital ($P = 0.38$) were not different between *primiparous* (24.8% (70/282), 13.62 ± 5.50 days, respectively) and *parous* animals (26.8% (80/328), 12.7 ± 8 days, respectively). The percentage of animals sent to hospital and number of days were not different for *primiparous* and *parous* between groups (Tables 7 and 8).

The percentage of animals sent to hospital and number of days they stayed there were greater for animals that had RA (40% (134/335) and 13.58 ± 7.07 days) when compared to animals that had not RA (8.7% (24/275) and 10.45 ± 6.06 days) ($P < 0.001$ and $P = 0.02$, respectively). Groups did not showed differences for percentage of animals sent to hospital and number of days.

Of the cows diagnosed as sick, 51% (150/294) was sent to hospital and stayed for 13.38 ± 6.99 days in average there, without differences between groups (C = 50% (75/150), CM = 52% (75/144), $P = 0.72$; C = 13.04 ± 6.2 , CM = 13.72 ± 7.72 , $P = 0.52$, respectively). For each disease, no differences between groups for percentage of cows sent to hospital were found, but cows with ketosis belonging to CM (85.7% (18/21)) tended to be sent to hospital more than cows from C group (60% (12/20)) ($P = 0.07$). For days staying at hospital the only difference found between groups corresponds to mastitis, where cows from CM group (15.28 ± 8.14 days) stayed longer than cows from C group (11.70 ± 3.92 days) ($P = 0.04$).

5.5.5. Treatments given to the animals

Table 5 summarizes the percentage of animals that receive treatment and number of treatments per cow. The percentage of animals that received any treatment was 57.2% (349/610), higher for animals from CM (61.4% (180/294)) than C group (53.3% (169/317)) ($P = 0.04$). The percentage of cows that received antibiotic, anti-inflammatory and support treatments were 23.9% (146/610), 25.7% (157/610) and 56.4% (344/610), respectively. No differences between groups were found for antibiotic ($P = 0.46$) and anti-inflammatory ($P = 0.16$) treatments. However, a higher percentage of cows from CM group (61.1% (179/293)) received support treatments when compared to C group (52% (165/317)) ($P = 0.02$). Average number of any treatments, antibiotic, anti-inflammatory and support treatments the cows received were 3.58 ± 2.39 , 1.37 ± 0.57 , 2.03 ± 0.78 and 2.12 ± 1.09 , respectively, without differences between groups ($P = 0.6$, $P = 0.87$, $P = 0.6$ and $P = 0.3$, respectively).

A higher percentage of *parous* (67% (220/328)) than *primiparous* (45.7% (129/282)) animals were treated ($P < 0.001$). Differences between groups were found for *primiparous*, where a higher percentage of animals from the CM (52.2% (71/136)) than the C (39.7% (58/146)) group were treated ($P = 0.03$). For *parous* animals, no differences between groups were found. For antibiotics, no differences between *primiparous* and *parous* animals were found. For anti-inflammatory treatments, no differences between *primiparous* and *parous* animals for percentage of cows that received treatment were found. However, a higher percentage of *primiparous* from the CM (35.3% (31/146)) than the C (21.2% (31/146)) group was treated with anti-inflammatories ($P = 0.01$). For support treatments, the percentage of treated animals was higher for *parous* (66.7% (219/328)) than for *primiparous* (44.3% (125/282)) ($P < 0.001$). For *primiparous*, a higher percentage of animals from the CM (51.4% (70/136)) than the C (37.6% (65/146)) group received support treatments ($P = 0.02$). Regarding to average number of treatments per cow, no differences were found for any treatment, antibiotics and anti-inflammatories. *Parous* animals received a higher number of support treatments than *primiparous* animals ($P = 0.03$), 2.25 ± 1.14 and 1.89 ± 0.96 , respectively. No differences for *primiparous* and *parous* were found between groups.

The percentage of animals that received any treatment ($P < 0.001$), antibiotics ($P < 0.001$), anti-inflammatories ($P < 0.001$) and support treatments ($P < 0.001$) was higher for animals with RA (71.6% (240/335), 37.9% (127/335), 40.6% (136/335), 71% (238/335), respectively) than animals that had not RA (39.6% (109/275), 6.9% (19/275), 7.6% (21/275),

38.5% (106/275), respectively). The number of any treatments and support treatments received by the cows was higher for those with RA (4.22 ± 2.43 and 2.3 ± 1.13 , respectively) than cows without RA (2.17 ± 1.56 and 1.7 ± 0.88 , respectively). However, no differences for antibiotics and anti-inflammatories were found.

Ninety two percent (270/ 293) of the cows diagnosed as sick received any treatment, and this was not different among groups ($P = 0.46$). For each disease, 100% of the cows that developed disease were treated, except for cows with metritis (98.8%) and “other diseases” (88.2%). No differences between groups were found for percentage of treated cows for each disease.

Table 5 - Percentage (%) of cows that received treatment and number of treatments per animal (mean \pm SD)

		Group			P-value
		Overall	C	CM	
All Treatments	Treated cows (%)	57.21 (349/610)	53.3 (169/317)	61.4 (180/294)	0.04
	Number of trt	3.58 ± 2.39	3.65 ± 2.43	3.51 ± 2.36	0.6
Antibiotics	Treated cows (%)	23.9 (146/610)	22.7 (72/317)	25.2 (74/293)	0.46
	Number of trt	1.37 ± 0.57	1.36 ± 0.56	1.39 ± 0.59	0.87
Anti-inflammatories	Treated cows (%)	25.7 (157/610)	23.3 (74/317)	28.3 (83/293)	0.16
	Number of trt	2.03 ± 0.78	2.09 ± 0.81	1.97 ± 0.76	0.6
Support Treatments	Treated cows (%)	56.4 (344/610)	52 (165/317)	61.1 (179/293)	0.02
	Number of trt	2.12 ± 1.09	2.20 ± 1.11	2.04 ± 1.07	0.3

All treatments, any treatment given during the first 30 DIM; Antibiotics, antibiotic treatments given during the first 30 DIM; Anti-inflammatory, anti-inflammatory treatments given during the first 30 DIM; Support Treatments, any support treatment (minerals such as calcium, potassium, magnesium, and sodium, propylene-glycol, amino acids, bismuth, vitamin B, dextrose, saline solution, mineral oil, rumen fluid, and intrauterine bolus containing urea) given during the first 30 DIM; Treated cows (%), percentage of cows that received treatment during first 30 DIM; Number of trt, number of treatments per cow (mean \pm SD) during the first 30 DIM; C, control group; CM, collar monitoring group.

5.5.6. Culling

The percentage of cows culled until 60 DIM and 150 DIM was 6% (37/610) and 10.5% (64/610), respectively. Average DIM at culling until 60 DIM and 150 DIM was 20.59 ± 16.33 and 52.82 ± 43.43 . No differences between groups were found for percentage of cows culled until 60 DIM ($P = 0.45$) and 150 DIM ($P = 0.73$), and for average DIM at culling until 60 DIM ($P = 0.55$) and 150 DIM ($P = 0.28$). A higher percentage of *parous* than *primiparous* animals were culled until 60 DIM (9.4% (31/328) and 2.1% (6/282), respectively; $P < 0.001$) and 150 DIM (14.3% (47/328) and 6% (17/282), respectively; $P < 0.001$). *Parous* animals tended to be culled earlier until 150 DIM than *primiparous* animals ($P = 0.08$), 47 ± 41.5 DIM and 68.94 ± 45.64 , respectively. No statistical differences between groups were found for *primiparous* and *parous* animals.

The percentage of culled cows until 60 DIM ($P = 0.01$) and 150 DIM ($P = 0.002$) was higher for cows that had RA (8.3% (28/275) and 14% (47/335), respectively) than for cows that had not RA (3.2% (9/275) and 6.2% (17/275), respectively). The percentage of culled cows was not different between groups for cows with RA and cows without RA. For average DIM at culling, differences were found only for cows that had not RA, where animals from CM group were culled earlier until 60 DIM than cows from C group ($P = 0.02$), 3.75 ± 2 and 27.6 ± 23.73 , respectively.

A higher percentage of cows that developed disease than healthy cows were culled until 60 DIM (10.6% (31/294) and 1.9% (6/316), respectively; $P < 0.001$) and 150 DIM (16% (47/294) and 5.3% (17/316), respectively; $P < 0.001$). Besides, sick cows tended to be culled earlier than healthy cows until 150 DIM ($P = 0.06$), 47.17 ± 41.44 and 68.47 ± 37.45 , respectively. No differences between groups were found for healthy and sick cows.

5.5.7. Reproductive performance

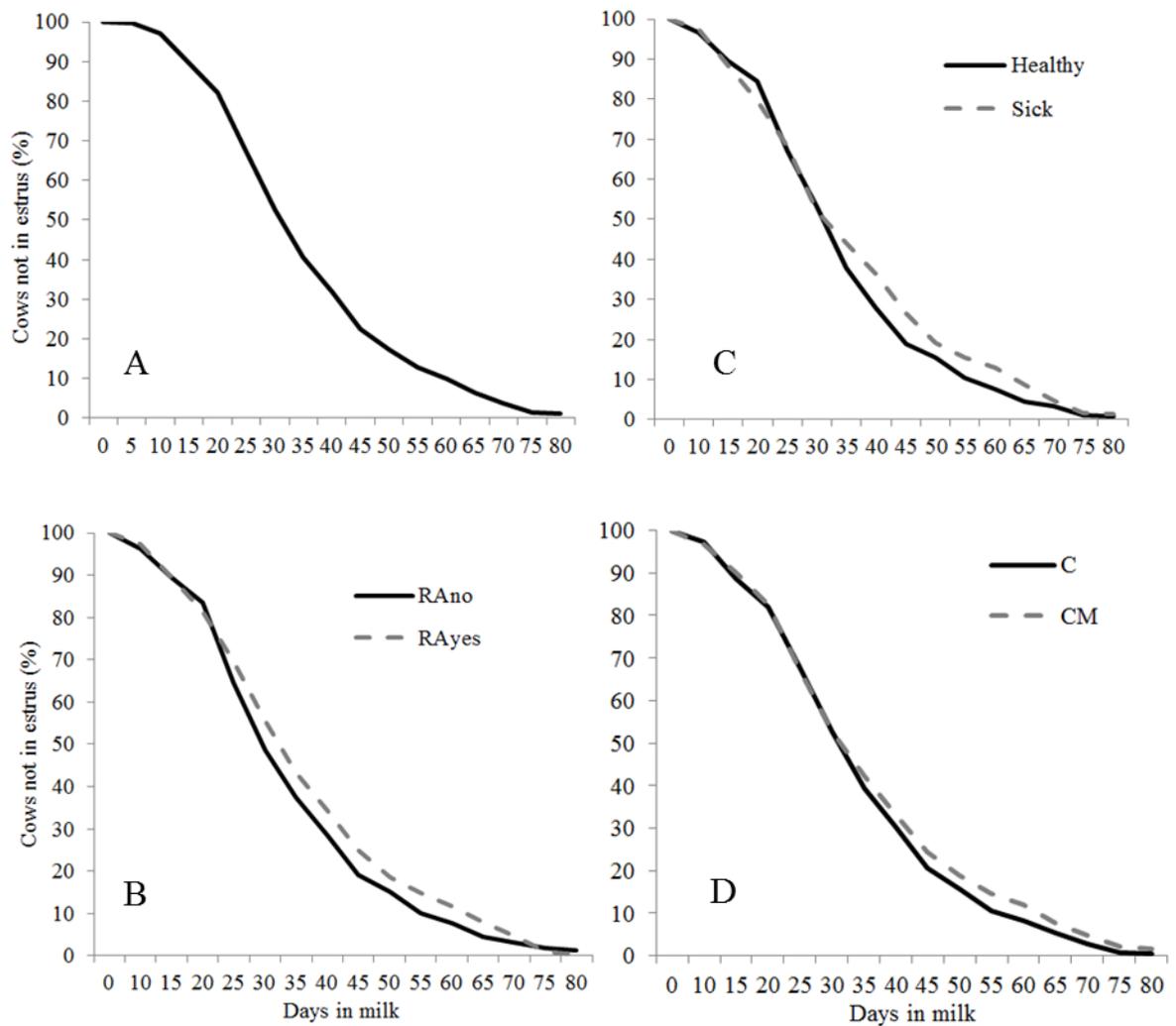
Cows displayed sign of estrus shortly after calving. By approximately 30 DIM 50% of cows had displayed estrus at least once and by 60 DIM 90% of cows had displayed estrus according to the data collected by the tags (Figure 17A). Kaplan-Meier survival analysis did not show differences for the appearance of sign of estrus between groups (Figure 17D). Speed

at which cows appeared in estrous was approximately 5 d slower for cows that had RA than cows that had no RA events (Figure 17B). The same occurred between healthy and sick cows (Figure 17C). Eighty-six percent of the animals (527/610) were inseminated until 150 DIM, and first service was performed at 75.64 ± 14.88 DIM in average. Fifty-nine percent (311/527) of first services were done based on heat detection and 41% (216/527) using fixed time artificial insemination protocols (FTAI). Pregnancy rate and pregnancy loss for first service were 35.1% (183/521) and 10.4% (19/182), respectively. Twenty-one (3.4%) and 48 cows (7.8%) were marked as Do Not Breed (DNB) by farm personnel until 60 DIM (DNB60) and 150 DIM (DNB150), respectively. For all parameters described, no statistical differences were found between groups.

The percentage of cows inseminated until 150 DIM was higher for animals that had not RA events (90.9% (250/ 275)) than for animals that had RA (82.6% (277/335)) ($P = 0.003$). Moreover, a higher percentage of animals with RA were marked as DNB until 60 DIM (3.8% (13/335)) and 150 DIM (8.6% (29/335)) when compared to animals without RA (2.9% (8/275) and 6.9% (19/275), respectively) ($P < 0.001$).

Cows diagnosed as sick had lower service rate until 150 DIM ($P < 0.001$), higher pregnancy loss rate for first service ($P = 0.02$) and higher incidence of DNB150 ($P = 0.008$) than healthy cows (sick = 79.8% (234/293), 16.2% (13/80), 10.9% (32/293); healthy = 92.4% (293/317), 5.8% (6/102), 5% (16/317), respectively). Incidence of DNB60 tended to be higher ($P = 0.09$) for sick (4.7% (14/293)) than healthy (2.2% (7/317)) animals. Differences between groups were observed only for DNB60 for sick animals, with higher values for cows from the group C (7.3% (11/149)) than the group CM (2% (3/144)). Additionally, a higher percentage of sick cows from the CM group (64.1% (75/117)) tended to be inseminated based on heat detection than sick cows from the group C (52.1% (61/117)) ($P = 0.06$).

Figure 17 - Kaplan-Meier survival analysis illustrating the occurrence of estrus after calving



Legend: Kaplan-Meier survival analysis illustrating the occurrence of estrus after calving for all cows (A), cows that had rumination alteration events (RAYes), and cows that had not rumination alteration event (RAno) (B), healthy and sick cows (C), and cows from C and CM groups. Source: Silva, 2017.

Table 6 - Incidence of disease and Rumination Alteration events, milk yield, percentage of cows sent to hospital and treated, culling rate, service and pregnancy rate and sensitivity of RA system

	Overall	Group		p-value
		C	CM	
Disease (%)	48 (293/610)	47 (149/317)	49.1 (144/293)	0.59
RA (%)	54.9 (335/610)	53.9 (171/317)	55.9 (164/293)	0.61
MY 30DIM	35.7 ± 0.35	36 ± 0.46	35.3 ± 0.54	0.31
MY 80DIM	42.3 ± 0.41	42.7 ± 0.52	41.8 ± 0.63	0.31
Hospital (%)	25.9 (158/610)	24.6 (78/317)	27.3 (80/293)	0.44
Treated cows (%)	57.21 (349/610)	53.3 (169/317)	61.4 (180/293)	0.04
Cull 60 DIM (%)	6 (37/610)	5.3 (17/317)	6.8 (20/293)	0.45
Cull 150 DIM (%)	10.5 (64/610)	10.1 (32/317)	10.9 (32/293)	0.73
Service 150 DIM (%)	86.3 (527/610)	86.4 (274/317)	86.3 (253/293)	0.97
Preg/1 service (%)	35.1 (183/521)	36.2 (98/270)	33.8 (85/251)	0.56

C, Control group; CM, Collar Monitor group; Disease, incidence of disease; RA, percentage of cows that showed Rumination Alteration; MY 30DIM, milk yield until 30 DIM (Kg, mean ± SEM); MY 80DIM, milk yield until 80 DIM (Kg, mean ± SEM); Hospital, percentage of cows sent to the hospital; Treated cows, percentage of cows that received treatment; Cull 60 DIM, culling rate until 60 DIM; Cull 150 DIM, culling rate until 150 DIM; Service 150 DIM, percentage of cows inseminated until 150 DIM; Preg/1 Service, pregnancy rate for first service.

Table 7 - Disease and Rumination Alteration incidence, milk yield, percentage of cows sent to hospital and treated, culling rate, service and pregnancy rate for *primiparous*

	Overall	Group		p-value
		C	CM	
Disease (%)	37.1 (106/282)	32.1 (47/146)	43.3 (59/136)	0.05
RA (%)	57.8 (163/282)	52.7 (77/146)	63.2 (86/136)	0.07
MY 30DIM	31.8 ± 0.32	32.3 ± 0.41	31.2 ± 0.49	0.67
MY 80DIM	37 ± 0.32	37.5 ± 0.38	36.5 ± 0.53	0.76
Hospital (%)	24.8 (70/282)	21.2 (31/146)	28.6 (39/163)	0.14
Treated cows (%)	45.7 (129/282)	39.7 (58/146)	52.2 (71/136)	0.03
Cull 60 DIM (%)	2.1 (6/282)	1.3 (2/146)	2.9 (4/136)	0.37
Cull 150 DIM (%)	6 (17/282)	4.1 (6/146)	8 (11/136)	0.16
Service 150 DIM (%)	93.6 (264/282)	95.9 (140/146)	91.1 (124/136)	0.11
Preg/1 service (%)	40.2 (105/261)	42.7 (59/138)	37.4 (46/123)	0.37

C, Control group; CM, Collar Monitor group; Disease, incidence of disease; RA, percentage of cows that showed Rumination Alteration; MY 30DIM, milk yield until 30 DIM (Kg, mean ± SEM); MY 80DIM, milk yield until 80 DIM (Kg, mean ± SEM); Hospital, percentage of cows sent to the hospital; Treated cows, percentage of cows that received treatment; Cull 60 DIM, culling rate until 60 DIM; Cull 150 DIM, culling rate until 150 DIM; Service 150 DIM, percentage of cows inseminated until 150 DIM; Preg/1 Service, pregnancy rate for first service.

Table 8 - Disease and Rumination Alteration incidence, milk yield, percentage of cows sent to hospital and treated, culling rate, service and pregnancy rate for *parous*

	Overall	Group		p-value
		C	CM	
Disease (%)	57 (187/328)	59.6 (102/171)	54.1 (85/157)	0.26
RA (%)	52.4 (172/328)	54.9 (94/171)	49.6 (86/157)	0.33
MY 30DIM	39 ± 0.53	39.2 ± 0.68	38.8 ± 0.83	0.97
MY 80DIM	46.8 ± 0.61	47.1 ± 0.78	46.5 ± 0.95	0.92
Hospital (%)	26.8 (80/328)	27.4 (47/171)	26.1 (41/157)	0.78
Treated cows (%)	67 (220/328)	64.9 (111/171)	69.4 (109/157)	0.38
Cull 60 DIM (%)	9.4 (31/328)	8.7 (15/171)	10.2 (16/157)	0.66
Cull 150 DIM (%)	14.3 (47/328)	15.2 (26/171)	13.3 (21/157)	0.63
Service 150 DIM (%)	80.2 (263/328)	78.3 (134/171)	82.1 (129/157)	0.38
Preg/1 service (%)	30 (78/260)	29.5 (39/132)	30.4 (39/128)	0.87

C, Control group; CM, Collar Monitor group; Disease, incidence of disease; RA, percentage of cows that showed Rumination Alteration; MY 30DIM, milk yield until 30 DIM (Kg, mean ± SEM); MY 80DIM, milk yield until 80 DIM (Kg, mean ± SEM); Hospital, percentage of cows sent to the hospital; Treated cows, percentage of cows that received treatment; Cull 60 DIM, culling rate until 60 DIM; Cull 150 DIM, culling rate until 150 DIM; Service 150 DIM, percentage of cows inseminated until 150 DIM; Preg/1 Service, pregnancy rate for first service.

6. DISCUSSION

Daily rumination time and activity patterns for the diagnosis of disease in animals with different health disorders were studied and described in the present study. Although rumination time within a day may not accurately reflect total DMI, decreases in DRT over time are observed when decreases in DMI are observed, particularly around the time of calving (SCHIRMANN et al., 2013). Thus, variations in DRT may be used as a proxy for variations in DMI.

Average daily rumination time and average daily activity were within the values found in other studies (LIBOREIRO et al., 2015; PAUDYAL et al., 2016; SORIANI; TREVISI; CALAMARI, 2012). However, some differences between our values and those reported by these authors can be explained by differences in chemical and physical characteristics of the diet, environmental and management conditions, period of lactation, animal factors, such as genetics, period of the year, and health status (BYSKOV et al., 2015; GRUMMER; MASHEK; HAYIRLI, 2004; LIBOREIRO et al., 2015; PAUDYAL et al., 2016; SORIANI; TREVISI; CALAMARI, 2012). As was expected, *parous* ruminated more than *primiparous* during prepartum and postpartum periods (SORIANI; TREVISI; CALAMARI, 2012). The correlation between prepartum and postpartum DRT was moderate ($r = 0.39$) but lower than the reported by LIBOREIRO et al. (2015) ($r = 0.60$) and the correlation between prepartum and postpartum DMI from several studies reviewed and published by Grummer et al. (2004). These differences with previous results may be related to the factors mentioned for differences in DRT between studies. Cows ruminated less during the postpartum than during the prepartum period in our study, supporting the notion of how composition and processing of the diet and stress may affect DRT (BRISTOW; HOLMES, 2007; BYSKOV et al., 2015). Prepartum and postpartum activity were highly correlated ($r = 0.62$) as Liboreiro et al. (2015) reported before ($r = 0.69$). Cows were more active after calving than during prepartum period, and this is possibly related to group movements and milking routine, since animals have to walk a considerable distance from the freestalls to the milking parlor three times a day. Additional experiments are necessary to determine what factors explain prepartum and postpartum DRT and activity and their relations. Milk yield was weakly correlated with prepartum DRT, however, a moderate correlation with postpartum DRT was observed. These results are in concordance with those

published by Liboreiro et al. (2015), supporting the idea that DMI and milk are associated, and their variations can affect the behavior of the other (WEISS, 2015).

All cows had a significant decrease in DRT and a significant increase in activity around calving (Figures 3A and 4A), as described by Schirmann et al. (2013). Following the routine of the farm, cows were moved from the close-up pen to the maternity pen at the first sign of imminent calving, and although movement to a different pen might have affected DRT and activity, it is likely that the variability in DRT and activity on the day of calving is explained mostly by the calving itself.

Average daily milk yield was within the values reported and expected for high yielding dairy farms (MOALLEM et al., 2010). As expected, sick cows (FOURICHON et al., 1999; RAJALA; GRÖHN, 1998b; RANDALL et al., 2016) and those with altered rumination (CALAMARI et al., 2014; SORIANI; TREVISI; CALAMARI, 2012) produced less milk than healthy ones and animals with unaltered rumination, respectively.

Cows were evaluated for signs of retained placenta, metritis, milk fever, displaced abomasum, clinical ketosis, mastitis, indigestion and other diseases, including lameness, vaginitis, etc. The incidence for each disease were within the values found in the literature (ALERI et al., 2016; FLEISCHER et al., 2001; LEBLANC; LESLIE; DUFFIELD, 2005; VANHOLDER et al., 2015). Culling rate during the first months of lactation was within the values expected for well managed herds (BRETT J, 2011; DECHOW; GOODLING, 2008; SALFER J, 2016).

6.1. Daily rumination time, activity, and milk production patterns for cows with clinical disease, subclinical disorders, calving problems, and regrouped cows

6.1.1. Retained Placenta and Metritis

Cows in our experiment that developed RP had a stronger and earlier decline in DRT than control animals. For animals that did not develop RP, rumination fell down one day before calving, however for cows with RP this decrement began as earlier as 4 to 5 days before parturition and reached a lower nadir than in healthy cows. In the current experiment, diagnosis of metritis was performed at 11 DIM in average, but cows with metritis ruminated

less than healthy cows from 7 d before diagnosis or maybe sooner, as has already shown by other authors (STANGAFERRO et al., 2016a). The fact that the animals ruminated less during the days preceding diagnosis or did not recover rumination after calving as healthy cows did may have caused immunosuppression through the increased severity and extent of negative energy balance, in addition to having increased the risk of RP and metritis (HAMMON et al., 2006; KIMURA et al., 2002b; LIBOREIRO et al., 2015). However, it was not able to distinguish if low rumination is a result of very early manifestation of the disorder or because these cows did not recover after calving. Moreover, the normal increase of activity observed for all cows around calving was smaller in animals with RP. Although no statistical differences for activity were found between animals with or without metritis, SickRAYes animals were numerically and graphically less active around diagnosis, and this can be supported by the results published by Liboreiro et al. (2015), as they found that metritic cows had reduced activity when compared to healthy cows during the first DIM. Milk production was lower for cows with RP and metritis when compared to healthy cows in this study, as had been previously described by several authors (GALVÃO et al., 2010; GIULIODORI et al., 2013; SHELDON et al., 2009). For metritis, it could be thought that the level of severity may be related to DRT, ACT, and milk production patterns because animals with metritis without RA did not show statistical differences for DRT, ACT, and milk production when compared to control animals. The degree of severity of metritis was not collected in this experiment, but probably metritis was more severe in animals from the SickRAYes group than animals from the SickRAno group. Supporting this hypothesis, a greater percentage of animals belonging to group SickRAYes stayed at the hospital, received antibiotic's treatments, and left the herd until 60 DIM and 150 DIM after than animals from the SickRAno group. Moreover, service rate until 150 DIM and pregnancy rate for first service were lower in the SickRAYes than in the SickRAno group animals.

6.1.2. *Clinical Ketosis*

The rumination and activity patterns for cows diagnosed with clinical ketosis found in this experiment are similar with those shown by (STANGAFERRO et al., 2016c). Cows with ketosis ruminated less than healthy cows during the period around diagnosis. Differences in

rumination were found between control animals and ketotic animals (SickRA_{no} and SickRA_{yes}), in addition to differences found between the both groups of cows with ketosis. Stangaferro et al. (2016) did not find differences between animals with ketosis not “flagged” by the system (without RA events) and healthy animals as we found in the current study. This may be due the different Health Index threshold used to flag cows in the two experiments. Cows were flagged when HI was < 86 AU/d by Stangaferro, and when HI was less than 80 AU/d in this experiment. These animals in the SickRA_{no} group probably ruminated less but were not flagged in our experiment would be flagged by Stangaferro’s criterion because it is possible their Health Index was between 80 and 86 AU/d. In a study published by Edwards et al. (2004), ketotic cows had lower activity than healthy cows, as was found in the present work. The main differences for activity were seen between control animals and those cows with ketosis and RA events during the period of interest around diagnosis (SickRA_{yes}). Activity levels for animals with ketosis without RA events around diagnosis (SickRA_{no}) were in the middle between the levels for healthy and SickRA_{yes} animals. However, no important statistical differences were found, and these findings are in concordance with those published by Stangaferro et al. (2015). Probably, this reflects the different impact that rumination and activity have on Health Index calculation by the system. The results of the present study showed a negative effect of ketosis on milk yield, as had been established before (EDWARDS et al., 2004; LUCEY; ROWLANDS; RUSSELL, 1986). These authors demonstrated how milk production is negatively affected even before the diagnosis of ketosis, as occurred with the cows in this experiment. Interestingly, although there was no effect of the occurrence of other disorders around the diagnosis of ketosis on DRT, ACT, and milk production patterns, sensitivity of RA to identify cows with ketosis was greater for cows that had other disorders than for cows that had ketosis only. Likely, the fact that a higher percentage of cows with other disorders were flagged by the system is related to the level of severity of ketosis and the associated diseases. Cows with a more severe case of ketosis are expected to have DRT and activity patterns more affected than cows with light or mild ketosis. The majority of health disorders accompanying ketosis in the present study were metabolic, digestive, and some metritis cases. It is possible that these effects in conjunction with a light or mild case of ketosis led DRT and activity patterns to alterations of a degree as observed in severe ketosis cases. Or probably, ketosis cases observed in this group of animals were secondary due to anorexia as a result of other diseases. These observations provided evidence that the sole occurrence of ketosis caused alterations to rumination and activity that in some cases are not enough to flag the cows. The fact that the Se was not different for cows with only ketosis and cows with other

disorders, additionally observed in the study performed by Stangaferro et al. (2016c), may be due to their threshold alert (HI = 86 AU/d) being higher than the used in this experiment (HI = 80 AU/d), flagging cows with smaller rumination alterations that would not be flagged in this trial.

6.1.3. *Milk Fever*

The occurrence of milk fever affected DRT patterns, leading rumination to a deeper and more prolonged decrease around calving than in healthy cows in the current experiment. For healthy cows, rumination recovered to normal levels quickly after calving, however, for cows with MLF, rumination's recovery only took place after diagnosis and treatment of this disorder. Although no statistical differences among groups SickRA_{no} and SickRA_{yes} were found, rumination's nadir for the last group was slightly lower and the recovery of rumination was 1 to 2 d slower than for the first group. Milk fever is a disorder of easy identification because signals are evident through simple visual inspection of the cow, however, this is not the same for the system because the proximity to calving can lead to misleading rumination behavior as a normal pattern around calving. The fact that some cows with MLF had RA and other did not, even with a very similar DRT pattern, could be explained by the fact that the diagnosis was performed during the first or second day after calving, and for this reason, it is possible that some cows actually showing RA events were ignored or assumed as part of the "normal" rumination curve around parturition. As a punctual example, one cow diagnosed with MLF at 3 DIM showed DRT of 170 min/d two days after calving, but the Health Index was higher than 80 AU on that day. Then, this cow was not flagged with RA, but she was actually sick. One day after diagnosis and treatment, rumination rose to 450 min/d, and the health disorder was ignored by the system for this cow. Authors showed that activity is lower in cows with MLF than in healthy cows (EDWARDS et al., 2004; TALUKDER et al., 2015b). Although the results in this trial did not show statistical differences among cows suffering from milk fever and control cows, the first ones were numerically less active around calving and this can be seen graphically. Probably, the small number of cows with MLF did not allow for finding statistical differences. Milk production was affected in cows that suffered from MLF, however, no important differences were observed between them and healthy cows in the current trial. This is in line with the results of Rajala-Schultz et al. (1999), who found that

despite the loss in milk production in cows with milk fever, these cows produced more milk than healthy cows. Although no statistical differences were observed on the first day of lactation, it may be because of the small number of sick animals, while cows with MLF showed numerically higher milk production than healthy cows. This finding could be related to the hypothesis that high milk yield is a risk factor for the occurrence of MLF (ERB, 1987). After diagnosis and treatment, milk production increased, and it seems to be that this increment was faster in cows from the SickRAno group. However, it should be mentioned that some cows from this group could have had RA, but the system was not able to flag them because of the proximity to calving.

6.1.4. Displaced abomasum

Displaced abomasum is very disruptive to cow's health and welfare, and this is reflected in rumination and activity patterns (TALUKDER et al., 2015b). In the present study, all cows that suffered from DA were flagged by the system because rumination and activity patterns were strongly altered. Stangaferro et al. (2016) had similar results with only one cow not being marked as sick. However, rumination and activity showed a marked decline in that cow around diagnosis, resulting in a reduction in HI that was not observed as an alert by the system because values were above 86 AU, the cut-off point those authors used in their experiment. Our data showed that rumination and activity were lower for sick cows from 7 d preceding diagnosis, as was already shown by Talukder et al. (2015). Besides, Stangaferro et al (2016) showed that cows with DA had lower rumination and activity 5 d before diagnosis when compared to healthy cows. Eighty percent of the cows in our experiment had other diseases from 7 d preceding up to 4 d after the diagnosis of DA, following the assumption that cows are more likely to develop DA if they have experienced one or more periparturient disorders (RAIZMAN; SANTOS, 2002). Among the cows suffering from other diseases in addition to DA in this study, 60% were diagnosed with clinical ketosis, supporting the idea that it is possible that cows which developed DA can also be ketotic prior to diagnosis (LEBLANC; LESLIE; DUFFIELD, 2005). Only one *primiparous* cow was diagnosed with DA in the present trial, reflecting how parity influences the incidence of DA, being *parous* in higher risk than *primiparous* cows (MARKUSFELD, 1986). All cases of displaced abomasum in this experiment were corrected by the toggle-pin suture technique (TPS; BARTLETT et al., 1995;

ZADNIK et al., 2011) on the same day of diagnosis or the next day. TPS is a procedure less invasive, less expensive, and faster than the traditional surgical abomasopexy or omentopexy via laparotomy resolution for DA, with no differences in return to normal milk production, return to normal feed intake, mortality, culling rate, tissue reaction at the surgical site, or redisplacement (KELTON et al., 1988). The TPS procedure has high success rates of up to 95% (ZADNIK et al., 2011). As graphed in Figure 7A, there was a fast and strong recovery in rumination time after treatment (“Day 2”), showing how fast cows recover after the toggle-pin procedure. As exposed by Raizman and Santos (2002), milk production was affected in cows that developed DA. A decrement in milk production was observed on the days before diagnosis until reaching its nadir on the day DA was diagnosed. As a consequence of treatment, milk production started to increase the day after DA was resolved. However, it has been reported that affected cows reach the production of the control healthy animals only 120 d after the correction of DA (BARTLETT et al., 1995).

6.1.5. *Indigestion*

Indigestion was disruptive to rumination, activity, and milk yield in the present study, as has been shown before (STANGAFERRO et al., 2016c). Although rumination and activity had patterns similar to those in Stangaferro’s work around diagnosis of indigestion, sensitivity of the system to flag sick cows was lower in the current study. This may be related to the cut-off point of HI used, the severity of the disease, and the diagnosis criterion in the two experiments. In example, one cow not flagged by the system in the current study showed a HI of 81 AU/d on the day of diagnosis, a value accepted as an alert in Stangaferro’s work. In both studies, the severity of the disorder was not established, but it can be thought that cows not flagged had a less severe episode of the disorder. In addition, diagnosis of indigestion is not as well established as other disorders leading to differences in the criterion for diagnosis. Moreover, among the cows with indigestion not flagged by the system in our experiment, one cow was diagnosed on the day of calving, and this could have been the reason why the system did not mark the drop in rumination as an alert, which happened with MLF cases very close or in the day of parturition. Additionally, the low number of cows with indigestion may have been the main contributor to the sensitivity obtained. Although there were no statistical differences for activity between healthy and sick cows in the current experiment, a tendency for animals

with indigestion to be less active was observed. Activity for sick animals showed a decrement beginning three days before diagnosis. Edwards et al. (2004) showed how cows with digestive disorders had lower activity than healthy cows from 2 d preceding diagnosis. Moreover, Stangaferro et al. (2016c) found that cows with indigestion were less active than control cows. Milk production was significantly lower in animals with indigestion than in control cows from 6 d before diagnosis, as has already been shown by Edwards et al. (2004).

6.1.6. Mastitis

Clinical mastitis, which is defined and characterized by udder inflammation and milk changes, ranges from light to severe cases that may be accompanied by systemic signs of illness as depressed attitude, anorexia, and fever (FUENZALIDA et al., 2015; NASH et al., 2002; WENZ; GARRY; BARRINGTON, 2006). Soriani et al. (2012) stated that one of the factors that reduces rumination time is inflammation, based on work from Borderas et al. (2008), who observed a reduction in rumination time in calves challenged with a low dose of lipopolysaccharides (LPS). Their results that showed that cows with decreased rumination time in early lactation were characterized by a greater increase of positive acute phase proteins after calving. Additionally, Fitzpatrick et al. (2013) found that cows spent less time ruminating in the hours following LPS infusion for induction of clinical mastitis. Our data support the notion that inflammation reduces rumination, since DRT of cows with mastitis was lower around diagnosis compared to healthy animals, as has already shown previously (SIIVONEN et al., 2011; STANGAFERRO et al., 2016b). Rumination for the cows with mastitis not flagged by the system (group SickRA_{No}) was lower than for cows in the Healthy group and similar to the cows with mastitis flagged by the system (group SickRA_{Yes}). However, DRT for the animals belonging to the SickRA_{Yes} group was lower than SickRA_{No} on the day of diagnosis. This difference on “Day 0” is possibly related to the severity of the disorder in both groups. Probably, although no statistical differences were found for rumination on the days preceding the diagnosis, the values for rumination and activity for the SickRA_{No} group were not low enough to “pull the trigger” to activate the alerts (DRT<200, Neg3dRV, and HI<80) as done for the SickRA_{Yes} group. It could be speculated that the severity of mastitis may be related to the degree of rumination change among groups. Stangaferro et al. (2015a) showed that rumination in cows with mastitis caused by *E. coli* was more negatively affected than in cows

with mastitis caused by *S. aureus*. This is supported by the notion that intramammary infections caused by *E. coli* are characterized by a severe inflammatory response whereas the majority of *S. aureus* infections cause light responses or go clinically unnoticed (SCHUKKEN et al., 2011). Activity was not affected by the occurrence of mastitis. In opposition, it had already been shown that cows with mastitis were less active than healthy cows (STANGAFERRO et al., 2016b). However, in the work of Stangaferro et al. (2016b), half of the cows that developed mastitis caused by gram-positive pathogens and *S. aureus* had activity patterns no different to healthy cows; in fact, these animals were not flagged as sick by the system. Possibly, most of the mastitis cases in our experiment were caused by this kind of pathogens and is the reason why activity was not affected. Interestingly, the effect of mastitis on milk production was not different between the SickRA_{no} and the SickRA_{yes} group, showing that milk yield was affected in both groups at the same level. This may indicate that the reduction in milk production for cows with mastitis was more a consequence of the direct effect of inflammation on the mammary gland than an overall effect of disease on cow health as happened for the other diseases in study (AKERS; NICKERSON, 2011). Based on rumination, activity and milk production data, in which almost no differences between SickRA_{no} and SickRA_{yes} were found, we could assume that the most of mastitis cases had a similar range of severity. However, some cows in the SickRA_{yes} group showed a lower DRT on the day of diagnosis that may be related to a more severe case of mastitis.

6.1.7. Lameness

It has been shown that rumination, activity, and milk production are affected in lame cows (HERTEM et al., 2011; MIGUEL-PACHECO et al., 2014). However, Thorup et al. (2016) found that these animals had a more variable feeding behavior (fewer feeding bouts with higher intakes, faster eating, and less time spent feeding) when compared to healthy cows, but no differences in rumination time and milk yield were observed by these authors. Thus, lame cows eat for less time and quicker than their healthy herd mates (GALINDO; BROOM, 2002; THORUP et al., 2016), but it seems to be that the feed intake is not affected (GONZÁLEZ et al., 2008; HERTEM et al., 2011). However, one work did not find changes in drinking, grazing, and rumination time and how these behaviors fluctuated throughout the day (WALKER et al., 2008). Moreover, a study suggested that healthy cows increased their daily

rumination time with increasing daily feed intake, whereas lame cows decreased their rumination time with increasing feed intake (THORUP et al., 2016). The results of the present experiment showed that rumination was strongly affected by the occurrence of lameness. The cows diagnosed as lame by the farm personnel and flagged by the system (SickRAyes group) showed a strong decrement in DRT as early as 5 d before the day of diagnosis of lameness. However, cows from the SickRAno group (lame cows but not flagged by the system) had a rumination pattern very similar to healthy cows. It could be related to the degree of severity of lameness. Or possibly, as was stated by some authors, the idea that cows, as being descendants from ranging wild cattle that were prone to predator attack, masked any signs of pain and its implied weakness (HERTEM et al., 2011; PHILLIPS, 2002). Activity was also altered in lame cows in our study, supporting the results published by HERTEM et al. (2011). However, activity was lower from 6 d before diagnosis in Hertem's study, differing from our results that showed a decrease in activity only one day before the day of diagnosis. This may be due the different severity and causes of lameness in both studies, besides the sample size used in our study could not been big enough to make assumptions and comparisons with a work with a much bigger sample size. Milk production was also strongly affected by lameness in the current study. Data indicated that milk yield was lower from at least 3 d before diagnosis. This is in accordance with Hertem et al. (2011) that found that lame cows produced less milk than healthy cows from days and even weeks before the diagnosis of this affection. These authors suggested that lameness may be a long-term disease that is subclinical until signs are visible, based on these findings. It cannot be assumed the same because we do not have enough data to support this notion, since the average DIM at diagnosis in our study was 9, while Hertem's average DIM at diagnosis was 166. Milk yield data support the idea that severity was related to cows flagging by the system. Cows from the SickRAno group produce more milk than cows from the SickRAyes group, and their production was not different from that of healthy cows. Based on rumination, activity, and milk production patterns observed in the different groups in study, we can hypothesize that the level of severity and possibly the cause or type of foot/leg affection may be related to the degree of alteration in these patterns. Cows from the SickRAyes group possibly suffered from more severe affections than cows from the SickRAno group, which had rumination, activity, and milk yield patterns no different to healthy cows. Thorup et al. (2016) has stated that lameness problems would require measurement of feeding as well as rumination to be noticed automatically, however, our results indicate that the used of rumination may be used as an early indicator for lame cows, as well as for milk production. It

is important to note that more studies with larger sample size would be important to support our findings.

6.1.8. *Other diseases*

“Other diseases” included respiratory problems, udder sore, injuries, lameness, vaginitis, vaginal laceration, rupture of uterus, septicemia, abscesses, intestine torsion, kidney failure, and fatty liver. Not all of these affections have the same severity and health compromise, and sorting them as being part of the same group of health disorders could lead to misinterpretation. However, they were grouped as “other diseases” because their incidence was too low for being studied separately, except for lameness that was studied for rumination, activity, and milk yield patterns but not for system capacity to detect sick cows. DRT time was affected in animals diagnosed with “other diseases”. Based on rumination time, it could be assumed that cows belonging to the SickRAYes group suffered from more severe disorders and had their health status more compromised because they showed the lowest DRT values, lower than those observed for cows from the SickRAno and the Healthy group, from 6 d before diagnosis. Cows from the SickRAno group had also lower rumination than healthy animals but from only 3 d before the day of diagnosis, and their DRT did not reach values as low as in SickRAYes group cows. Animals belonging to the SickRAYes group suffered mostly from severe health disorders such as fatty liver, serious injury, respiratory affections, rupture of uterus, septicemia, toxic mastitis, and kidney failure. On the other hand, severe disorders diagnosed in the SickRAno group were less common, such as injury, intestine torsion, and fatty liver. Vaginitis, udder sore, and less severe injuries occurred in both groups. Activity was also affected in cows from the SickRAYes group. The decrease in activity occurred later compared to rumination; activity was lower for the SickRAYes group from 3 d before diagnosis compared to the healthy cows but, interestingly, lower than the SickRAno group animals from 5 d preceding diagnosis. This is a consequence of a slightly increase in activity observed for the SickRAno group on the days preceding diagnosis, however, there is not a clear explanation for this rise. Milk production was also affected in animals belonging to the SickRAYes group, but not in animals from the SickRAno group. Animals from the SickRAYes group produced on average 13 and 12 kg/d less milk than animals from the Healthy and SickRAno group, respectively. It seems to be that the level of severity and health compromise

of the diseases are related to the patterns of rumination, activity, and milk yield alteration. It could be thought that health problems such as vaginitis, non-severe injuries, and udder sore affect rumination less than severe disorders such as fatty liver, kidney failure, or septicemia. Supporting this, milk production was strongly affected by these severe disorders but not by the less concerning health problems in previous works (BOBE et al., 2004; FOURICHON et al., 1999). In addition, cows that suffered from ketosis, metritis, RP, and mastitis from 7 d preceding to 4 d after the diagnosis of “other diseases” had their rumination pattern more affected. Thus, it is likely that non-severe “other diseases”, such as vaginitis, have been detected because another more concerning disorder, like metritis, was also present.

6.1.9. Regrouping

Regrouping strategy potentially has an influence on cow productivity, welfare, and health, because it can have a significant impact on the feeding behavior and feed intake of dairy cattle (GRANT; ALBRIGHT, 2001). Indeed, cows that experience abrupt environmental and social changes during the periparturient period often exhibit aberrant feeding behavior and are more susceptible to metabolic disorders (BAZELEY; PINSENT, 1984). In this regard, a decrease in feed intake can lead to reduced rumination time. The results indicate that rumination time suffered a reduction on the day and days after cow’s regrouping, with different degree for *parous* and *primiparous* cows. This had been previously shown by Schirmann et al. (2011), who found that rumination time decreased on the same day and one day after regrouping, returning to previous values 2 days after the animals were moved. Interestingly, DRT was more affected in *parous* than in *primiparous* in the current study with a greater and more prolonged reduction in rumination observed in the first ones. A more severe decrement in rumination in first lactation cows would be expected because it was stated that *primiparous* are more sensible to social stress (BACH et al., 2006) and abrupt environmental changes after calving than *parous* (SORIANI; TREVISI; CALAMARI, 2012). In the current study, the animals were not separated by parity, and *primiparous* and *parous* were housed in the same group during the first 30 DIM, and it has been documented that separating *primiparous* cows from *parous* cows after calving positively affected production and health of *primiparous* (ØSTERGAARD; THOMSEN; BUROW, 2010). Probably, the drop in rumination in *primiparous* was attenuated because these animals were moved from a pen where they were

mixed with *parous* cows to a new pen with only *primiparous*. Possibly, the new environment was less challenging and this minimized the negative effect of regrouping on rumination with an attenuation of the decrease observed and a fast recovery after regrouping. In the case of *parous*, an important drop of approximately 70 min/d on the same day the cows were moved to the new pen was observed, and these lower DRT values were maintained on the following days. When cows are grouped or regrouped, social behavior and hierarchy can modify eating behavior and DMI (GRANT, 1991). Mixing cows in different stages of lactation may lead to reductions in DMI in cows with less DIM because these cows could be rationally less active in eating compared to cows in more advanced phases of lactation (NIKKHAH; KOWSAR, 2012). Cows recently introduced to a new group might be vulnerable to reductions in DMI because of the competition imposed by the group. Dominant cows with high social rank and those that stay longer in the group demand priority at feeding and may spend more time ruminating after rapidly consuming their meals before the new herdmates (GRANT, 1991; NIKKHAH; KOWSAR, 2012), which are displaced more often from the feeding area (SCHIRMANN et al., 2011b). Moreover, by evolutionary definition, rumination occurs when ruminants are superior in psychological status and feel socially secure (NIKKHAH; KOWSAR, 2012); thus, social stress can be linked to a decrease in rumination time (BRISTOW; HOLMES, 2007). So, moving *parous* cows to a new group may have challenged them to compete for hierarchy and dominance, which may have led to shorter DRT in the present study. This probably did not happen with *primiparous* cows, since social dominance is strongly related to age, body size, and seniority in the herd (ARAVE et al., 1976; DICKSON et al., 1970), and the hierarchical competition was less important between first lactation cows. However, Hasegawa et al. (1997) showed that the dominance decreased in *primiparous* cows moved to a new pen. Activity showed an increase on the day the animals were moved to the new group. Contrasting what happened with rumination, the degree of changes in activity on the day before and the day the animals were moved was similar for *primiparous* and *parous*. Activity was statistically different on “Day 0” for *parous*, but it was not different in the case of *primiparous*. This occurred because control *primiparous* cows had high activity levels during the entire period, for some reason cannot be explained. When animals are introduced to a new group they compete for a new social niche and hierarchy, as was stated previously, and this involves social stress. It has been shown that cows modify their lying down behavior, increasing the number of lying bouts, possibly, as a sign of restlessness (HASEGAWA et al., 1997; VON KEYSERLINGK; OLENICK; WEARY, 2008). Previous studies have shown that milk yield is reduced after moving animals, especially in those with lower dominance rank

(ARAVE et al., 1976; HASEGAWA et al., 1997; VON KEYSERLINGK; OLENICK; WEARY, 2008). However, the cows in our experiment, neither *primiparous* nor *parous*, showed any reduction in milk production after they were moved to another group, as was shown by other authors (CLARK; RICKETTS; KRAUSE, 1977; GRANT; ALBRIGHT, 2001). This observation was not expected, because cows that ruminated longer had greater milk production than cows with shorter DRT (SORIANI; TREVISI; CALAMARI, 2012). Besides, these authors also observed a significant correlation between milk yield and the average DRT of the previous 3 days. As rumination time suffered minor reductions on the day *primiparous* cows were moved to another group in our study, the magnitude of the reduction in DRT was not big enough to determine a negative drop in milk production. It is important to note that *primiparous* were separated from *parous* after regrouping cows in our trial, and this may carry benefits, as has been shown in one study, in which heifers were separated from older cows and positive feeding behavioral changes, such as increased eating time, number of meal per day and increased DMI. (GRANT; ALBRIGHT, 2001). *Parous* animals had a reduction in DRT after they were moved, likely due to the distress of regrouping, but probably with no great diminutions in DMI that could lead to decreased milk yield.

6.1.10. Sub-clinical ketosis

The incidence of subclinical ketosis in our study (14.5% (86/593)) was within the lower limit of the values found in the literature (KAUFMAN et al., 2016; MCART et al., 2012; SCHIRMANN et al., 2016; SUTHAR et al., 2013). This low incidence may be due to the adopted schedule in our experiment, which may result in some inaccuracies in the diagnosis of SCK and might not be sufficient to identify sudden changes in concentrations of BHBA. This is because only 2 blood samples were used in comparison with the cited studies in which cows were sampled at least three times. In addition, lower BHBA concentration were related to cows calving during the second part of the year when compared to cows calving during the first months, winter being a risk for SCK and clinical ketosis (SUTHAR et al., 2013; VANHOLDER et al., 2015). It was stated that SCK can result in lower milk production and increased risk of other diseases (SUTHAR et al., 2013); indeed, *primiparous* and *parous* cows without SCK had lower incidence of disease ($P < 0.001$) and higher daily milk production mean during the first 30 DIM ($P = 0.01$) than cows with SCK in the current experiment. The risk of

SCK increases with the lactation number, milk production and BCS losses (KAUFMAN et al., 2016). Regarding to parity, the incidence of SCK is higher for *parous* than for *primiparous* cows (VANHOLDER et al., 2015), as happened with the animals in our trial ($P < 0.001$). Moreover, alteration in feeding behavior and reduction in DMI are risk factors for the development of SCK (GOLDHAWK et al., 2009). Our results support the notion that cows with SCK have lower rumination time than healthy cows after calving (LIBOREIRO et al., 2015; PAUDYAL et al., 2016; SORIANI; TREVISI; CALAMARI, 2012) and that the risk of developing SCK decreases in relationship with higher rumination time in the week previous to parturition (KAUFMAN et al., 2016). Interestingly, cows diagnosed with SCK but not clinically sick from any disease (SCKonly) had higher rumination time compared to control cows during the first 14 DIM, except on the day after calving when they ruminated less than their healthy herd-mates. This higher DRT found in these animals may be related to their higher milk production compared to healthy cows, since increased milk yield after calving is expected to be associated with increased DMI (NRC, 2001). The lowest postpartum DRT values were found for cows with SCK and diagnosed as sick by the farm personnel, suggesting that the decreased rumination may be more related to clinical diseases than to the SCK itself. Liboreiro et al. (2015) showed that cows with SCK had lower DRT and activity than healthy cows after calving. However, these authors did not specify if the cows had suffered from any other health disorder, which may have had a further effect on rumination time. Moreover, Kaufman et al. (2016) found that *parous* cows with only SCK tended to ruminate less during the week before and the week after parturition. Activity was also affected in cows with SCK, as had already been shown by other authors (EDWARDS et al., 2004; GOLDHAWK et al., 2009; LIBOREIRO et al., 2015). The cows in our experiment had lower activity as early as one day after calving, and this reduction in activity was related to the occurrence of a clinical disease because cows from the SCKdisease group showed the lowest values. Daily milk production during the first 30 DIM was lower in cows with SCK for *primiparous* and *parous* and tended to be lower during the first 80 DIM in *parous* when compared to healthy animals. However, it seems to be that these lower values are more related to clinical events affecting the cows than with SCK alone, as seen with DRT. The cows from the SCKonly group had higher daily milk yield than healthy cows. The association between milk production and SCK is ambiguous, as both positive and negative associations have been reported (OSPINA et al., 2010b; RABOISSON; MOUNIÉ; MAIGNÉ, 2014; VANHOLDER et al., 2015). The time of diagnosis for SCK seems to be related to the effect of this disease on milk production. Duffield et al. (2009) found higher milk production at the second and third dairy herd improvement

(DHI) tests and greater projected milk yield in cows with SCK diagnosed at week 2 after calving than that for healthy cows. However, the results in this study were negative for cows diagnosed with SCK at week 1 of postpartum. Ospina et al. (2010) found a differential effect of SCK over milk production among parities, showing a positive effect in heifers and negative in cows. However, this phenomenon was not observed in our experiment. Moreover, some studies have identified previous lactation milk yield (FLEISCHER et al., 2001) and length (VANHOLDER et al., 2015) as risk factors for SCK and clinical ketosis. This could be explained by the theory that higher producing cows are at greater risk of ketosis. The degree of severity of SCK may be involved in its effect on milk production. It is likely that cows with moderate SCK are able to produce more milk than cows without SCK (DUFFIELD et al., 2009). Supporting this, when the cows from our study were divided according to the maximum BHBA concentration levels ($>$ or $<$ 1300 $\mu\text{mol/L}$), milk production of healthy cows was not different from milk production of the animals with lower BHBA concentration ($<$ 1,300 $\mu\text{mol/L}$). However, cows with higher BHBA levels ($>$ 1,300 $\mu\text{mol/L}$) produced less than healthy cows and those with BHBA lower than 1,300 $\mu\text{mol/L}$. When the same analysis was performed including only cows that had SCK but did not have any clinical disorder (SCKonly), cows with BHBA $<$ 1300 $\mu\text{mol/L}$ had higher milk production than healthy and cows with higher BHBA concentrations. For the cows with SCK and clinical disease (SCKdisease), all cows with SCK had lower milk production than healthy animals. However, milk yield in the cows with BHBA $<$ 1,300 $\mu\text{mol/L}$ showed an earlier recovery when compared with those cows with higher BHBA concentrations. We could speculate that cows can utilize ketone bodies to attend the high milk production demands. Although, as BHBA concentrations get higher the animal cannot deal with them, leading to negative effects on milk yield. Furthermore, our results showed that rumination time, activity and milk production are affected by the occurrence of SCK, but the patterns and degree of affection are related to the presence of clinical disorders in conjunction with SCK. SCKdisease animals (SCKdisease) showed the lowest DRT, activity, and milk yield levels. However, when cows did not suffer any clinical affection (SCKonly), DRT and milk production were higher than in healthy cows, suggesting that moderate SCK may be beneficial for milk production.

6.1.11. Sub-clinical hypocalcemia

After calving, cows require about 1 to 2 days to maximize calcium inflow from the gastrointestinal tract and from bones to the mammary gland. Consequently, most cows experience some degree of hypocalcemia during the first days of postpartum, however, calcium concentration returns to normal within 2 to 3 days (HORST et al., 1994). In our study, 77% of the animals had calcium concentration below 8.55 mg/dL, the chosen cut-off point to determine if the animal has or does not have subclinical hypocalcemia, being the same between *primiparous* (76.8%) and *parous* (78.3%) ($P = 0.66$). Although these results are above the prevalence of sub-clinical hypocalcemia of 25% and 47% for *primiparous* and *parous*, respectively, reported by Reinhardt et al. (2011), Martinez et al. (2012) had similar prevalence to ours, reporting 65.5% of the cows with hypocalcemia using a similar cut-off point. The significant difference between our prevalence and that one reported by Reinhardt et al. (2011) might be influenced by the fact that the cows in the current study were originated from only one herd, and it is known that the prevalence of postpartum problems, such as SCHC, varies among dairies. In our study, the DRT pattern in cows with SCHC showed interesting differences among *primiparous* and *parous*. *Primiparous* diagnosed with SCHC but without any clinical disorder (group SCHConly) ruminated less than healthy *primiparous* 6 days after calving only and tended to do the same on “Day -1” and “Day 7”. In the case of *parous*, cows with SCHC without a positive diagnosis for clinical events ruminated less than healthy cows during the 2 first days after calving. However, these animals achieved the DRT level of healthy controls as early as “Day 4”, also showing a positive numerical difference during the following days. Differently, *primiparous* from the group SCHConly matched the healthy DRT levels later than *parous*, on “Day 11”. Liboreiro et al. (2015) reported that cows with SCHC ruminated less than healthy cows on the day of calving and three days after with small differences in DRT mean, similar to what happened with *parous* in our experiment. In addition, Hansen et al. (2003) showed that induced SCHC (by Na₂EDTA) depressed feed intake and rumination of dairy cows. Contrary to these findings, Jawor et al. (2012) had a tendency for greater intake after calving in cows with SCHC (Ca <7.2 mg/dL) when milk production was increased, and this is that could have happened to *parous* after day 4 of postpartum in our study, which had an increased milk production also. It is important to note that in these referenced studies the presence and incidence of health disorders that could influenced rumination and intake are not fully explained. Our results for cows with only SCHC

and no clinical disorders suggest that rumination in *parous* is more affected than in *primiparous* during the first days of postpartum, and this could be related to the severity of hypocalcemia because the calcium concentration mean in animals with SCHC was lower for *parous* (7.29 mg/dL) than for *primiparous* (8.03 mg/dL) ($P < 0.001$). Regarding the animals with SCHC and clinical disorders (group SCHCdisease), it seems to be that *parous* had DRT values more affected than *primiparous*. DRT in *parous* with clinical problems was lower during the last week of the prepartum period, while *primiparous* showed differences with healthy and cows from the SCHConly group on the day preceding calving only. Additionally, the degree of affection of DRT was greater in *parous* from the SCHCdisease group because the drop in rumination on the day of calving was deeper in these animals when compared to *primiparous*. Besides this, no differences on the day of calving were found in *primiparous* animals. These findings could be related to the type and severity of clinical problems that affected each group. *Primiparous* cows suffered mostly from metritis, few mastitis cases, and only one case of milk fever, while *parous* had more severe affections such as MLF, clinical ketosis, and DA that may have greater negative effects on DRT (SORIANI; TREVISI; CALAMARI, 2012; STANGAFERRO et al., 2016c). Cows with SCHC were less active after calving than healthy cows in our study. The negative effect on activity was greater in cows suffering from clinical disorders than in cows with SCHC only, especially in *parous*, in which activity was stronger and longer affected, as happened with rumination, and this could be related to the type and severity of the clinical affections in these animals. Cows with SCHC but without clinical disorders had lower activity than healthy cows one day before and two days after calving in *primiparous* and *parous* animals, respectively. These findings go hand in hand with those reported by Jawor et al. (2012), in which cows with SCHC made fewer visits to the water and feeding bin during the two weeks after calving and spent less time standing the day after parturition. The lower activity in cows with SCHC after calving may be explained by muscle weakness due to calcium deficiency, since low plasma calcium concentrations can affect contraction of smooth and skeletal muscle (MURRAY et al., 2008). Interestingly, SCHConly *primiparous* cows were more active than healthy cows the day before calving. This agrees with the longer standing time in cows with SCHC the day preceding parturition in the Jawor et al. (2012) study. As a speculation, the prolonged labor generally found in *primiparous* (ATASHI et al., 2012) may be even longer due to hypocalcemia, since the contraction of calcium-dependent smooth muscle is compromised, leading to longer second and third stage labor and prolonged discomfort (MURRAY et al., 2008), which could explain this finding. The lower activity during the first days after calving showed by *parous* from the SCHConly group

could be related to the higher milk production of these animals. Animals with increased milk yield have greater udder fill before parturition, leading to more discomfort while resting that would result in longer standing times (JAWOR et al., 2012). In our study, *parous* cows with only SCHC produced more milk than healthy cows and those with SCHC and clinical disorders during the first days of postpartum, supporting the notion that cows with SCHC produced more milk than healthy cows during the first 4 weeks of lactation, as reported by Jawor et al. (2012). Contrary to Martinez et al. (2012), who showed a greater milk production (numerical but not statistical) in *primiparous* hypocalcemic cows, *primiparous* animals with only SCHC in our study did not produce differently to healthy animals. Cows with clinical disorders and SCHC produced less milk than healthy cows and those with only SCHC, regardless of parity, in our experiment. Based on the findings of this study, the presence of Ca concentrations below 8.55 mg/dL alone does not appear to be a risk factor for decreased milk yield. Further, it could bring beneficial milk production outcomes in *parous* cows. It is important to note that cows of parity 3 or higher received sub-cutaneous and oral calcium supplementation at calving that could have had effects on the studied parameters. This preventive treatment may have helped the cows utilizing more endogenous calcium reserves to avoid clinical milk fever, maintain high milk yield, or even improve their milk production. However, the reported effects of calcium supplementations on milk production are confounding. Martinez et al. (2016) found that the response of milk production during the first 30 DIM to Ca supplementation is conditional to parity and production potential of the cows because it was positive in *parous* cows with greater production potential, negative in *parous* with lower production potential, and null in *primiparous*. In one study, no differences between cows supplemented with Ca and control animals were found for milk yield at the first DHI test (STEVENSON; WILLIAMSON; HANLON, 1999). Amanlou et al. (2016) showed no effect from different doses and timing of Ca supplementation at and after calving on milk yield. Contrary to what happened when cows had only SCHC, milk production decreased when this last condition was accompanied by clinical events. Hypocalcemia is referred to as a risk factor for the development of other subclinical and clinical disorders (MARTINEZ et al., 2012; MURRAY et al., 2008) related to lower rumination, activity, and milk production. In our experiment, 17% of the cows with hypocalcemia had SCK, while only 6% of the animals with Ca concentration greater than 8.55 mg/dL had this condition. Almost all cows with metritis had subclinical hypocalcemia in the work of Martinez et al. (2012). In our study, 84.9%, 100%, 83.3%, 92.3% and 94.4% of the cows with metritis, MLF, ketosis, DA, and mastitis had SCHC, respectively, and the incidence of these diseases was higher in cows with hypocalcemia

than in those without hypocalcemia. It seems to be that the occurrence of clinical disorders is related to the degree of severity of SCHC because the Ca concentration mean for cows from the SCHCdisease group (7.48 mg/dL) was lower than for cows belonging to the SCHConly group (7.77 mg/dL) ($P < 0.001$). Furthermore, the effects of SCHC on DRT, activity, and milk productions were conditioned by parity and the presence of clinical disorders. DRT and activity were strongly affected in cows with SCHC and clinical events but almost not altered by SCHC alone, and the degree of the affect was greater in *parous* than in *primiparous* animals. Milk production was increased in *parous* cows with only SCHC but unaltered in *primiparous*. However, animals with SCHC and clinical diseases had lower milk production, regardless of parity.

6.1.12. Calving problems: dystocia, twins, stillbirth

6.1.12.1. Dystocia

Dystocic calving, those that require assistance, have generally labor, production, reproduction, welfare, and economic implications (MEE, 2008). “Calving difficulty” scales are used to quantify the degree of assistance needed at calving. In the present work, the scale used for “calving difficult” adopted by the farm ranges from 1 to 5, with 1 as normal calving without any help, 2 for calving with some and easy assistance, 3 for considerable assistance, 4 for calving with more than 1 person and strong help, and 5 for extreme difficult with C-section surgery needed. It is important to note that the determination of calving difficulty has a high degree of subjectivity and may vary among people who determine it. As the calving difficulty score was given by the farm personnel for each cow in our trial, we defined dystocia for cows with a calving difficult of 3 or higher. For the Control group, only cows with calving difficulty of 1 were chosen. Although around 40% to 50% of calving need some assistance in dairy farms (LOMBARD et al., 2007), the incidence of dystocia may vary from 5% to 23%, and it is higher for *primiparous* than *parous* (JOHANSON; BERGER, 2003; MEE, 2008). In our experiment, 7.5% of the cows had dystocia with similar results in *primiparous* (7.9%) and *parous* (7.1%) ($P = 0.72$). Dystocia is a risk factor for stillbirth, retained placenta, metritis, mastitis, and metabolic problems (DUBUC et al., 2010; LOMBARD et al., 2007). Among the cows that had

dystocia in our study, 68% suffered from clinical problems, mostly metritis, ketosis, and vaginitis during the first 2 weeks of postpartum. However, half of the cows that delivered stillborn calves had calving difficulty of 1 or 2, not being considered as dystocia. On the other hand, only one cow that delivered twins had calving difficult of 4, and the remaining had 1 and 2, not being considered as dystocia neither. These findings, stillborn and twins from non-distotic cows, may be related to the small size of the twins and stillborn calves (JOHANSON; BERGER, 2003) reported in this farm. Dystocia was ranked by cattle practitioners as one of the most painful conditions for cows (HUXLEY; WHAY, 2006), and based on the notion that cows ruminate while they are at ease (BRISTOW; HOLMES, 2007), we could assume that dystocia can affect rumination negatively. In fact, Paudyal et al. (2016) found that cows with dystocia had lower DRT pre and postpartum, depending on the season (hot and cool). Additionally, DMI and feeding time were reduced during the day before calving in cows with dystocia compared to cows with normal calving (PROUDFOOT; HUZZEY; VON KEYSERLINGK, 2009). Moreover, cows with difficult calving began to decrease their intake compared with cows with eutocia by 11 h before parturition in this last referenced work. However, these authors found no differences in DMI on the first two days after calving. In our experiment, cows that had dystocia ruminated less than cows with normal calving from the day before parturition until 12 days after. In figure 10D, it can be seen that there was no difference in rumination the day before calving between cows that had dystocia but did not get sick (Dystociadisease) and cows without dystocia, in contrast to Proudfoot et al. (2009) results, who also used cows without any other health disorder in their trial. Our results for prepartum DRT does not converge with those found by Paudyal et al. (2016) for cows during the hot season, however, these authors used only 3 cows and did not specify if they had other concomitant disorder. On the other hand, our results agree with Wehrend et al. (2006) that found no differences in feeding behavior. We cannot explain the reduction in DRT in cows with dystocia 4 days before calving, but it seems not to be related with dystocia itself. Our dystocic cows ruminated less than eutocic cows during the first 12 days of the postpartum period, in partial agreement with Paudyal et al. (2016) and in disagree with Proudfoot et al. (2009). Cows that had dystocia but had no other disorders ruminated less than the control cows during the first week of postpartum, and surprisingly, they had no differences with cows with dystocia and other health problems after calving. The only difference between these two groups of cows occurred the day before calving because cows that got sick after calving ruminated less than those cows with dystocia that did not develop any disease. These findings suggest that the alterations in rumination time are related with the occurrence of dystocia itself. However, we

can observe that cows with disease had numerically lower rumination time and that the differences to the control cows lasted longer (14 DIM) than the differences among the control and cows with dystocia and no other disorder (8 DIM). The differences between our results of DRT and those of DMI published by Proudfoot et al. (2009) could be related to sample size, diets, and managements of both experiments, and/or by the notion that the reduction of 24% of DMI found by these authors the day before calving, may not be enough to cause important reductions in DRT (CLÉMENT et al., 2014; HASEGAWA et al., 1997). Cows that had dystocia were less active during 2 weeks after calving, contrary to the findings of Proudfoot et al. (2009), who found no differences in standing time between cows with and without dystocia. Moreover, contrary to our findings during the prepartum period, these same authors found that cows with dystocia showed transitions from standing to lying positions more often than cows without dystocia. Interestingly, cows that had dystocia and did not get sick after calving showed no differences with cows with dystocia and some health disorder. Even so, cows from the Dystociaonly group were less active than control cows during the first days of postpartum. It should be noted that even though there were no statistical differences among these groups of cows on the day of calving, cows with dystocia without clinical health disorders had numerically higher rumination with greater data dispersion. This could be related to the fact that cows with dystocia are submitted to discomfort and restlessness because of some factors, such as the size and malposition of the calf, in addition to pain (BARRIER et al., 2012). The lowest rumination and activity levels during the prepartum and on the day of calving found in animals from the Dystociadisease group may be not only related with the clinical affections they developed after calving but with subclinical ketosis also. Twenty two percent (22%) of these cows had BHBA concentrations greater than 1000 $\mu\text{mol/L}$ during the days following parturition, and it has been shown that cows diagnosed with SCK during the first week of lactation have lower dry matter intake, fewer visits to the feeder, and spent less time at the feeder than healthy animals (GOLDHAWK et al., 2009). Milk production is the most affected parameter (over health and reproduction) by dystocia in dairy cows (MEE, 2008). Cows with dystocia have lower milk production than cows with normal calving, and production losses are greatest in high yielding cows in early lactation (LOMBARD et al., 2007; RAJALA; GRÖHN, 1998b). However, some authors found no effects of dystocia on milk yield (FOURICHON et al., 1999). Cows with dystocia had lower daily milk production mean during the first 30 DIM ($P < 0.001$) and 80 DIM ($P = 0.05$) than cows without dystocia in our study. Regarding the first 14 DIM, differences among cows with normal calving and cows with dystocia appeared as early as 3 DIM. Although cows from the Dystociaonly group produced statistically less milk

than the control cows only on the days 6 and 12 after calving, the numerical difference mean was ~4 kg/d approximately from 3 to 14 DIM. The same numerical difference (~4 kg/d) was observed between the cows from the Dystociaonly and Dystociadisease groups, but milk production was statistically different on 8 and 11 DIM only between these groups. Cows that had normal calving produced statistically more milk than cows with dystocia and some disease from 3 to 14 DIM with a mean difference of ~8 kg/d. This means that the presence of disease doubles the milk losses in cows that have dystocia, however, the occurrence of a difficult calving merely makes the cow produce on average 4 kg/d less milk than a cow with a normal calving.

6.1.12.2. Twins

Seventeen cows delivered twins in our experiment (2.8%). As was expected, the incidence of twinning was greater in *parous* (4.3%) than in *primiparous* (1%) ($P = 0.02$) (BICALHO et al., 2007b). Although the occurrence of twinning is a risk factor for dystocia (MEE, 2008) only one cow that delivered twins was considered with dystocia in our study (calving difficult of 4). This could be related to the small size of twin calves reported in the farm (MEE, 2004) and with the prepartum and maternity pen management or possibly with an underestimation of calving difficulty by the farm personnel. As was mentioned before, farm workers were responsible to assign the calving difficulty score, and this has a great subjective factor and may be the reason of the low incidence of dystocia among twin births and overall dystocia incidence, compared to those values found in the literature (JOHANSON; BERGER, 2003; LOMBARD et al., 2006; MEE, 2008). However, half of the cows that delivered twins needed some assistance at calving (calving difficult of 2). As only 2 cows delivering twins were not diagnosed as sick after calving, they were excluded for the analysis to analyze rumination, activity, and milk production patterns. Rumination time was strongly affected by the occurrence of twins in our study. Our findings are in accordance with Liboreiro et al. (2015), who found no differences for prepartum, but significant differences after calving in DRT. The unaltered rumination before calving was unexpected because it could be thought that cows carrying twins would have less rumen fill and capacity (BURFEIND et al., 2011), higher incidence of metabolic problems (FRICKE, 2000), and more discomfort (MEE, 2004) that would affect rumination. However, Silva-del-Rio et al., (2010) demonstrated that despite

the fact that cows that delivered twins had reduced energy balance during the prepartum period compared with cows that delivered singletons, only minor differences in DMI were observed during the prepartum period. It is possible that the small number of cows delivering twins in the current experiment was insufficient to detect statistical differences in prepartum DRT between cows delivering twins and singletons. The reduced postpartum DRT among cows that delivered twins compared with cows delivering singletons was somewhat expected because occurrence of twins increases the risk of metritis (GIULIODORI et al., 2013) and metabolic diseases (FRICKE, 2000). Indeed, the incidence of metritis was higher ($P < 0.001$) in cows delivering twins (76%) than in cows delivering singletons (27%) in our study. This is the reason why 70% of the cows delivering twins in our study stayed at the hospital and received antibiotic treatment. Moreover, 29% of the cows that delivered twins were diagnosed as having SCK during the first days of postpartum and tended to be higher than the 14% of the cows with SCK that delivered singletons ($P = 0.08$). Moreover, all of these cows were given some treatment (fluids and drench in combination with antibiotics). Cows delivering twins were less active during the postpartum period than cows delivering singletons, as was stated previously by Liboreiro et al. (2015). It seems to be the reduced postpartum activity of cows that delivered twins may be related with the infectious and metabolic diseases that affected them, as had been suggested by Liboreiro et al. (2016). Our results related to prepartum activity differ with those found by these authors, in that we did not find any statistical difference between cows delivering twins and cows delivering singletons, as they did. In this referenced work, the authors suggested that the reduced activity of cows carrying twins during the prepartum period may indicate discomfort. However, it could be hypothesized that cows with discomfort would switch from stand up to lying down positions more often (PROUDFOOT; HUZZEY; VON KEYSERLINGK, 2009). Moreover, although we did not find statistical differences in prepartum activity, cows carrying twins had numerically greater activity than cows carrying singletons. Milk production was lower in cows that delivered twins compared to cows that delivered singletons in our experiment, as had already shown by some authors (BICALHO et al., 2007b; SYRSTAD, 1977). We speculate that the lower milk production in cows delivering twins in our experiment could be related not only to the occurrence of twinning, but to the health status of the cows, since almost all cows delivering twins got sick after calving in our study. Bicalho et al. (2007b) found that those cows that delivered at least one dead twin had lower milk production than cows delivering live twins, and it is known that the occurrence of stillborn is a risk factor for health problems (BICALHO et al., 2007a).

6.1.12.3. Stillbirth

The incidence of stillbirth in our experiment was 4.4%, which is below values found in several studies (LOMBARD et al., 2006; MEYER et al., 2000, 2001). This lower incidence of stillbirth could be related to the incidence of dystocia found in our study, since dystocia is the greatest risk factor for stillbirth (LOMBARD et al., 2007), as well as in the work done by trained personnel at the maternity pen. It is important to note that the definition of stillbirth was different among our work and those referenced studies. Lombard et al. (2016) and Meyer et al. (2000) included calves that died within 24 and 48 hours after birth in their definition of stillbirth, while only calves born dead were defined as stillborn in our study. It could be argued that some calves that died within 24 to 48 hours of birth would have been defined as stillborn by the others authors, leading to an underestimation of the incidence of stillbirth in our work. However, Bicalho et al. (2008) reported that the incidence of stillbirth by farm ranged from 4% to 14.3%, using data from 20 dairy farms in the United States. Note that the management practices (breeding, genetic selection, husbandry etc.) adopted by individual farms may influence the incidence of stillborn calves (BICALHO et al., 2008). Besides this, our data are not in line with the notion that *primiparous* are in higher risk for stillbirth (BICALHO et al., 2008; LOMBARD et al., 2006; MEYER et al., 2000) because the incidence was numerically higher in *parous* (5.4%) than in *primiparous* (3.1%) ($P = 0.17$). This finding could be related to the lack of difference in the incidence of dystocia between parities. Although it has been determined that *primiparous* are at higher risk for stillbirth than *parous*, severe dystocia in *parous* was more likely to result in stillbirth than severe dystocia in *primiparous* in a previous study (LOMBARD et al., 2006). This may be an effect of fetal malposition as a cause of severe dystocia occurring more frequently in *parous* than a mismatch of fetal and maternal size (LOMBARD et al., 2006). Our data support the idea that the occurrence of dystocia influences stillbirth (MEE, 2008; MEYER et al., 2001) because the risk of stillbirth was 12.8 times higher for calves born from dystotic cows than from eutotic cows. Forty-five percent (45%) of the cows that delivered stillborn had dystocia in our experiment. However, 90% of the cows that had dead calves needed some help (calving difficult = 2), not being considered as dystotic. This could be related with the increased odds of stillbirth by 2.91 in *primiparous* and 4.67 in *parous* in animals with slight calving problems, as found by Meyer et al. (2001). Twinning was related with stillbirth in our study, 17.7% of twin calvings had at least one stillborn calf, while only 3.4% of singletons were born dead. It has been shown that cows delivering stillborn

calves are at higher risk of developing metritis and retained placenta (BARTOLOME et al., 2014; BICALHO et al., 2007a), and this was supported by our data because 48.1% and 11.1% of the cows that delivered stillborn calves suffered from metritis and RP, respectively. And these values were higher than the 27.6% and 2% found for cows delivering live calves, respectively ($P = 0.02$ and $P = 0.008$). Rumination time was affected by the occurrence of stillbirth in our experiment, as had been previously published by Liboreiro et al. (2015). Although these authors suggested that differences in DRT between cows delivering stillborn and live calves may be a consequence of other diseases associated with stillbirth, the cows that delivered stillborn calves but had no disease during postpartum (group Stillbornonly) ruminated less around calving than cows delivering live calves. However, statistical differences between cows from the Stillbornonly group and cows that delivered stillborn calves and got sick (group Stillborndisease) were only observed at 2, 5, and 11 DIM; numerical differences were observed during the first two weeks of postpartum. This possibly means that the negative effect of stillbirth on DRT could be greater in cows that developed disease. The majority of the cows from the Stillborndisease group developed RP, metritis, ketosis, and vaginal lacerations, and our results and results from other authors (LIBOREIRO et al., 2015; STANGAFERRO et al., 2016a, 2016c) revealed that cows suffering from these effects have DRT altered. However, it is possible that cows from the Stillbornonly group could have suffered from vaginitis or uterine/vaginal lesions not perceived by the farm personnel. Activity was lower in cows that delivered stillborn calves than live calves. Our results did not show differences in prepartum activity, however, cows from the Stillbornonly group were numerically more active than control cows during this period. This could be related to the restlessness due to discomfort in cows that deliver stillborns (PROUDFOOT; HUZZEY; VON KEYSERLINGK, 2009). This could be the same reason why cows with stillbirth had lower activity than cows with live calves on the day of calving. The reduced activity after calving seems to be linked with the occurrence of disease, however, cows that delivered stillborn calves but did not develop any disease showed activity levels as low as those found for cows from the Stillborndisease group. Nevertheless, activity from the Stillbornonly group recovered earlier than the Stillborndisease group. This reduced activity in cows without disease goes hand in hand with the low DRT, and they could be related to the calving difficulty and the possible vaginal and uterine lesions that could have been ignored by the farm personnel. Stillbirth was related to lower milk production in a review by Fourichon et al. (1999). Bicalho et al. (2008) showed that the losses attributed to stillbirth calvings were much higher in the beginning of the lactation and greater in *parous* than in *primiparous* cows. The biology of how

stillbirth calvings may affect milk production is thus far unknown. It has been suggested that milk losses related with stillbirth calvings could be a consequence of health problems that affect cows since stillbirth is a risk factor for metritis and retained placenta, as was mentioned before in this text. However, cows from the groups Stillbornonly and Stillborndisease showed no differences in milk production during the first 14 DIM. Possibly, the small sample size of these groups did not allow detecting statistical differences. The mean difference for daily milk production between Control and Stillbornonly and between Stillborndisease and Control groups was 7.33 kg/d and 8.33 kg/d, respectively. This happened because milk production from the Stillborndisease group cows seems to begin a recovery at 10 DIM approximately, and the differences with control cows get numerically smaller after this point. It would be interesting to follow milk production longer during lactation in order to see if the trend of this pattern continues over time and if there would be differences between the Stillbornonly and the Stillborndisease group later in lactation because of an earlier recovery from the first of these groups, and to find if the lower milk production found in cows with stillbirth is compensated later (RAJALA; GRÖHN, 1998b). The daily milk production difference mean between cows with and without stillbirth found in our study is much higher than that found by Bicalho et al. (2008) (1.1 kg/d). However, they compared milk production over 10 months. The lack of difference in cows with stillbirth with and without disease may suggest that stillbirth *per se* is detrimental for milk production during the first 2 weeks in lactation. However, the slight recovery observed in cows without disease may indicate that the negative effect on milk production could be longer in cows with disease. We should remember that the cows from the Stillbornonly group may have had vaginal or uterine lesions ignored by the farm personnel that could have had a detrimental effect on milk production. More controlled works with larger sample size and longer period of study would be necessary to better determine how stillbirth calvings affect milk production.

6.2. Addition of data supplied by the collars to fresh cows health monitoring

A fresh cow monitoring program during the first days of postpartum is important for dairy farms because cow health and comfort are crucial for future production and reproduction performances. An early and accurate detection of cows with health disorders during the early postpartum period is needed for a comprehensive and efficient health monitoring program.

Dairy farms use different strategies, tools and, parameters to screen and check fresh cows during the first days in milk. Depending on the necessity of each farm, cows are checked with different intensity or frequency and based on several different parameters, such as number of days in milking, milk production, or in farms with a more intensive and aggressive program, all cows are checked every day regardless milk production or DIM. In this regard, this study evaluated the practicability of the addition of an automated rumination and activity monitoring system to identify fresh dairy cows with health disorders.

6.2.1. Collars performance to detect sick cows and anticipate diagnosis of disease

The use of the data supplied by the collars was able to detect 56% of health events diagnosed by the farm personnel from 7 days preceding to 2 days after diagnosis. The greater sensitivity of the system to identify cows with more than one health disorder than cows with only one disorder suggests that the ability of the system is related to the health compromise of the animals, detecting those cows with poorer health status, as was already suggested by Stangaferro et al. (2016a). The Se varied according to the disease with the lowest and highest values for metritis and DA, respectively. The Se for cows suffering from metritis, ketosis, and “other diseases” that developed other health disorders from -7 to 4 d relative to diagnosis was higher than for cows that had only these diseases. Stangaferro et al. (2016a) had similar results for metritis, and they suggested that the major reason for this finding was the range of severity of the disorder, which, in turn, caused a wide range of alterations to the rumination and activity patterns. For ketosis, we had higher Se for cows that developed other disorders around the diagnosis, different from the results of Stangaferro et al. (2016b), who had no differences in this regard. Interestingly, when rumination and activity around diagnosis were analyzed by ANOVA for repeated measures, the results showed that rumination and activity were not different between cows suffering from ketosis alone and cows that developed other disorders additionally. These observations provided evidence that the sole occurrence of ketosis caused alterations to rumination and activity that in some cases are not big enough to flag the cows. We can speculate that this may be related to the severity of the disorder affecting the animals. The fact that the Se was not different for cows with only ketosis and cows with other disorders additionally in the study performed by Stangaferro et al. (2016b), may result because their threshold alert (HI > 86 AU/d) was higher than ours (HI > 80 AU/d), flagging cows with

smaller rumination alterations that would not be flagged by us. For RP, DA, MLF, and mastitis, Se showed no differences for cows with the disease of interest only and cows with additional disorders. The highest Se found for cases of DA was a reflection of its severity, and our results are in concordance with those published by Stangaferro et al. (2016b). It would be expected that cows with MLF have the highest Se because this is a disorder that strongly affects rumination. However, around 40% of the cases of MLF were not flagged by the system. We can speculate that this happened because the diagnosis of this disease was performed during the first 2 DIM, and the system was not able to differentiate between the normal decrease in rumination around calving and the decrement because of this disturbance. The sensitivity to detect mastitic cows was not statistically different between cows with only mastitis and cows with mastitis plus another disorder. In the work of Stangaferro et al. (2016c), the sensitivity for cows with mastitis and other disorder tended to be greater than for cows with mastitis only. When they divided the cases by pathogen, the Se of the system flagged cows with *E. coli* was greater than for the other pathogens (*S. aureus*, *Klebsiella spp.*) together, and this was related to the severity of the mastitis caused by each microorganism by the authors. We did not have information about the etiology of mastitis in our experiment. As was stated by these researchers, we suggest that not all cows that develop a case of mastitis will be detected by the system in dairy farms, and it could be used to detect severe cases that affect the cow systemically or those in combination with other disorders.

The system was able to anticipate all health disorders, except for RP and MLF. This may be due to the proximity of the day of calving and the diagnosis of these diseases because we started to flag the cows on the day of parturition. Stangaferro et al. (2016a, b, c) were able to anticipate the diagnosis of metritis, DA, ketosis, and mastitis. However, the intervals between the first RA event and the diagnosis found in our experiment were numerically greater than those published by these authors, except for ketosis that was similar. This may be because the cows were checked every day by the farm personnel in the study of Stangaferro, and the cows in our experiment were checked in certain days of postpartum but not in all.

The overall sensitivity in the current experiment can be considered low and undesirable for farm monitoring system implementation, and it was similar to that shown by Stangaferro et al. (2016a) (59%). Metritis was the disorder for which the system had the lowest Se among all the disorders in study, and it was the disease had the highest incidence. Conversely, the highest Se was found for DA, which showed one of the lowest incidences among the disorders. These facts, in addition to the wide range of Se for the different health disorders (40% - 100%), are the reasons for the low overall Se and PPV obtained. The Se and PPV results may have been

influenced by the window adopted to define positive and false alarms (-7 d to 2 d after relative to diagnosis). It is possible that sick cows could show altered rumination outside this range of days, leading to misclassification of the alarm. This window for alarm classification was chosen based on the evidence that sick cows have reduced rumination and activity even before clinical signs are visible (LIBOREIRO et al., 2015; STANGAFERRO et al., 2016a, 2016b, 2016c). Stangaferro et al. (2016a, b, and c) used a more restricted window around diagnosis (-5 to 2 d after relative to diagnosis) to consider a positive alarm. However, their PPV (58%) was considered low and not much different to ours, assuming this restricted window as a reason and mentioning that some cows presented a positive RA event up to 6 to 8 d before diagnosis. For this reason, we decided to enlarge the window in two days from -5 to -7 relative to diagnosis. However, we could not improve the results for Se and PPV obtained by these authors, and this may be related to the parameters and thresholds used for flagging cows, as well as the frequency for checking cows adopted in each experiment. Generating the fewest false-positive alerts is important to avoid the unnecessary inclusion of cows without a health disorder in reports created to select cows for clinical examination. Among all RA alerts, more than a half (53.6%) were false alarms in our experiment. This means that most of the times the farm workers received cows based on RA, they found no signs of disease or considered them as healthy. The Sp obtained in the current experiment was relative low (74.5%) and lower than that in Stangaferro's work (97.6%). This is in relation to the differences observed in NPV among studies (57.6% in ours, and 97.7% in Stangaferro). These observations suggest that the parameters and thresholds adopted for flagging cows were not good indicators of cows not affected by a health disorder, conversely to the HI higher than 86 arbitrary units in Stangaferro's work.

6.2.2. *Fresh cow health monitoring farm performance*

The overall incidence of disease was statistically higher in *primiparous* from the CM group compared to *primiparous* from the C group. However, when analyses were run for each disease, only numerical differences were found, probably because the available sample size. The incidence of metritis, mastitis, and "other diseases" was numerically higher for the group CM than for the group C in *primiparous*. This may indicate that the addition of rumination and activity data helped to detect sick heifers that otherwise would be ignored following the farm

routine only. The RA events incidence, hospital visits, percentage of treated animals, and culling rate in *primiparous* from the CM group that were higher than the C group may reflect the higher incidence of disease in heifers from the CM group.

Regarding to *parous*, the only statistical difference was observed for MLF with higher incidence in the group C. However, this finding should be taken with care because this is a disorder of easy diagnosis for the farm and maybe should not be included for analysis, as was done by Stangaferro et al. (2016). In addition, the incidence of metritis tended to be higher in the C group compared to the CM group. The lack of differences for the rest of the diseases could suggest that the addition of rumination and activity data may have not influenced in the detection of cows suffering from these disorders.

The diagnosis of ketosis occurred ~3 days earlier in the group CM than in the group C. However, no differences for incidence, sensitivity of RA to detect ketotic cows, and days from the first RA event to diagnosis were found between groups. Possibly, the difference of average DIM at diagnosis between groups was a coincidence and had no relation to the system performance. The higher sensitivity of the system to detect cows with clinical ketosis and other health problems compared to animals with ketosis only was observed, meaning that as the health compromise of the animals was greater, greater were the chances of being flagged by the system (STANGAFERRO et al., 2016c). However, this statistical difference for Se between cows with ketosis only and ketosis plus another disorder was not observed in each group, possibly because the small sample size to perform the analysis. Additionally, a higher percentage of ketotic cows from the CM group tended to stay at the hospital than cows from the C group, and this may be due the earlier diagnosis and/or the degree of health compromise. However, 100% of the cows with ketosis from both groups received treatment.

Mastitis was diagnosed earlier in cows from the C group compared to the CM group. However, RA anticipated the diagnosis in the group CM only. This could lead to the thinking that the difference in DIM at diagnosis was related to the efficiency of the farm workers to check the cows flagged by the system and those sent for a check by our research group. The longer interval from the first RA and the diagnosis of mastitis in the group CM may be because farm personnel did not pay much attention to the cows sent for a check based on collar's data, and this could be the reason of the later diagnosis. Farm workers were instructed to check the cows sent by our research group following the same procedures as they always do. Additionally, it has been shown that decreased rumination time was observed previous to clinical signs of mastitis (SORIANI; TREVISI; CALAMARI, 2012), which could make difficult an early clinical diagnosis.

The only statistical difference between groups for sensitivity of RA system to detect sick cows was observed for “other diseases”, specifically when these were accompanied by other health problems. It seems to be that sending cows to be checked based on collar’s information improved the detection of sick animals suffering from these health alterations. Although no statistical differences were found for the interval in days between the first RA event and the diagnosis, this was numerically shorter (1.3 d) in cows from the CM group, suggesting that sending animals to be checked allowed an earlier diagnosis of these kind of health disorders.

For metritis, although no statistical differences for the sensitivity of the system to detect cases accompanied by other health affections between groups were found, this was numerically higher in the CM group than the C group. This means that sending cows to check may improve the detection of animals with this condition, which can be supported by the tendency for higher incidence of metritis in *primiparous* from the CM group. The mentioned differences in the sensitivity of the system to detect cows suffering from metritis and “other diseases” accompanied by other health affections led to a higher overall sensitivity to detect animals suffering from more than one disorder in the CM group compared to the C group.

The prevention treatment routine in the current farm involves the use of fluids, drenching (support treatments in our description), and anti-inflammatories in animals with “poor” health status or low milk production performance. Additionally, cows in the first days of lactation with suspicion of metritis are given intrauterine boluses containing urea, based on its antibacterial properties (REDDY et al., 2011). Interestingly, a higher percentage of healthy *parous* cows from the CM group received support treatments than healthy *parous* from the C group (C = 24.6%, 44.4%, $P = 0.01$). This could be related to the lower incidence of MLF and the tendency for lower incidence of metritis found in the CM group, as well as to the numerically lower culling rate found in these animals (C = 1.3%, CM = 4.3%, $P = 0.3$). Healthy animals from the CM group, possibly, were sent to the hospital based on RA in early stages of disease or even before the signs of health problems were evident, and for this reason, they were not diagnosed as sick but treated with drenching and fluids support therapies. These findings suggest that the use of the collars could be positive in animals not diagnosed as sick but needing some support therapy to prevent health disorders.

The incidence of disease, culling rate, and reproductive parameters performance, such as the early resumption of cyclicity, suggest that health and comfort are very good and that the farm schedule and methodology seems to be efficient to diagnose disease, detect sick cows, and implement the proper treatments. Checking cows three to four times during the first 10

DIM when the actual milk production does not meet the expected or when strong milk production drops occur can be categorized as an intensive monitoring system (GUTERBOCK, 2004). Farm workers check general appearance, uterine discharge, udder fill, milk aspect, and ketone bodies in urine every time they check the cows in the current farm. Additionally, every fresh cow of 3 or more lactations and those with difficult calving receive a drench in the day of calving as a preventive tool. We could not obtain a greater improvement in the detection of sick cows in a farm with an intense fresh cow monitoring system like this, but, maybe, the addition of rumination and activity data would improve the detection of sick cows in farms with a less intensive program. Our results suggest that the addition of the data supplied by the collars could be useful to improve the detection of sick *primiparous* animals. This finding could be related to the fact that *primiparous* are more susceptible and suffer more from environmental changes than *parous* cows, especially around calving that leads to reduced rumination and a slower increase of DRT at the beginning of lactation (SORIANI; TREVISI; CALAMARI, 2012). We could speculate that the negative effects of calving, milking, and re-grouping over rumination time during the first weeks of lactation (SCHIRMANN et al., 2011a; SORIANI; TREVISI; CALAMARI, 2012) were reinforced by the occurrence of health disorders, like metritis, which affected *primiparous* in our experiment. For this reason, it could be suggested that reductions in DRT were more accentuated and easily detected by the system in *primiparous* than in *parous* cows. However, Wittrock et al. (2011) showed that healthy and metritic *primiparous* cows did not differ in feed intake during the 3 weeks after calving. Besides, Stangaferro et al. (2016a) observed a reduction in rumination time around the diagnosis of metritis, but no effect of parity was found.

On the other hand, our results indicate that the use of collars can be useful to prevent health problems in cows showing altered rumination needing therapy support before clinical signs are evident, reducing the incidence of disease as occurred with *parous* cows in our experiment. Stangaferro et al. (2016) have shown that cows suffering from metabolic and infectious disorders had reduced rumination prior to the diagnosis made by the farm personnel. In the same way, Soriani et al. (2012) found that mastitic cows had decreased rumination time previous to clinical signs of mastitis. And Huzzey et al., (2007) showed that metritic cows had lower DMI even before parturition. The fact that clinical signs are visible few days after the rumination reduction could be a difficulty for the diagnosis. As reductions in rumination are not specific for each disorder and sometimes clinical signs are not evident yet, we cannot treat cows specifically, and for this reason, palliative treatments are a good alternative. Drenching and fluids are used because they support for energy and prevent from ketosis, aid in

dehydration, regulate the rumen microflora, help in hypocalcemia and milk fever prevention, and supply vitamins and minerals involved with the immune system (GUTERBOCK, 2004; THILSING-HANSEN; JØRGENSEN; ØSTERGAARD, 2002). Anti-inflammatories are used for fever reduction and pain, and they can contribute to improve rumination time since rumination is affected by these factors (ANDERSON; MUIR, 2005). However, while some authors found the positive effect of AINE's administration on rumen motility (BANTING et al., 2008; VANGROENWEGHE et al., 2005), an indirect rumination measurement, others researchers showed that rumination time did not change after treatment (FITZPATRICK et al., 2013; ZIMOV et al., 2011). Detecting cows at risk for health disorders before clinical signs are evident or get worse can prevent cows from suffering from disease by giving prevention and palliative treatments to them.

7. CONCLUSIONS

Our findings showed that the patterns of rumination, activity, and milk production around diagnosis in cows with calving problems, RP, metritis, ketosis, MLF, DA, indigestion, lameness, and “other diseases”, such as fatty liver, respiratory problems, and rupture of uterus, showed differences up to 7 days previous to diagnosis compared to healthy animals and that the degree of these patterns alteration seems to be related to the severity of the disease. In addition, cows suffering from subclinical metabolic disorders, such as SCK and SCHC, have disrupted rumination and activity patterns around calving, and this disruption is greater when clinical problems are present. Moreover, our results demonstrated that regrouping cows has consequences on rumination and activity but not on milk production.

Our results showed that the collar system was able to identify more than a half of sick cows around diagnosis and did it earlier than the farm personnel diagnosis for most of the health disorders in study, excluding RP and MLF.

The addition of rumination and activity monitoring by collars to on-farm system for checking fresh cows in a commercial dairy farm with an intense health monitoring program may be useful for improving the detection of sick cows and to prevent the occurrence of health problems in cows showing altered rumination needing support therapy before clinical signs of disease are evident, and this seems to depend on the number of lactation of the animals and on the disease in study.

In the current study, rumination and activity monitoring was added to the on-farm fresh cow's health check program in a dairy farm with an intense screening of the animals. Thus, future research is needed to evaluate the efficiency of rumination and activity data in dairies with different degrees of intensity for checking cows. It would be expected that the usefulness of the collars may be greater in farms with a less intense screening schedule, allowing for the checking of potentially sick cows that otherwise would not be checked.

One problem we faced in the current experiment was sending cows not needing attention or not being sick (false positives). This is not desirable because it represents extra work and time for the farm workers that could be used for other activities. This issue could be improved by working on the selected parameters and parameters threshold we used to send cows for checking (daily rumination time, rumination variation related to three days before, and Health Index). New research should evaluate variation in thresholds and possibly new

parameters provided by the company software in order to minimize the occurrence of false positives, which maximizes the system efficiency.

In conclusion, rumination and activity are affected around diagnosis in sick cows, and this can be used as a tool for detecting cows suffering from disease and cows needing attention before clinical signs appear. The usefulness may vary according to parity, disease, and the intensity of the farm system for checking cows.

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