UNIVERSIDADE FEDERAL DO PARANÁ

ALINE MILANI FILLUS

CALCEMIA E RUMINAÇÃO EM VACAS JERSEY RECÉM PARIDAS EM REBANHO COM ORDENHA ROBOTIZADA

CURITIBA

2021

ALINE MILANI FILLUS

CALCEMIA E RUMINAÇÃO EM VACAS JERSEY RECÉM PARIDAS EM REBANHO COM ORDENHA ROBOTIZADA

Dissertação apresentada ao Programa de Pós-Graduação em Zootecnia, Área de Concentração Nutrição e Produção Animal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial para obtenção do título de Mestre em Zootecnia.

Orientador: Prof. Dr. Rodrigo de Almeida

CURITIBA

2021

Fillus, Aline Milani
Calcemia e ruminação em vacas Jersey recém paridas em rebanho com ordenha robotizada. / Aline Milani Fillus. - Curitiba, 2021.

Dissertação (Mestrado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia.
Orientação: Rodrigo de Almeida.

1. Jersey (Bovino). 2. Bovinos de leite - Digestão. 3. Cálcio na nutrição animal. I. Almeida, Rodrigo de. II. Título. III. Universidade Federal do Paraná.

Sistema de Bibliotecas/UFPR
Guilherme Luiz Cintra Neves - CRB9/1572

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ZOOTECNIA -
40001016082P0

ATA Nº092021

ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM ZOOTECNIA

No dia vinte e seis de março de dois mil e vinte e um às 10:00 horas, na sala Microsoft Teams, Remoto, foram instaladas as atividades pertinentes ao rito de defesa de dissertação da mestranda **ALINE MILANI FILLUS**, intitulada**: Calcemia e ruminação em vacas Jersey recém paridas em rebanho com ordenha robotizada**, sob orientação do Prof. Dr. RODRIGO DE ALMEIDA. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em ZOOTECNIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: RODRIGO DE ALMEIDA (UNIVERSIDADE FEDERAL DO PARANÁ), SIMONE GISELE DE OLIVEIRA (UNIVERSIDADE FEDERAL DO PARANÁ), LUCIANO SOUZA CAIXETA (UNIVERSITY OF MINNESOTA).
A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, RODRIGO DE ALMEIDA, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 26 de Março de 2021.

Assinatura Eletrônica
26/03/2021 17:04:27.0
RODRIGO DE ALMEIDA
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
27/03/2021 16:16:31.0
SIMONE GISELE DE OLIVEIRA
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
29/03/2021 19:32:45.0
LUCIANO SOUZA CAIXETA
Avaliador Externo (UNIVERSITY OF MINNESOTA)

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ZOOTECNIA -
40001016082P0

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **ALINE MILANI FILLUS** intitulada: **Calcemia e ruminação em vacas Jersey recém paridas em rebanho com ordenha robotizada**, sob orientação do Prof. Dr. RODRIGO DE ALMEIDA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 26 de Março de 2021.

Assinatura Eletrônica
26/03/2021 17:04:27.0
RODRIGO DE ALMEIDA
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
27/03/2021 16:16:31.0
SIMONE GISELE DE OLIVEIRA
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
29/03/2021 19:32:45.0
LUCIANO SOUZA CAIXETA
Avaliador Externo (UNIVERSITY OF MINNESOTA)

Dedico à Deus por abençoar a minha vida todos os dias, me dando força e sabedoria, e aos meus pais Elio Fillus e Emely Milani Dolla, que não pouparam esforços para a realização deste meu sonho.

AGRADECIMENTOS

Agradeço a Deus por estar sempre presente, e me dando forças para continuar.

Aos meus pais, minha família, e ao meu namorado Raphael por serem pacientes, me darem todo apoio e entenderem minha ausência.

Aos meus colegas de trabalho na EXIAN, pelo apoio e pela evolução diária.

Ao meu orientador Rodrigo de Almeida pelo tempo dedicado aos ensinamentos e orientação durante este período, e por me auxiliar no amadurecimento profissional.

Aos professores do Programa de Pós-graduação em Zootecnia, e aos técnicos dos laboratórios envolvidos nas análises imprescindíveis para o desenvolvimento deste trabalho.

Aos colegas de Pós-graduação e do Grupo do Leite da UFPR pela parceria; as meninas Isabela, Eloize, Milaine e Dani pelos momentos de apoio e troca de experiências.

Ao Nico Biersteker e Ellen Salomons Biersteker por disponibilizarem seu rebanho para realização deste trabalho.

E por fim à Cooperativa Capal e ao técnico Rodrigo Navarro por toda assistência prestada.

Meu muito obrigada.

RESUMO

Vacas leiteiras, principalmente as de alta produção, enfrentam um período desafiador após o parto e início da lactação, comumente classificado como período de transição. A hipocalcemia é uma das doenças que mais acometem as vacas nesse período, e é relatado na literatura que vacas da raça Jersey são mais propensas a apresentar esta doença que pode apresentar-se de duas formas; na forma clínica (HC) ou na forma de hipocalcemia subclínica (HSC). Como protocolo muitas fazendas têm tratado as vacas com HSC da mesma forma, mas trabalhos recentes mostram que vacas com HSC podem lidar de forma diferente com esta homeostase do cálcio nos primeiros dias após o parto. É fisiológico que o Ca tenha seu nadir ao redor das 12 às 36 horas após o parto, mas o que parece ser mais importante é quão rapidamente essas vacas retornam a normocalcemia. Este estudo ocorreu em uma fazenda comercial, que possui rebanho formado apenas por vacas da raça Jersey, mantidas em *compost barn,* sistema de ordenha robotizada (SOR), com 6 robôs, em fluxo livre. O estudo foi dividido em duas partes. Na primeira parte realizamos um estudo retrospectivo observacional, foram avaliadas 116 vacas recém paridas, e as classificamos em grupos de normocalcêmicas (NC) com concentração de Ca> 2,15 mmol/L nos 4 primeiros dias após o parto, e HSC em três grupos: HSC transitória que apresenta a concentração de Ca ≤ 2,15 mmol/L no 1 dia após o parto, mas consegue recuperar a partir do 2, 3 e 4 dias; HSC atrasada que não obteve queda de Ca> 2,15 mmol/L no primeiro dia mas sim a queda ≤ 2,15 mmol/L ocorreu a partir do segundo dia em diante; e a HSC persistente que teve a concentração de Ca ≤ 2,15 mmol/L em todos os 4 dias após o parto. Após os resultados da análise de Ca, definimos a classificação das vacas em categorias de cálcio e relacionamos com produção de leite e os metabólitos: cálcio plasmático, cálcio sérico, haptoglobina, BHB, AGNE, albumina, bilirrubina, colesterol, AST e glicose. O objetivo desse trabalho foi relacionar as categorias de HSC com produção e saúde em vacas Jersey, e na segunda parte do estudo avaliamos a ruminação de 377 vacas em 895 lactações, que correspondem a todo o período de funcionamento do SOR. Utilizamos a ruminação dessas vacas nos primeiros 15 dias após o parto, comparando-a com dados da lactação até os 60 dias, gerados pelo robô. Avaliamos as variáveis produção de leite, indicações de gordura e proteína, número de ordenhas e ingestão de concentrado no robô, para verificar qual o impacto que vacas de alta, média ou baixa ruminação tem sobre esses parâmetros de produção. Os resultados observados no presente estudo demonstram que vacas que apresentaram HSC transitória produziram mais leite que vacas que apresentaram HSC persistente, e obtiveram um perfil metabólico semelhante a NC. Foi possível observar também que vacas classificadas com alta ruminação produziram mais leite, com menores teores de gordura e de proteína, foram ordenhadas mais frequentemente e consumiram maior quantidade de concentrado no robô, em comparação as vacas de baixa ruminação.

Palavras chave: vacas leiteiras, cálcio, ruminação, período de transição

ABSTRACT

Dairy cows, especially the high-producing ones, face a challenging period before and after calving, commonly called the transition period (21 days before and 21 days after calving). Hypocalcemia is one of the diseases that affect cows in this period, especially Jersey cows. Which can present itself in two ways: clinical (HC) and subclinical (SHC). As a protocol, many farms have treated cows with SHC in the same way, but recent studies show that cows with this disorder may deal differently with calcium homeostasis in the first days of lactation. It is physiological that Ca has its nadir around 12 to 36 h after calving, but is interesting how well these cows return their Ca concentration to the ideal level. This experiment was carried out on a commercial farm in Southern Brazil, Paraná, which has a herd composed of Jersey cows that were housed in a compost barn with 6 automated milking systems (AMS) with free-flow. This study was divided into two parts. In the first one, we used a study retrospective cohort, divided 116 recently calved cows into four groups of calcemia: normocalcemic (NC; Ca> 2.15 mmol/L in the first 4 days after calving); transient SHC (Ca ≤ 2.15 mmol/L at 1st day after calving and recovery to Ca> 2.15 mmol/L in the other 3 days after calving); delayed SHC (Ca> 2.15 mmol/L at the first days and a drop to ≤ 2.15 mmol/L in the other 3 days after calving); persistent SHC (Ca ≤ 2.15 mmol/L in the first 4 days after calving). This study aimed to investigate the association between calcemia group and with milk yield and blood metabolites, plasma calcium, serum calcium, haptoglobin, BHB, NEFA, albumin, bilirubin, cholesterol, AST, and glucose. In the second part of the study, we evaluated the rumination of 377 cows in 895 lactations and related with milk yield, fat and protein indication, number of milkings and concentrate intake, to see what impact the level of rumination (high, medium and low) have on these production parameters. The results demonstrate transient SCH cows had a higher milk yield than persistent SCH and similar metabolic profile to normocalcemic cows. It was also possible to observe that cows having a high level of rumination until 15 days in milk, produced more milk, with lower milk fat and protein contents, were milked more frequently and showed a higher concentrate intake compared with cows with medium and low levels of rumination.

Keywords: dairy cows, calcium, rumination, transition period

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE SIGLAS

AGNE – Ácidos graxos não esterificados

AST – Aspartato Aminotransferase

BHB – Beta-hidroxibutirato

HC – Hipocalcemia clínica

HSC – Hipocalcemia subclínica

HSCa – Hipocalcemia subclínica atrasada

HSCp – Hipocalcemia subclínica persistente

HSCt – Hipocalcemia subclínica transitória

NC - Normocalcêmica

SOR- sistema de ordenha robotizada

TR – Tempo de ruminação

LIST OF ABBREVIATIONS

ADF – Acid detergent fiber

AST – Aspartate aminotransferase

BHB – Beta-hydroxybutyrate

Ca – Calcium

CH – Clinical hypocalcemia

DCAD – Dietary cation anion difference

DIM – Days in milk

DM – Dry matter

DMI – Dry matter intake

dSCH – delayed subclinical hypocalcemia

HRT – high rumination time

LRT- low rumination time

MRT- medium rumination time

NC – normocalcemic

NDF – Neutral detergent fiber

NEFA – Non-esterified fatty acids

PMR – Partial mixed ration

pSCH – Persistent subclinical hypocalcemia

RT – rumination time

SCH – subclinical hypocalcemia

SD – standard deviation

tCa – total calcium

TMR – Total mixed ration

tSCH – transient subclinical hypocalcemia

SUMÁRIO

Capítulo 1

Hypocalcemia in Jersey cows: effects on health and milk yield *by Fillus et al.* High producing dairy cows face a challenging period when transitioning from late gestation to early lacation. Hypocalcemia is a common disease of periparturient cows. Jersey cows with subclinical hypocalcemia only on the first day, produced more milk than persistent subclinical hypocalcemia cows and their metabolic profile are similar to normocalcemic cows.

Running Head: HYPOCALCEMIA IN JERSEY LACTATING COWS

Persistent, transient or delayed hypocalcemia in Jersey cows: association on metabolites and milk yield

A. M. Fillus[1], A. C. Neves, I. F. Carrari[1], C. Baccili[2], V. Gomes[2], R. B. Navarro[3], R. Almeida[1†]

[1]Departmento de Zootecnia, Universidade Federal do Paraná, Curitiba, PR, 80035050 Brazil
[2]Universidade de São Paulo, São Paulo, SP, Brazil
[3]Capal Cooperativa Agroindustrial, Arapoti, PR, Brazil

[†]Corresponding author: ralmeida@ufpr.br

1.1 ABSTRACT

The aim of this study was to determine the types of subclinical hypocalcemia (SCH) and relate it to milk yield (10, 30 and 60 d postpartum) and blood metabolites (1, 2, 3 and 4 postpartum). Lactating Jersey cows (n=116; 31 primiparous and 85 multiparous), averaging (mean ± standard deviation) 480 ± 50 kg of BW and 30.0 ± 6.5 kg of milk/d, were housed in a compost barn with an automatic milking system. A retrospective cohort study was developed, where pimiparous cows were categorized in 4 early postpartum Ca status groups: normocalcemic (NC; > 2.15 mmo/L at 1 and 2 DIM), transient SCH (tSCH; ≤ 2.15 mmo/L at 1 Days in milk - DIM and > 2.15 mmol/L at 2 DIM), delayed SCH (dSCH > 2.15 mmol/L at 1 DIM and ≤ 2.15 mmol/L at 2 DIM), and persistent SCH (pSCH; ≤ 2.15 mmol/L at 1 and 2 DIM). The plasma Ca thresholds for multiparous were the same described above, but the second blood collection was at 4 DIM. Average milk yield in the first 10 d was greater for tSCH than pSCH cows (23.24 vs. 18.96 kg/d, respectively), while NC and dSCH cows showed intermediate yields (21.66 and 20.54 kg/d, respectively). No differences on average milk yield in the first 30 and 60 d were observed. Haptoglobin concentrations were higher for pSCH and dSCH cows than for tSCH ones; 24.5 and 22.8 vs. 14.9 mg/dL, respectively. NEFA concentrations were higher for pSCH cows than NC ones; 0.43 vs. 0.25 mmol/L, respectively. Bilirubin concentrations were higher for pSCH than NC cows (0.33 vs. 0.17 mmol/L, respectively). Albumin concentrations were lower in pSCH and dSCH than for NC cows (3.14 and 3.18 vs. 3.40 g/L, respectively). No differences were found in BHB, AST, cholesterol, and glucose concentrations when comparing the postpartum Ca status groups. In conclusion, transient SCH Jersey cows produce more milk than persistent SCH at 10 DIM, and their metabolic profile are similar to normocalcemic cows.

Key words: calcium, milk fever, postpartum

1.2 INTRODUCTION

Dairy cows, particularly the high-producing ones, face a challenging period in the last 3 weeks before calving and 3 first weeks of lactation, a phase known as the transition period (Drackley, 1999). The demand for energy increases by 2.5 times (Reynolds et al., 2003) and the requirements for minerals, especially calcium, are also increased to support calving, colostrogenesis and lactogenesis (Degaris, 1997). Mineral demands vary according to the animal's production phase. The calcium requirement for the production of one kg of milk is 1.45g for Jersey cows and 2.1g to produce one kg of colostrum (NRC, 2001). This Ca requirement triple in one week, the sudden increase in the demand for Ca occurs in a moment when typically there is a 30% reduction in feed intake around calving, which reinforces the deficit of this micromineral during this critical period (Hayirli et al., 2002).

Hypocalcemia is one of the most common diseases of periparturient dairy cows. This metabolic disorder is often categorized as clinical (CH; cow in decubitus with locomotor difficulties, commonly called milk fever) and subclinical (SCH; low concentration of blood calcium). Cows are categorized as SCH when total calcium concentration is ≤ 2.15 mmol/L (Martinez et al., 2012) and as CH or milk fever when the concentration is < 1.25 mmol/L (Goff, 2008). Traditionally, hypocalcemia has been associated with the occurrence of other diseases such as dystocia, uterine prolapse, retained placenta, metritis, mastitis, and displaced abomasum (Goff, 1996), in addition to decrease milk yield (Chapinal et al., 2011) and losses in reproduction (Ospina et al., 2010).

The blood Ca concentrations reach a nadir around 12-36 h after calving (Goff, 2008), but recent work showed that the diagnosis of SCH based on a single sample within 24h after calving, is considered a poor indicator of postpartum diseases (Neves et al., 2008a). Caixeta et al. (2017) and McArt and Neves (2020) suggest that it is not the nadir of blood Ca concentration that increases the risk of diseases development and negatively impacts reproduction and milk yield, but the persistency of SCH.

These findings suggest that a drop in Ca concentration just after calving followed by an increasing recovery of this mineral may be part of the normal adaptation of dairy cows that have high demands in the beginning of lactation. Meantime, animals that remain with low calcium concentration or even when this drop is delayed (2 to 4 d after calving), it can represent a greater damage to health, leading to an increased risk of diseases, decreased milk production and increased culling (McArt and Neves, 2020).

So, the aim of this study was to determine the types of subclinical hypocalcemia (SCH) among fresh Jersey cows and relate it to milk yield and blood metabolites.

Our hypothesis is that there is a difference between the groups of SCH. And that cows that have SCH with low calcium levels only on the first day after calving, have higher milk production, without impairing metabolic parameters.

1.3 MATERIAL AND METHODS

1.3.1 Cows, experimental design and treatments

The Animal Research Ethics Committee of the Federal University of Paraná approved all experimental procedures, protocol number 085/2019.

A retrospective cohort study was carried out with a total of 131 cows, which calved during the experimental period, from January 4th to April 5th, 2020. Fifteen animals were removed from the study for the following reasons: a blood collection missing in any of the 4 d after calving, application of oral or intravenous Ca in the postpartum (4), sale (6) or death (5). Therefore, data from 116 cows (31 primiparous and 88 multiparous) were used for analysis.

These 116 Jersey cows from a commercial farm were housed in a compost barn with 6 automatic milking systems. All cows used in this study should not be supplemented with oral or injectable calcium in the postpartum period. Prepartum cows were housed in a compost barn at 21d before expected calving date, and they were fed a TMR diet with -10 meq/100g DCAD, which it was offered twice daily (0800 and 1700 h). Postpartum cows were housed in a compost barn with automatic milking system, in free flow (Lely®, Astronaut A4 - AMS®) with PMR (forage + part of the concentrate) offered three times a day (0800, 1200, and 1700 h) with feed push ups between time, and part of the concentrate in the robot during milkings. Ingredients and nutrients composition of the diet fed during the experimental period are shown in Table 1-1.

TABLE 1-1. Ingredient and nutrient composition of experimental diets[1].

Item	Diet	
	Prepartum	Postpartum
Ingredient, % of DM		
Corn silage	63.7	47.0
Barley straw	13.0	0
Soybean meal	18.8	0
Prepartum premix[2]	4.5	0
Ryegrass haylage	0	8.9
Whole cottonseed	0	6.4
Concentrate (PMR)	0	14.8
Concentrate (Milking)	0	22.9
Nutrient, % of DM		
DM	43.6	38.1
NDF	41.4	41.4
ADF	22.1	19.3
CP	17.1	13.7
DCAD	- 10.0	-

[1] Nutrient composition was determined from feed ingredients sampled monthly during the trial.
[2] Bovigold Prepartum Plus (DSM®)

1.3.2 Sample collection and analysis

Samples of PMR and dietary forages were collected monthly to determine their chemical composition. All samples were frozen after collection until analysis. Samples were dried in a forced-air oven (135°C for >72h) before grinding through a Willey mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA). Diets and forages samples were analyzed for DM (Goering and Van Soest, 1970; AOAC International, 2000, method 930.15), CP (AOAC International, 2000, method 990.03), ADF (AOAC International, 2000, method 973.18), NDF (Van Soest et al., 1991), ether extract (AOAC International, 2000, method 2003.05), and ash (AOAC International, 2000, method 942.05).

Daily milk yield and average yields at 10, 30 and 60 d were recorded by Lely – T4C® (Time for Cows) automatic milking management software. Body condition score was determined by one evaluator on the 4th day in milk, on a 5-point scale with 0.25 increments, where 1 = thin and 5 = fat, as described by Wildman et al. (1982).

Blood samples were collected on 1, 2, 3, and 4 d to measure metabolites on postpartum, every day at the same time (0900h), by venipuncture of the caudal vessels into a vacuum tube without anticoagulant and another tube with lithium heparin (Vacuette do Brasil, Campinas, SP, Brazil). All metabolites were analyzed by Veterinary Clinical Pathology Laboratory at Federal University of Paraná (Curitiba, PR, Brazil), using the automatic biochemical analyzer (BS-200; MINDRAY; Shenzhen; China). NEFA levels were determined by colorimetric enzymatic assay (Ref.: NEFA FA115; RANDOX Laboratories - Crumlin, UK). Albumin (Ref.: K040-3.1), glucose (Ref.: K082-5.1), bilirubin (Ref.: K107-2.2), cholesterol (Ref.: K083-5.1), and aspartate aminotransferase enzyme (AST) (Ref.: K048-6.1) were determined by colorimetric assays (commercial kits from Bioclin, Belo Horizonte, MG, Brazil). Total calcium in plasma and serum was determined by the ARZENAZO III method (Ref.: K051-3.1). Haptoglobin were analyzed by Laboratory FMVZ/USP. (methodology colorimetric method by spectrophotometry, adopted by Jones and Mould, 1984, and adapted by Bastos et al., 2013). Blood BHB was measured at 4 and 10 d postpartum using a handheld device (Precision Xtra, Abbott Laboratories, IL, United States). Subclinical hyperketonemia was defined as blood BHB concentration > 1.2 mmol/L in either measurement. (day 4 and/or 10).

1.3.3 Subclinical hypocalcemia definition.

To categorize the cows into groups based on blood calcium concentration in the first week postpartum we used a modified classification described by McArt and Neves (2020). After determining the total plasma Ca concentration, the primiparous cows were divided in 4 groups: normocalcemia (NC) > 2.15 mmo/L at 1 and 2 DIM), tSCH (transient SCH; ≤ 2.15 mmo/L at 1 DIM and > 2.15 mmol/L at 2 DIM), pSCH (persistent SCH; ≤ 2.15 mmol/L at 1 and 2 DIM), and dSCH (delayed SCH; > 2.15 mmol/L at 1 DIM and ≤ 2.15 mmol/L at 2 DIM). Multiparous cows were grouped in: NC (> 2.15 mmo/L at 1 and 4 DIM), tSCH (≤ 2.15 mmo/L at 1 DIM and > 2.15 mmol/L at 4 DIM), pSCH (≤ 2.15 mmol/L at 1 and 4 DIM), and dSCH (> 2.15 mmol/L at 1 DIM and ≤ 2.15 mmol/L at 4 DIM). According to the previously mentioned study, primiparous cows deal better with calcium homeostasis after calving, so the evaluation option on days 1 and 2, compared to multiparous cows that may take longer and extend hypocalcemia longer, from day 1 until day 4 for normalization of the calcium level.

1.3.4 Statistical Analysis

The FREQ procedure of SAS (v.9.4) was used for descriptive statistic. The CORR procedure of SAS (v.9.4) was used to determine Pearson's correlation coefficients between plasma Ca concentration and metabolites. The experimental design was a retrospective cohort. We use parity, postpartum Ca group (NC, tSCH, dSCH, and pSCH), time (days 1, 2, 3, and 4 after calving), and the interaction between time and Ca status group as fixed effects. For the variables without repeated measures over time statistical analyzes were performed using the GLM procedure of SAS (v.9.4). For repeated measures over time, statistical analyzes were performed using the MIXED procedure of SAS (v.9.4). The covariance structure was defined according to the lowest value obtained for "Akaike's Information Criterion Corrected" (AICC). For results interpretation and discussion, effects were declared significant when $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$.

1.4 RESULTS

1.4.1 Milk yield

We found 52.6% NC cows (n = 61), 26.7% tSCH (n = 31), 12.1% dSCH (n = 14), and 8.6% pSCH (n =10) to primiparous and multiparous. The lactation curves for milk yield up to 60d for each Ca status group are shown in Figure 1. In Figure 2, the averages for milk yield at 10, 30, and 60 d for each group of calcemia are shown. Average milk yield in the first 10 d was greater (*P*=0.04) for tSCH than pSCH cows (23.24 vs. 18.96 kg/d, respectively), while NC and dSCH cows showed intermediate yields (21.66 and 20.54 kg/d, respectively). No differences on average milk yield in the first 30 and 60 d were found (*P*>0.10) among early postpartum Ca status groups.

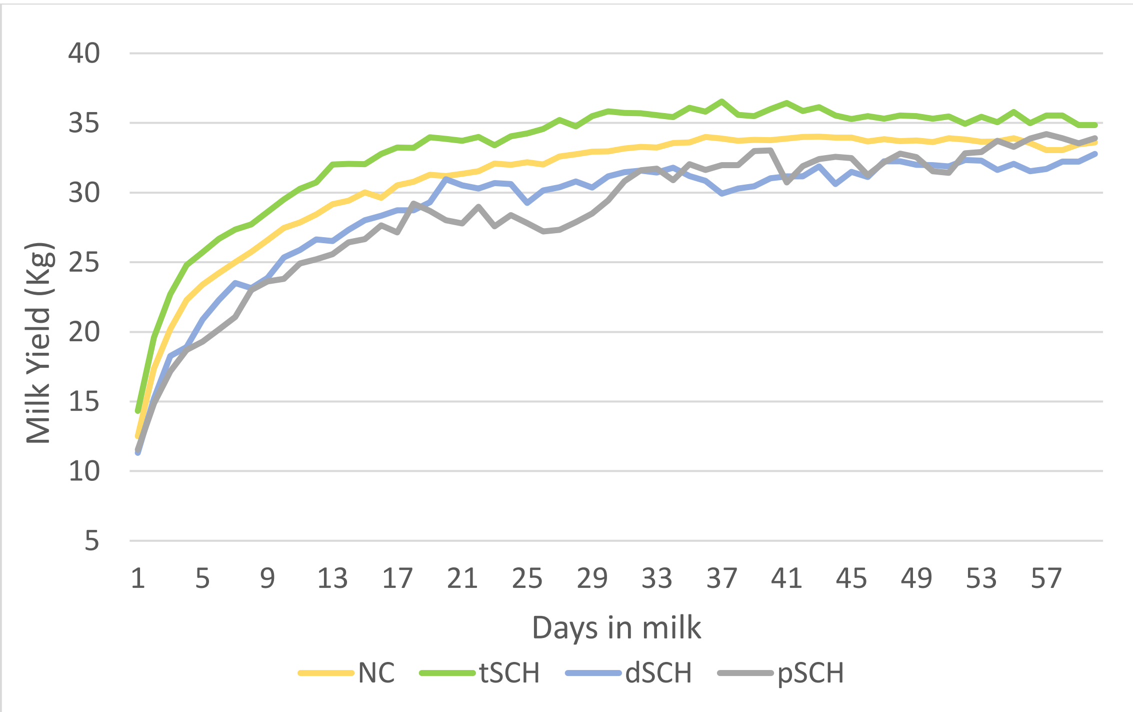


FIGURE 1-1 Daily milk yield in the first 60 days of lactation based on postpartum Ca status group. nº 116 cows. Yellow line: normocalcemic (NC); green line: transient

subclinical hypocalcemia (tSCH); blue line: delayed subclinical hypocalcemia (dSCH); grey: persistent subclinical hypocalcemia (pSCH).

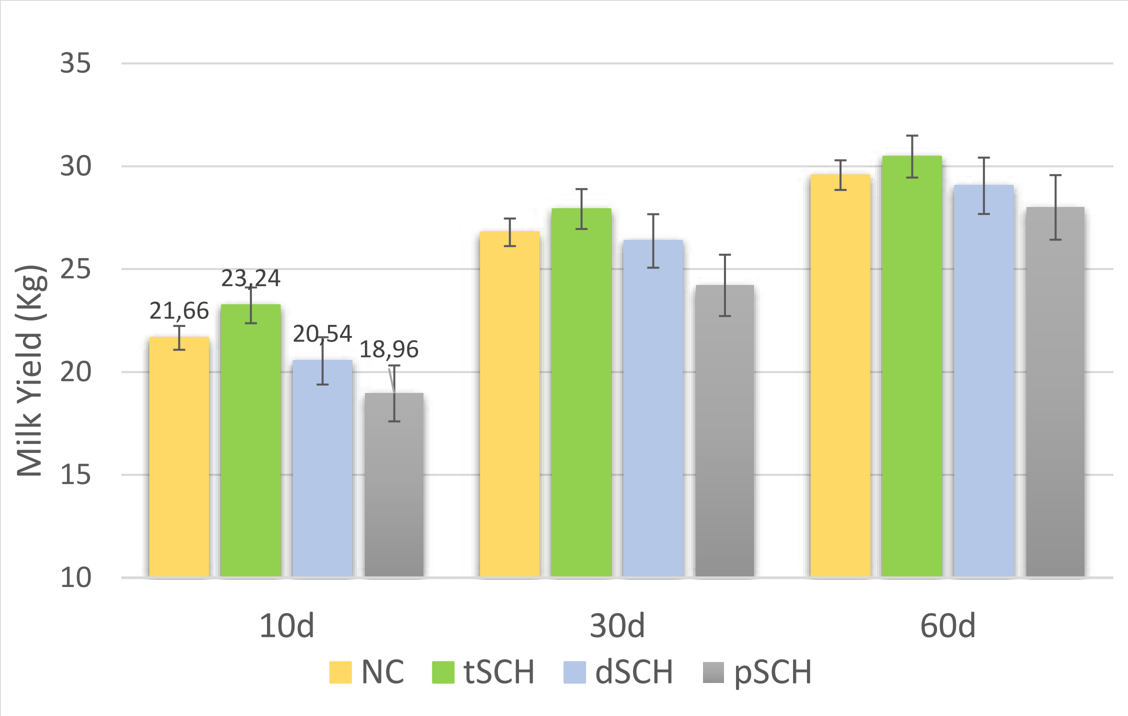


FIGURE 1-2. Average milk yield from cows classified into postpartum Ca status group at 10, 30, and 60 d postpartum. Yellow: normocalcemic (NC); green: transient subclinical hypocalcemia (tSCH); blue: delayed subclinical hypocalcemia (dSCH); grey: persistent subclinical hypocalcemia (pSCH).

1.4.2 Metabolites

Table 2 shows the Pearson's correlation between plasma Ca concentrations and the other evaluated metabolites. For AST and BHB, no statistically significant correlations were found (P=0.24 and P=0.16, respectively). As expected, high and positive correlations were found (P>0.01) between plasma and serum Ca in 1, 2, 3, and 4 d after calving (0.82, 0.81, 0.64, and 0.75, respectively). For NEFA, a moderate negative correlation was found (P=0.01) on day 1 (-0.45). Moderate negative correlations (P=0.01) also were found for bilirubin on days 1, 2, and 4 (-0.57, -0.43, -0.32, respectively).

Table 3 shows the adjusted means of each metabolite for each Ca status group. As expected, NC cows had high plasma Ca concentrations during the 4 days (2.37, 2.34, 2.48, and 2.45 mmol/L). Transient SCH cows had lower plasma Ca on day 1 (1.963 mmol/L), but they recovered in the following three days (2.146, 2.362, and 2.466 mmol/L). Delayed SCH cows had lower plasma Ca concentrations than tSCH cows on days 2, 3, and 4 (2.043, 2.085, and 2.027 mmol/L). And persistent SCH cows showed lower plasma Ca concentration during the 4 days (0.1920, 1.979, 2.006, and 1.985 mmol/L).

Haptoglobin concentrations were higher (*P*<0.05) on day 2 for dSCH cows than for NC and tSCH ones; 20.46 vs. 19.03 and 16.66 mg/dL, respectively. NEFA concentrations tended to be higher (*P*<0.07) at 4 DIM for pSCH cows than NC ones; 0.45 vs. 0.20 mmol/L, respectively. Glucose tended to be lower (*P*<0.07) at 3 DIM for tSCH cows than NC ones; 59.0 vs. 64.2 mg/dL, respectively. Bilirubin concentrations were higher (*P*<0.01) for pSCH cows on days 1, 2, and 4 than NC ones; 0.20, 0.26, and 0.21 mg/dL vs. 0.16, 0.21, and 0.18 mg/dL, respectively. Albumin concentrations were lower (*P*<0.05) on day 3 for pSCH and dSCH cows than for NC ones; 3.14 and 3.18 vs. 3.40 g/L, respectively. No differences were found (*P*>0.05) for BHB, AST, and cholesterol concentrations among postpartum Ca status groups.

Figure 3 shows the adjusted means of the evaluated metabolites in the first 4 DIM. The Ca category effect was significant (P <0.04) for Haptoglobin, (P <0.03) NEFA, (P <0.02) Albumin, (P <0.01) Bilirubin. For the other metabolites, Cholesterol, AST and Glucose there was no significant effect (P> 10) for the Ca category.

TABLE 1-2. Pearson's correlation coefficients among plasma Ca concentrations and metabolites up to four days after calving.

	SCa[1]d1	Hap[2] d1	BHB[3] d1	NEFA[4] d1	Alb[5] d1	Bil[6] d1	Chol[7] d1	AST[8] d1	Glu[9] d1
Plasma Ca d1	0.82	0.04	-	-0.45	-0.02	-0.57	0.04	-0.13	0.07
P value	<0.01	0.70	-	<0.01	0.79	<0.01	0.64	0.17	0.47
	SCa d2	**Hap d2**	**BHB d2**	**NEFA d2**	**Alb d2**	**Bil d2**	**Chol d2**	**AST d2**	**Glu d2**
Plasma Ca d2	0.81	-0.09	-	-	0.13	-0.43	0.06	-0.13	0.12
P value	<0.01	0.37	-	-	0.19	<0.01	0.51	0.17	0.24
	SCa d3	**Hap d3**	**BHB d3**	**NEFA d3**	**Alb d3**	**Bil d3**	**Chol d3**	**AST d3**	**Glu d3**
Plasma Ca d3	0.64	0.07	-	-	0.28	-0.06	0.24	0.08	-0.05
P value	<0.01	0.50	-	-	0.01	0.59	0.02	0.43	0.64
	SCa d4	**Hap d4**	**BHB d4**	**NEFA d4**	**Alb d4**	**Bil d4**	**Chol d4**	**AST d4**	**Glu d4**
Plasma Ca d4	0.75	-0.17	-0.15	-0.19	-0.15	-0.32	0.09	-0.13	0.08
P value	<0.01	0.09	0.16	0.11	0.13	<0.01	0.35	0.18	0.44

[1]SCa = serum Calcium; [2]Hap = Haptoglobin; [3]BHB = Beta-hydroxybutyrate; [4]NEFA= Non-esterified fatty acids; [5]Alb = Albumin; [6]Bil = Bilirubin; [7]Chol = Cholesterol; [8]AST = Aspartate Aminotransferase; [9]Glu = Glucose.

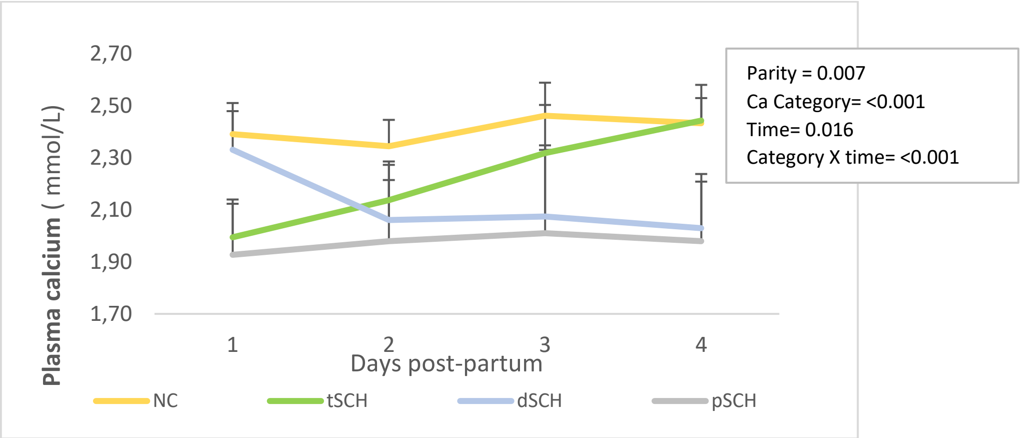
TABLE 1-3. Least square means for daily analysis of all metabolites according to postpartum Ca status groups.

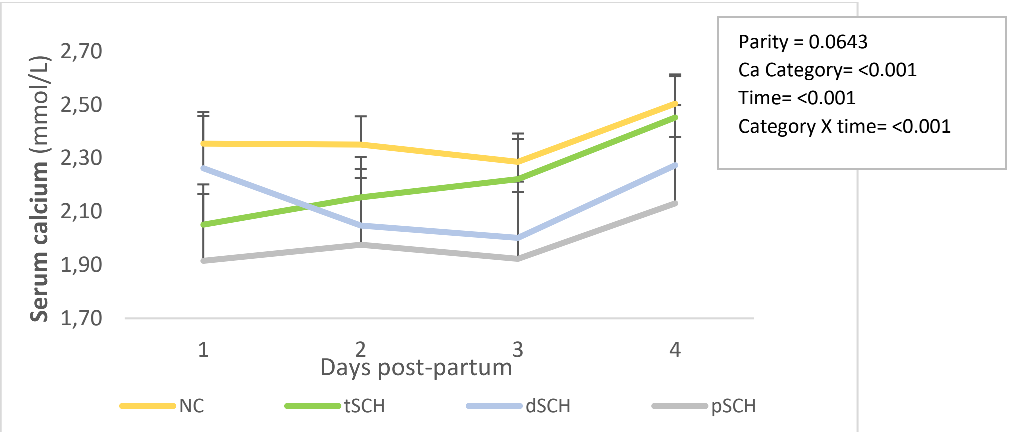
Metabolite	**NC[1]**	**tSCH[2]**	**dSCH[3]**	**pSCH[4]**	**SEM**	***P* value**
Plasma calcium (mmol/L)						
d1	2.375[a]	1.963[b]	2.336[a]	1.920[b]	0.153	<0.01
d2	2.339[a]	2.146[b]	2.043[b]	1.979[b]	0.177	<0.01
d3	2.476[a]	2.362[a]	2.085[b]	2.006[b]	0.235	<0.01
d4	2.445[a]	2.466[a]	2.027[b]	1.985[b]	0.172	<0.01
Serum calcium (mmol/L)						
d1	2.347[a]	2.037[b]	2.264[a]	1.912[b]	0.202	<0.01
d2	2.355[a]	2.159[b]	2.047[b]	1.977[b]	0.203	<0.01
d3	2.283[a]	2.210[a]	2.003[b]	1.921[b]	0.168	<0.01
d4	2.521[a]	2.478[a]	2.285[b]	2.136[b]	0.159	<0.01
Haptoglobin (mg/dL)						
d1	14,867	11.925	16.026	21.002	3.219	0.37
d2	19.031[b]	16.664[b]	20.462[a]	19.649[ab]	3.089	0.02
d3	23.195	18.260	22.140	18.578	4.240	0.39
d4	18.909	13.306	23.470	29.281	4.358	0.11
Albumin (g/dL)						
d1	3.298	3.287	3.192	3.221	0.054	0.43
d2	3.328	3.270	3.179	3.289	0.060	0.27
d3	3.403[a]	3.319[ab]	3.182[b]	3.135[b]	0.052	<0.01
d4	3.357	3.252	3.196	3.217	0.062	0.09

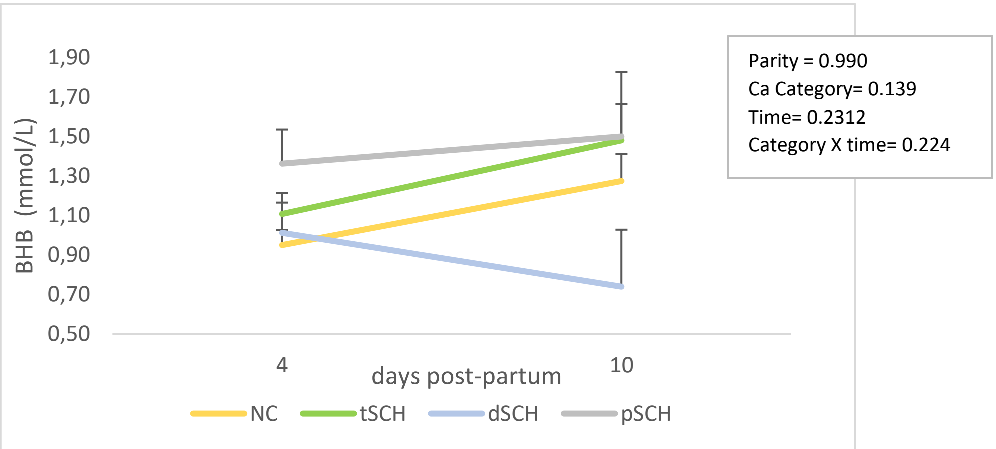
Bilirubin (mg/dL)						
d1	0.165^{b}	0.250^{a}	0.201^{ab}	0.230^{a}	0.026	<0.01
d2	0.208^{b}	0.296^{ab}	0.258^{ab}	0.443^{a}	0.051	0.01
d3	0.132	0.141	0.185	0.217	0.032	0.24
d4	0.185^{b}	0.182^{b}	0.211^{ab}	0.341^{a}	0.031	<0.01
Cholesterol (mg/dL)						
d1	54.322	54.663	54.827	55.792	2.180	0.97
d2	56.299	54.739	51.894	53.859	2.270	0.47
d3	58.819	56.441	55.896	53.685	2.295	0.36
d4	68.477	64.059	65.467	61.712	2.671	0.17
Glucose (mg/dL)						
d1	71.425	68.236	74.618	77.565	3.302	0.30
d2	64.335	63.012	67.168	62.065	2.286	0.56
d3	64.209^{a}	59.007^{b}	62.722^{ab}	62.989^{ab}	1.972	0.07
d4	62.984	57.774	62.392	56.034	9.103	0.19
AST (U/L)						
d1	90.078	99.666	88.171	91.850	6.284	0.44
d2	93.223	98.692	85.035	111.621	6.210	0.09
d3	94.765	103.832	94.415	110.290	4.692	0.05
d4	95.662	101.010	94.133	111.471	4.877	0.12
NEFA (mmol/L)						
d1	0.280	0.412	0.315	0.466	0.054	0.02
d4	0.203^{b}	0.274^{ab}	0.266^{ab}	0.454^{a}	0.065	0.07
BHB (mmol/L)						
d4	0.952	1.115	1.008	1.363	0.128	0.13
d10	1.248	1.424	0.783	1.500	0.247	0.30

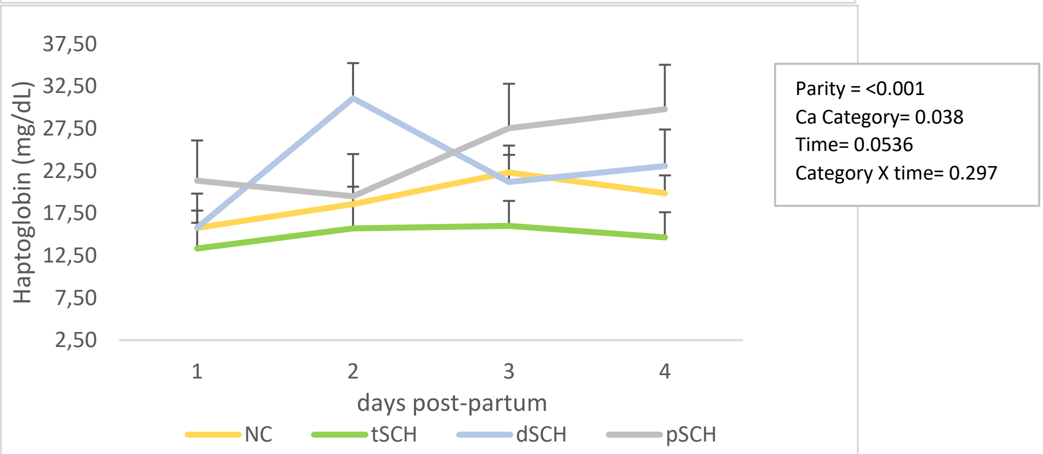
Means followed by the same letter in one line do not differ from each other with 5% of probability according to the Tukey test.

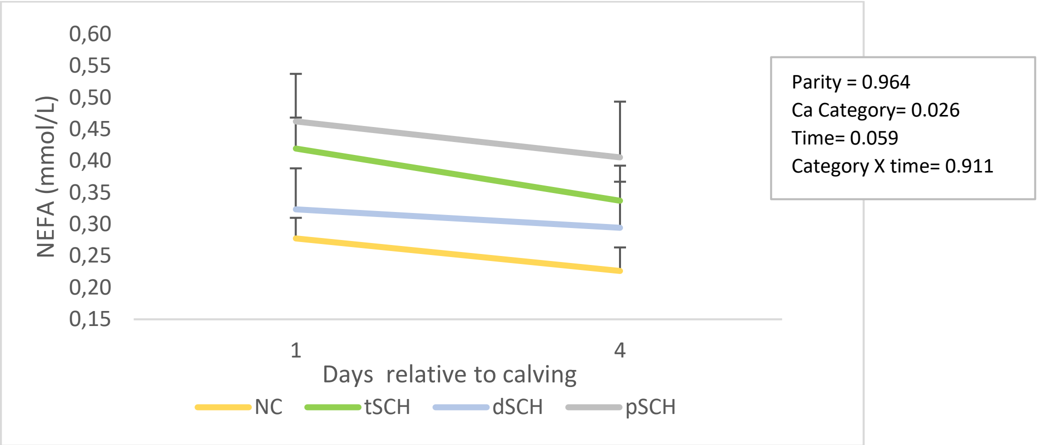
[1]Normocalcemic; [2]transient subclinical hypocalcemia; [3]delayed subclinical hypocalcemia; [4]persistent subclinical hypocalcemia.

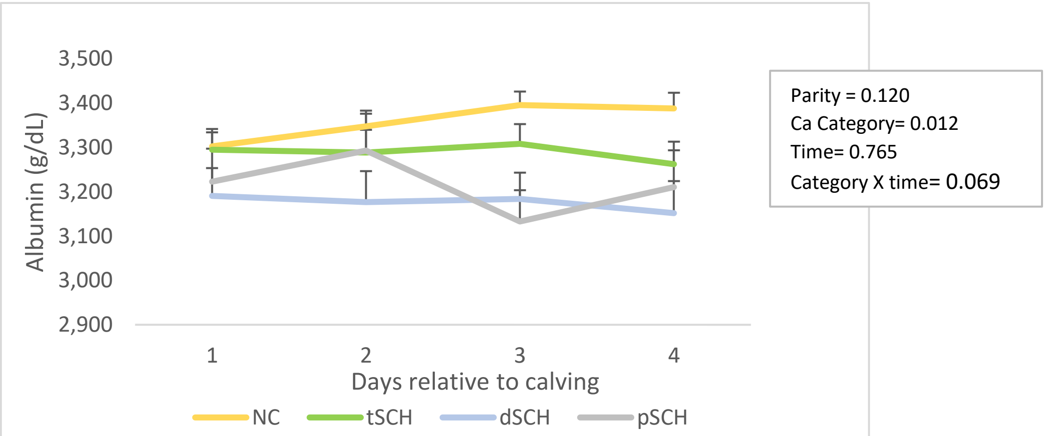

Plasma calcium (mmol/L)
2,70
2,50
2,30
2,10
1,90
1,70
1
2
3
4
Days post-partum
NC
tSCH
dSCH
pSCH
Parity = 0.007
Ca Category= <0.001
Time= 0.016
Category X time= <0.001

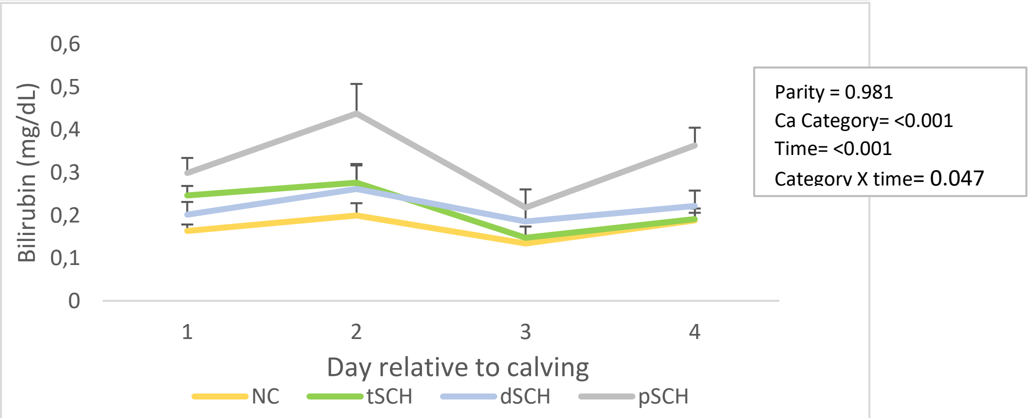

Serum calcium (mmol/L)
2,70
2,50
2,30
2,10
1,90
1,70
1
2
3
4
Days post-partum
NC
tSCH
dSCH
pSCH
Parity = 0.0643
Ca Category= <0.001
Time= <0.001
Category X time= <0.001

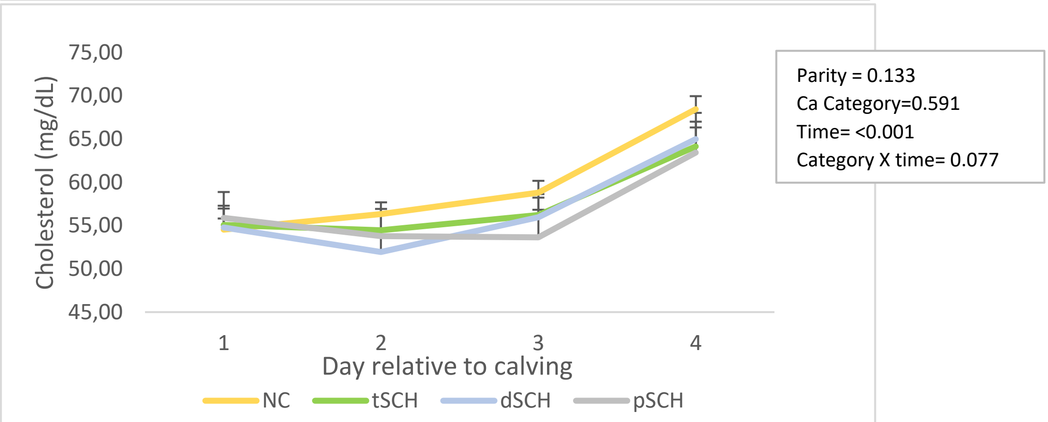

BHB (mmol/L)
1,90
1,70
1,50
1,30
1,10
0,90
0,70
0,50
4
10
days post-partum
NC
tSCH
dSCH
pSCH
Parity = 0.990
Ca Category= 0.139
Time= 0.2312
Category X time= 0.224


Haptoglobin (mg/dL)
37,50
32,50
27,50
22,50
17,50
12,50
7,50
2,50
1
2
3
4
days post-partum
NC
tSCH
dSCH
pSCH
Parity = <0.001
Ca Category= 0.038
Time= 0.0536
Category X time= 0.297


0,60
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
NEFA (mmol/L)
1
4
Days relative to calving
NC
tSCH
dSCH
pSCH
Parity = 0.964
Ca Category= 0.026
Time= 0.059
Category X time= 0.911


3,500
3,400
3,300
3,200
3,100
3,000
2,900
Albumin (g/dL)
1
2
3
4
Days relative to calving
NC
tSCH
dSCH
pSCH
Parity = 0.120
Ca Category= 0.012
Time= 0.765
Category X time= 0.069


0,6
0,5
0,4
0,3
0,2
0,1
0
Bilirubin (mg/dL)
1
2
3
4
Day relative to calving
NC
tSCH
dSCH
pSCH
Parity = 0.981
Ca Category= <0.001
Time= <0.001
Category X time= 0.047


75,00
70,00
65,00
60,00
55,00
50,00
45,00
Cholesterol (mg/dL)
1
2
3
4
Day relative to calving
NC
tSCH
dSCH
pSCH
Parity = 0.133
Ca Category=0.591
Time= <0.001
Category X time= 0.077

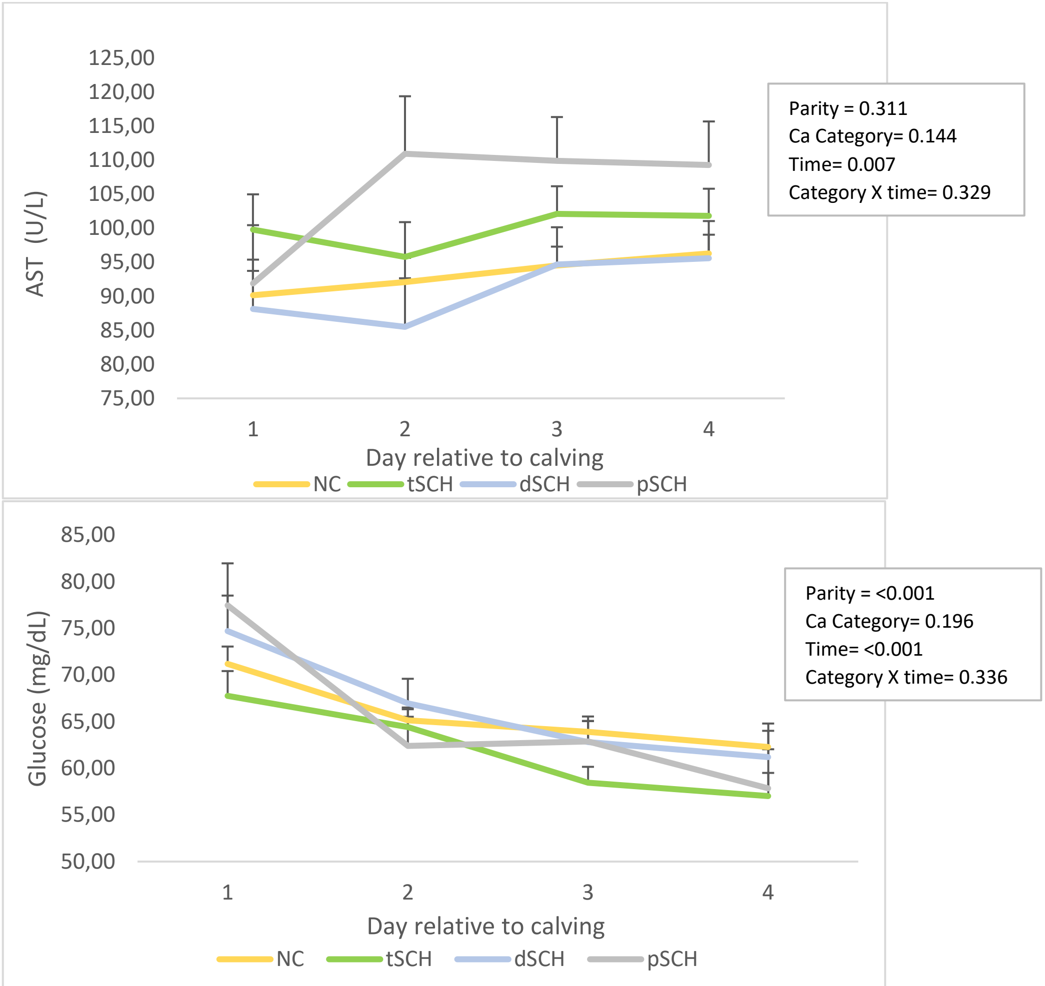


FIGURE 1-3. Least square means of each evaluated metabolite based on postpartum Ca status group. Yellow: normocalcemic (NC); green: transient subclinical hypocalcemia (tSCH); blue: delayed subclinical hypocalcemia (dSCH); grey: persistent subclinical hypocalcemia (pSCH).

1.5 DISCUSSION

We adopted the categorization of postpartum Ca status groups suggested by McArt and Neves (2020), but our calcemia values for each group were slightly different from those by the authors, due to the difference in the population used in our study, which has a distinct environment and breed described. McArt and Neves (2020) used the work of Neves et al. (2018) which results justify the adoption of different criteria for primiparous and multiparous, about the days in milk.

Neves et al. (2018), reports that primiparous who presented Ca ≤ 2.15 mmol / L on the 1st day after calving produced 2.9 kg more milk than those who presented this high value, and on the 2nd day when checking the Ca concentration ≤ 2.15 mmol / L, cows were 4X more likely to develop diseases such as metritis, abomasum displacement or both. For multiparous, when identifying Ca concentration ≤ 1.77 mmol / L on the 1st day, the cows produced 2.6 kg more milk than cows with higher Ca concentration, and when observing the Ca concentration ≤ 2.20 mmol / L on the 4th day, these cows produced 1.8 kg less milk and were 3X more likely to develop the aforementioned diseases.

For this reason, we chose days 1 and 2 for primiparous and days 1 and 4 for multiparous, for classification by Ca categories but adopted the cutpoint to all categories ≤ 2.15, mmol (Martinez et al., 2012), already consolidated in the literature, and not the thresholds mentioned in Neves et al. (2018), for the reason that our study population is quite diverse than the one studied by these authors.

Furthermore, our experimental cows received a diet with negative DCAD during prepartum and this probably reduced the occurrence of clinical and subclinical hypocalcemia, since a diet with a DCAD between -5 to -15 meq/100g can impact the plasma Ca concentrations postpartum and reduce the incidence of hypocalcemia (Leno et al., 2017; Santos et al., 2019). According to DeGroot et al. (2010), dairy cows not supplemented with acidogenic diets have an earlier drop of serum blood Ca compared with cows that received a negative DCAD diet at prepartum.

In the present study, 47.4% of cows were categorized as SCH, but this value not count supplemented cows with oral or intravenous Ca. A similar incidence of 51% of cows with SCH at calving was found by Chamberlin et al. (2013), having restored the Ca levels until the third day after calving. The prevalence of SCH in the United States was estimated between 50 and 70% (Goff, 2008; Santos, 2006; Moore et al., 2000). In Brazil, the prevalence of SCH in two high-producing Holstein herds was 78% (Carneiro et al., 2016).

The dynamics of the drop in blood Ca during postpartum is a natural and physiological finding for this period. It seems that the real problem is related to a pronounced drop of Ca concentration or the non-restoration of normal calcium levels, and with that, there are undesirable effects by decreasing muscle contraction and immune response (Goff and Horst 1997; Goff, 2008; Martinez et al., 2012). Thus, fresh cows are more prone to developed diseases such as downer cow syndrome, retained placenta, uterine prolapse, and displaced abomasum, as well as have lower reproductive performance and mastitis (Martinez et al., 2012; Chamberlin, et al., 2013).

A study by McArt and Neves (2020) found higher milk yields on tSCH cows compared with pSCH during the first week of lactation, and tSCH produced more milk for 10 weeks compared with dSCH. In our study, tSCH cows produced more milk than pSCH ones at the first 10 DIM, but we didn't find difference at 30 and 60 DIM. Martinez et al. (2012) did not find difference in milk yield between normocalcemic and SCH cows, probably because in this study the SCH cows weren't classified into persistent, transient and delayed hypocalcemic groups. But our work corroborates the finding that the calcium dynamic is different for each group of calcemia.

Cows with SCH shows higher NEFA concentrations at parturition and a higher proportion of lipids in the liver at 7 and 35 d compared with NC cows (Chamberlin et al., 2013). Prolonged episodes of SCH can cause a reduction of DMI due to the role of Ca in the contraction of muscles, decreasing ruminal motility, and increasing lipolysis, and NEFA concentrations as a consequence (Chamberlin et al., 2013). NEFA concentrations above 0.7 mmol/L in the postpartum period are related to a higher occurrence of several diseases during transition period (Chapinal et al., 2011). In our study, pSCH cows had higher concentrations of NEFA compared with NC cows (0.54 vs. 0.20 mmol/L) at 4 DIM. We believe that the pSCH group, having low Ca concentration for a longer time, faced a more pronounced negative energy balance than the NC group, resulting in greater NEFA concentrations. NEFA concentrations lower than 0.37 mmol/L at 3 DIM were associated with increased milk yield in the first 9 weeks of lactation (Menta et al., 2021). Different breeds or genetic groups can also result in variation in these results, as Jersey cows have adipose tissue mobilization in the postpartum for a shorter period compared to Holstein cows (Rastani et al., 2001), while using more energy for milk production (Olson et al., 2010).

Postpartum cows with negative energy balance and showing an inflammatory process have higher levels of bilirubin due to impaired liver function. Lower bilirubin concentration is an indicative of efficiency in the elimination of undesirable substances from the liver and this is associated with a better energy balance and a better metabolic status at the beginning of lactation (Assenat et al., 2004; Bertoni et al., 2008). In our study, NC cows showed lower levels of bilirubin compared with delayed and persistent SCH cows and that may be associated with well hepatocyte functionality. NC cows may experience a slight increase immediately before calving until 7 DIM in bilirubin levels (peak of 0.35 mg/dL) followed by a reduction after 4 to 5 weeks of lactation (0.12 mg/dL) (Bertoni et al., 2010).

The mean serum cholesterol values of healthy cows at calving are 85-96 mg/dL and these values change to 174-193 mg/dL at the end of the first month of lactation, due to the growing DM intake (Bertoni and Trevisi, 2010). In our study, cholesterol concentrations were lower (55-65 mg/dL) after calving, but it was not correlated with the Ca status groups or with DIM.

A recent study showed that a reduction in glucose concentration (≤2.96 mmol/L) may be associated with higher milk yield (Menta et al., 2021). In our work, tSCH cows showed a tendency for lower glucose concentration (3.27 mmol/L) and they showed higher milk yields. Glucose is known to be the main precursor of lactose synthesis, responsible for maintaining osmolarity, directly affecting the volume of produced milk (Cant et al., 2002).

1.7 CONCLUSIONS

In conclusion, transient SCH Jersey cows produce more milk at the first 10 DIM than persistent SCH cows and their metabolic profile are similar to normocalcemic cows. Therefore, even with higher milk yields, tSCH cows do not have major performance losses. Subclinical hypocalcemia is a natural and physiological part of postpartum and its occurrence at the first day of lactation can be non-detrimental.

1.8 ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the farmers who let this trial be carried out in his farm.

1.9 REFERENCES

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165–175. https://doi:10.4141/a04-043.

Assenat, E., S. Gerbal-Chaloin, D. Larrey, J. Saric, J. Fabre, P. Maurel, M. Vilarem, and J. M. Pascussi. 2004. Interleukin 1β inhibits CAR-induced expression of hepatic genes involved in drug and bilirubin clearance. Hepatology 40:951–960. https://doi:10.1002/hep.20387.

AOAC International. 1990. Official methods of analysis. Journal of Pharmaceutical Sciences, v. 1, n. Volume 1, p. 30.http://sutlib2.sut.ac.th/sut_contents/H125800.pdf%5Cnhttp://doi.wiley.com/10.1002/jps.260

Bertoni, G.; Trevisi, E. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Veterinary Clinics of North America - Food Animal Practice, v. 29, n. 2, p. 413–431. https://doi:10.1016/j.cvfa.2013.04.004.

Bertoni G, Lombardelli R, Piccioli-Cappelli F, et al. 2010. Main endocrine-metabolic differences between 1st and 2nd lactation of the dairy cows around calving. J. Dairy Sci. 93, E-Suppl.1:116.

Bertoni G, Trevisi E, Han X, et al. 2008. Effects of inflammatory conditions on liver activity in the puerperium and consequences for performance in dairy cows. J. Dairy Sci. 91:3300–10. https://doi: 10.3168/jds.2008-0995.

Carneiro, E. W.; Ichikawa, E. E.; Carneiro, D. M. V. F.; Almeida, R. D. 1225 Effects of oral calcium formate supplementation in peripartum dairy cows. J. Dairy Sci. 94, Suppl.5:589–590. https://doi:10.2527/jam2016-1225.

Caixeta, L. S., P. A. et al. 2017. Association between subclinical hypocalcemia in the first three days of lactation and reproductive performance of dairy cows. Theriogenology 94:1–7. 2017. https://doi.org/10.1016.01.039.

Chamberlin, W. G., J. R. et al. 2013.Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. J. Dairy Sci. 96:7001–7013. https://doi:10.3168/jds.2013-6901.

Chapinal, N., M. et al. 2011 The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897–4903. https://doi:10.3168/jds.2010-4075.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259–2273. https://doi:10.3168/jds.0022-0302(99)75474-3.

DeGaris P. J.; I. J. Lean. 1997. Milk fever in dairy cows: a review of pathophysiology and control principles. Vet. J. 2008:176:58e69. London, England https://doi:10.1016/j.tvjl.2007.12.029.

DeGroot, M. A.; Block, E.; French, P. D. 2010. Effect of prepartum anionic supplementation on periparturient feed intake, health, and milk production. J. Dairy Sci. 93:5268–5279. https://doi.org/10.3168/jds.2010-3092.

Goff, J. P., Horst, R. L., Jardon, P. W., Borelli, C., & Wedam, J. 1996. Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows. J. Dairy Sci, 79(3), 378–383. doi:10.3168/jds.s0022-0302(96)76375-0.

Goff, J. P., Horst, R. L. 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders. J. Dairy Sci, 80(7), 1260–1268. doi:10.3168/jds.s0022-0302(97)76055-7.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176:50–57. https://doi.org/10.1016/j.tvjl.2007.12.020.

Goff, J. P., A. Liesegang, and R. L. Horst. 2014. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. J. Dairy Sci. 97:1520–1528. https://doi.org/10.3168/jds.2013-7467.

Leno, B. M.; Ryan, C. M.; Stokol, T.; Kirk, D.; Zanzalari, K. P.; Chapman, J. D.; Overton, T. R. 2017. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. J. Dairy Sci. 100:4604-4622. https:// doi:10.3168/jds.2016-12221.

Martinez, N. et al. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158–7172. https://doi:10.3168/jds.2012-5812

McArt, J. A. A.; R. C. Neves. 2020. Association of transient, persistent, or delayed subclinical hypocalcemia with early lactation disease, removal, and milk yield in Holstein cows. J. Dairy Sci. 103:690–701. https://doi:10.3168/jds.2019-17191.

P.R. Menta; L. Fernandes; D. Poit; V.S. Machado; M.A. Ballou; R.C. Neves. 2021 Association of blood calcium concentration in the first 3 days after parturition and energy balance metabolites at day 3 in milk with disease and production outcomes in multiparous Jersey cows. J. Dairy Sci. https://doi.org/10.3168/jds.2020-19189

Neves, R. C. et al. 2018a Association of immediate postpartum plasma calcium concentration with early-lactation clinical diseases, culling, reproduction, and milk production in Holstein cows. J. Dairy Sci. 101:547–555. https://doi:10.3168/jds.2017-13313

Neves, R. C. et al. 2018. Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production. J. Dairy Sci. https://doi:10.3168/jds.2018-14587

Ospina P. A. et al. 2010. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546e54. https://doi: 10.3168/jds.2009-2277.

Reynolds, C. K. et al. 2003 Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201–1217. https://doi:10.3168/jds.0022-0302(03)73704-7.

Trebicka, J., A. Krag, S. Gansweid, B. Appenrodt, P. Schiedermaier, T. Sauerbruch, and U. Spengler. 2011. Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. Eur. J. Gastroenterol. Hepatol. 23:1218–1225. https:// doi:10.1097/meg.0b013e32834a75dc.

Van Soest, P.J.; Robertson, J. B.; Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Capítulo 2

Rumination time in early lactation Jersey cows: association on milk yield and composition, number of milkings and concentrate intake *by Fillus et al.* Monitoring rumination time after calving for lactating cows can bring interesting information about how well cows is performing, in an AMS systems cows with high rumination time produce more milk, higher number of milkings, and concentrate intake, but produces less fat and protein content.

Running Head: RUMINATION TIME IN JERSEY LACTATING COWS

Rumination time in early lactation Jersey cows: association on milk yield and its composition, number of milkings and concentrate intake

A. M. Fillus[*], R. Almeida[*†]

[*]Departmento de Zootecnia, Universidade Federal do Paraná, Curitiba, PR 80035-050 Brazil

†Corresponding author: mailto:ralmeida@ufpr.br

2.1 ABSTRACT

This study aimed to measure the association of rumination time up to 15 DIM on milk yield, milk fat and protein contents, number of milkings, and ingestion of concentrate on milking box, in the first 60 DIM of Jersey cows in an automated milking system (AMS). Three groups of rumination time were created by parity: high (HRT), medium (MRT), and low rumination time (LRT). Data were collected through the AMS system, from March 2016 to March 2020, totaling 895 lactations from 377 cows. From these 895 lactations, 31.8% were from primiparous (P1, n=285), 23.5% from second lactation cows (P2, n=210), and 44.7% were from cows with three or more lactation (P3, n=400). For the categorization of rumination groups, parity was taken into account, because previous analysis demonstrated that calving order impacts RT in the first 15 DIM. The adopted categorization was division into tertiles, wich divided third of the population by each parity. P1: High RT > 438 min/d; Medium RT 438 - 390 min/d; Low RT < 390 min/d; P2: High RT > 462 min/d; Medium RT 462 - 421 min/d; Low RT < 421 min/d; P3+: High RT > 450 min/d; Medium RT 450 - 403 min/d; Low RT < 403 min/d. Average rumination time in the first 15 DIM of P2 cows was greater (P<0.01) than on P1 and P3 cows; 435.05 vs. 422.59 and 411.50 min/d, respectively. HRT cows produced more milk (P<0.01) in the first 60 DIM than MRT and LRT cows; 29.41 vs. 28.13 and 27.47 kg/d, respectively. LRT cows showed greater (P<0.01) milk fat and protein indications than HRT and MRT cows; 4.24 vs. 4.12 and 4.16% milk fat, respectively, and 3.76 vs. 3.72 and 3.75% milk protein, respectively. HRT cows were milked more frequently (P<0.01) in the first 60 DIM than MRT and LRT cows; 3.66 vs. 3.56 and 3.52 times/d, respectively. And in the same manner, HRT cows ingested more concentrate in the robot (P<0.01) in the first 60 DIM than MRT and LRT cows; 5.87 vs. 5.80 vs. 5.81 kg/d, respectively. Cows that ruminated more during the 15 DIM produced more milk, in contrast cows with low rumination time produced higher estimated levels of fat and protein.

Key words: automatic milking, parity, rumination.

2.2 INTRODUCTION

Rumination is a cyclic process modified by regurgitation, remastigation and reswallowing (Bauchemin, 1991). The time spent for rumination in healthy ruminants is approximately 8 or 9 hours a day (Welch, 1982). This process is natural and biological and is influenced by several factors, like the ones related to the diet, in which the proportion of physically effective fiber is able to stimulate rumination (Kononoff et al., 2003a; Beauchemin and Yang, 2005). The composition and quality of forage and season of the year when heat stress occurs, can also affect rumination (Welch and Smith, 1970; Tapk and Sahin, 2006; Moallem et al., 2010).

Aikman et al. (2008) reported differences in chewing behavior between Holstein and Jersey cows. Jersey cows spends more time ruminating per unit of feed ingested and the volume of the regurgitated boluses in Jersey is lower when compared with Holstein cows (17.5 vs. 26.0 g of DM, respectively).

In dairy cattle herds, automated monitoring of the behavior of cows using non-invasive techniques is already a reality. For example, indirect methods make it possible to measure the daily rumination pattern and time through the movements of the jaw (Kononoff et al., 2002; Braun et al., 2015) or through the sound of chewing (rumination monitor Hr-Tag system, SCR Engineers Ltd., Netanya, Israel). These methods are an easy alternative to visual observations, which take time and manpower. These devices collect and store information on an ongoing basis, making it a useful management tool. In addition, the sensors can be associated with other technologies such as the automated milking system (AMS). This system integrates sensors that store different variables associated with milk quality and composition, number of milkings and feed intake in the milking unit (De Koning, 2010).

Important events can be predicted using RT, such as calving (Pahl et al., 2015; Schirmann et al., 2016), indicated by a reduction in the daily mean RT approximately 4 hours on the day of the partum (Soriani et al., 2012; Kaufman et al., 2016). Other studies also indicate a reduction on RT related to the appearance of diseases related to the digestive tract or metabolic disorders, such as subclinical ketosis, displaced abomasum, and ruminal acidosis (DeVries et al., 2009; Kaufman et al., 2016, Stangaferro et al., 2016a). Likewise, RT decrease during lameness and pneumonia events (King et al., 2017; King and DeVries, 2018). Also, cows that experienced a severe inflammatory response with a higher concentration of acute-phase proteins in the peripartum had a reduced RT at the beginning of lactation (Bertoni and Trevisi, 2013). Hypocalcemic cows at calving also have lower RT on first day of lactation compared to healthy cows (Liboreiro et al., 2015).

Most of the diseases mentioned above occur mainly in the beginning of the lactation, also known as the transition period for dairy cows. These health disorders at the beginning of the postpartum period affect a substantial portion of the lactating dairy cows, from 30 to 50% of fresh cows, with negative impact for their health, well-being and performance (Ingvarsten, 2006). Another consequence of low RT around calving is related to milk yield; in several studies it was found a relationship between RT and milk yield. In general, cows with lower RT in the first 10 DIM have less milk production and more health problems (DeVries et al., 2009; Soriani et al., 2012; Kaufman et al., 2018; Peiter et al., 2021).

Rumination patterns were evaluated in relation to the diets fed to dairy cows by Nørgaard et al. (2003) and with several diseases by Stangaferro et al. (2016 a,b,c), but there is little information about how a rumination pattern is influenced by environment, housing and management system. When cows are milked there is an activation of the brain center that stimulate hormonal release of oxytocin and increases motivation for rumination (Andersson, 1958).

As far as we know, few studies in Jersey cows relate and identify the behavior and the relationship of the rumination time after calving with some productive variables. The aim of this study was to measure the effects of rumination time up to 15 DIM in relation to milk yield and its solids contents, number of milkings, and concentrate intake of Jersey cows maintained in an AMS.

2.3 MATERIAL AND METHODS

2.3.1 Cows, experimental design and treatments

The study was conducted in a commercial farm in Southern Brazil. The herd had 330 lactating Jersey cows housed in a compost barn with six automated milking system (AMS) (Lely® model Astronaut - AMS®) with free flow to milking units, resting area and feeding area. Mean BW and milk yield of cows (mean ± SD) were 480 ± 50.0 kg of BW, and 30.0 ± 6.5 kg of milk/d, respectively. The cows had access to partial mixed ration (PMR - forage sources + 2 kg of the concentrate) offered three times a day (0800, 1200 and 1700 h) and the rest of the concentrate was offered during milking.

The AMS software provided daily averages of milk yield, predicted fat and protein percentages, number of visits to the milking unit and the concentrate intake in the milking unit up to 60 days of lactation. For rumination, we used the average RT up to the first 15 DIM. Data of 895 lactations from 377 cows were retrieved from the beginning of the activities on the farm, from March 2016 to March 2020.

The analysis of milk composition was performed through the Milk Quality Control system (MQC-2) that is integrated to the AMS. The system, when carrying out fat and protein analyzes, considers the term “fat indication” and “protein indication”, because the system does not directly measure the actual contents of milk fat and protein, but it does provide an indication of these levels. From a bulk tank sample carried out in the Centralized Milk Analysis Laboratory of the Associação Paranaense de Criadores de

Bovinos da Raça Holandesa (APCBRH – Paraná, Brazil) which calibrates weekly the equipment.

For rumination data, a monitoring collar (Hi-Tag SCR, Allflex® and Lely®, validated by Schirmann et al., 2009) was used in this farm. The collars are composed of microphones that identify the rumination sound of the cows through lactation and dry periods. The data collected is also integrated to the AMS and recorded by Lely's operational management software – Time for Cows (T4C®).

2.3.2 Study groups

The cows were grouped into high, medium and low rumination time by calving order - primiparous (P1), secondiparous (P2), and three calvings or more (P3+). We used the rumination data up to the first 15 DIM and the groups were defined by the tertiles-two points that divide an ordered distribution into three parts, each containing a third of the population divided by each parity.

- P1: High RT > 438 min/d; Medium RT 438 - 390 min/d; Low RT < 390 min/d;
- P2: High RT > 462 min/d; Medium RT 462 - 421 min/d; Low RT < 421 min/d;
- P3+: High RT > 450 min/d; Medium RT 450 - 403 min/d; Low RT < 403 min/d.

2.3.3 Statistical Analysis

The FREQ, MEANS, and CORR procedures of SAS (v.9.4) were used to perform descriptive statistics. In order to verify if parity impacts RT up to 15 DIM, statistical analysis was performed by the GLM procedure of SAS (v.9.4). For the analysis of the remaining variables with repeated measures over time (from parturition until 60 DIM), the MIXED procedure of SAS (v.9.4) was used, with the inclusion of parity and RT category as fixed effects and the inclusion of cow within RT category as random effect. The covariance structure was defined according to the lowest value obtained by "Akaike's Information Criterion Corrected" (AICC). For results interpretation and discussion, a significant effect was adopted when $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ was considered a tendency.

2.4 RESULTS

From the 895 lactations data set, 31.8% were from primiparous (P1 n=285), 23.5% from secundiparous (P2 n=210), and 44.7% were from cows with three or more parities (P3+ n=400). Descriptive statistics showed in Table 2-1.

TABLE 2-1. General means, SD and quantiles values <10% and >90% for the studied variables up to 60 DIM.

Variable	n	Mean	SD	<10%	>90%
RT[1]	14,284	422.34	107.39	272	541
MY[2]	54,496	28.60	8.35	17.5	38.8
Fat Ind[3]	54,327	4.18	1.03	3.16	5.18
Prot. Ind[4]	54,320	3.74	0.47	3.31	4.25
Milkings[5]	54,369	3.59	1.11	2.0	5.0
Conc Intake [6]	54,471	5.85	1.99	2.92	8.20

[1]RT = rumination time (min/d); [2]MY = milk yield (kg/d); [3]Fat Ind = milk fat indication (%); [4] Prot. Ind = milk protein indication (%); [5]Milkings = number of milkings on AMS (n/d); [6] Conc Intake = concentrate intake on AMS (kg/d).

Table 2-2 shows that P2 cows ruminated more (*P*<0.01) than P1 and P3+ cows; 435.04 vs. 422.59 and 411.80 min/d, respectively. As expected, multiparous P3 cows produced more milk (*P*<0.01) up to 60 DIM than P2, and P2 cows have higher (*P*<0.01) milk yields than P1 cows; 31.67 vs. 29.80 vs. 23.40 kg/d, respectively. Multiparous P3+ cows produced more (*P*<0.01) milk fat and protein than P1 cows; 4.26%F and 3.75%P vs. 4.07%F and 3.71%P, respectively. The average number of milkings in the first 60 DIM was lower (*P*<0.01) for P1 cows than P2 and P3+; 2.96 vs 3.92 and 3.86, respectively. In the same way, the intake of concentrate in the robot was lower (*P*<0.01) for P1 cows compared to P2 and P3+ cows (5.05 vs. 6.25 and 6.23 kg/d, respectively; *P*<0.01).

TABLE 2-2. Test of comparison the adjusted means of variables according parity order.

Parity	RT[1] (min/d)	MY[2] (kg/d)	Fat. Ind[3] (%)	Prot. Ind[4] (%)	Milkings[5] (n/d)	Conc Intake[6] (kg/d)
P1	411.80 ^{b}	23.40 ^{c}	4.07 ^{b}	3.71 ^{b}	2.96 ^{b}	5.05 ^{b}
P2	435,05 ^{a}	29.80 ^{b}	4.17 ^{ab}	3.73 ^{ab}	3.92 ^{a}	6.25 ^{a}
P3+	422.59 ^{b}	31. 67 ^{a}	4.26 ^{a}	3.75 ^{a}	3.86 ^{a}	6.23 ^{a}

* All variables had P-value <0.01.
Means followed by the same letter in the column do not differ by Tukey's test (P <0.05)
[1]RT = rumination time (min/d); [2]MY = milk yield (kg/d); [3]Fat Ind = milk fat indication (%); [4] Prot. Ind = milk protein indication (%); [5]Milkings = number of milkings on AMS (n/d); [6] Conc Intake = concentrate intake on AMS (kg/d).

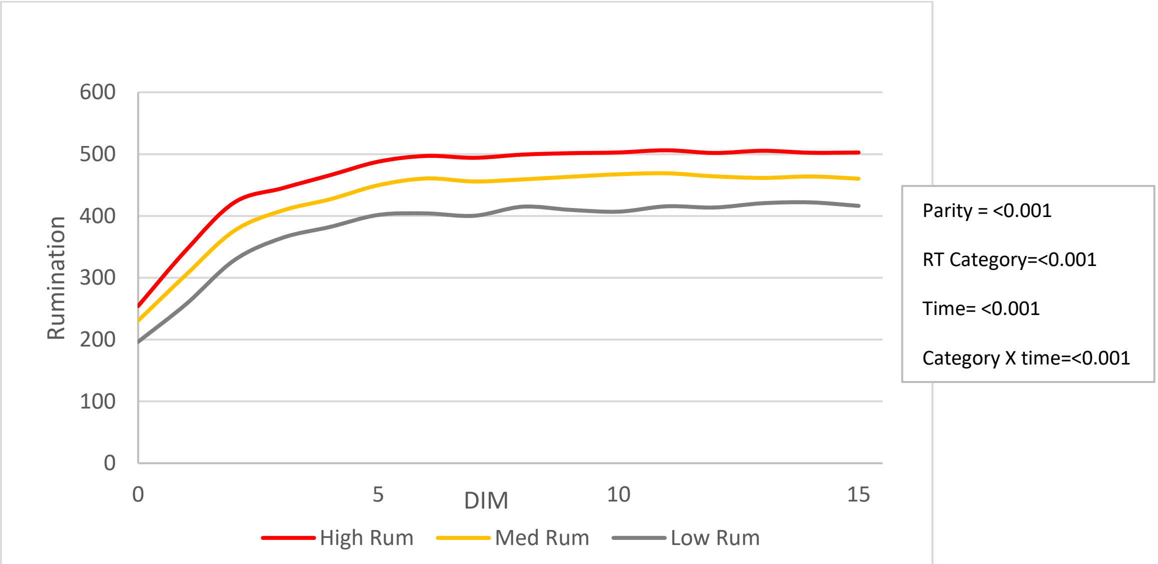
Table 2-3 and Figure 2-1 show the adjusted means for each group of rumination time. HRT cows produced more milk (*P*<0.01) than MRT and LRT cows; 29.41 vs. 28.13 and 27.47 minutes, respectively. LRT cows had greater (*P*<0.01) milk fat and protein indications than HRT and MRT cows; 4.24%F and 3.76%P vs. 4.12%F and 3.72%P, and 4.16%F and 3.75%P, respectively. HRT cows showed a greater (*P*<0.01) number of milkings than MRT and LRT cows; 3.66 vs. 3.56 and 3.52 milkings per day, respectively. And following the same pattern, HRT cows had a greater (*P*<0.01) concentrate intake than MRT and LRT cows; 5.87 vs. 5.80 and 5.81 kg/d, respectively.

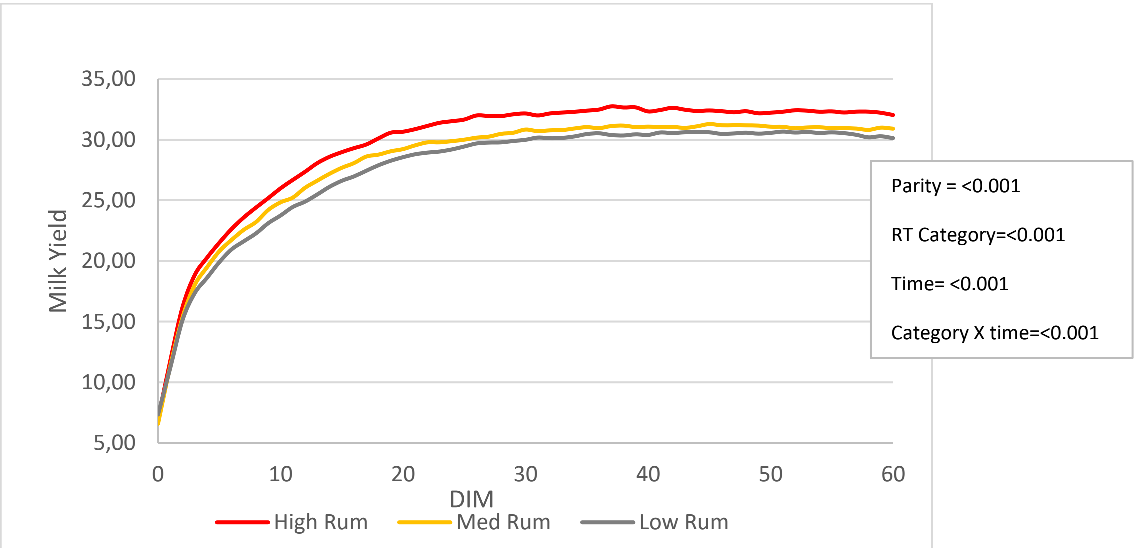
TABLE 2-3. Least square means ± standard error, for daily analysis of all variables according to rumination status groups.

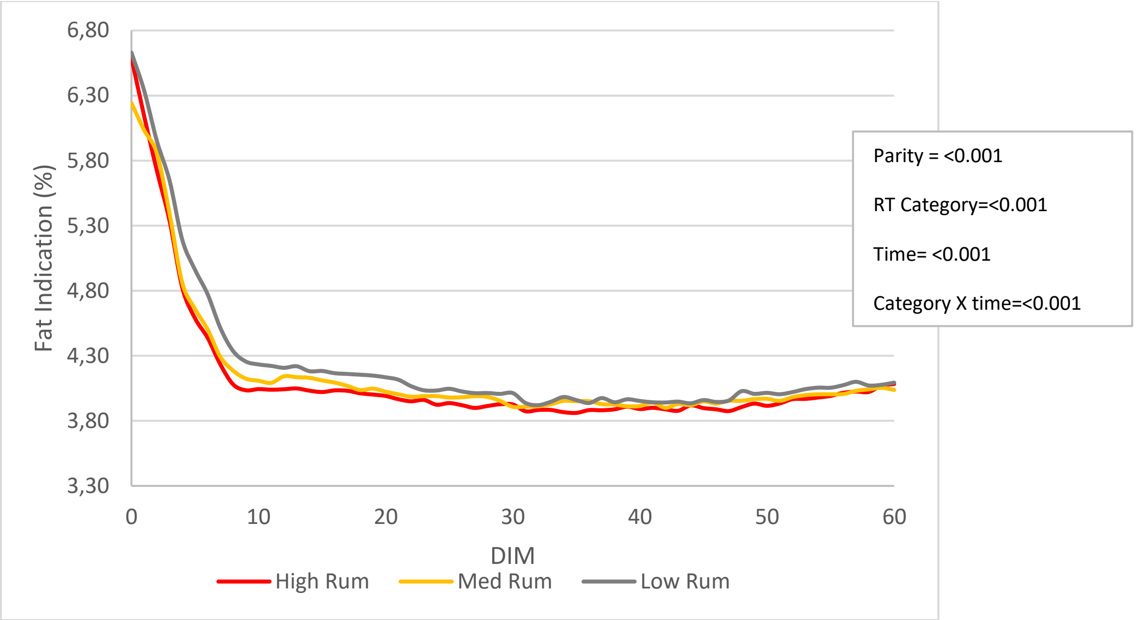
RT Category	RT[1] (min/d)	MY[2] (kg/d)	Fat Ind[3] (%)	Prot. Ind[4] (%)	Milkings[5] (n/d)	Conc Intake[6] (kg/d)
High RT (n=300)	464.55 ± 1.56	29.41 ± 0.32	4.12 ± 0.01	3.72 ± 0.01	3.66 ± 0.01	5.87 ± 0.01

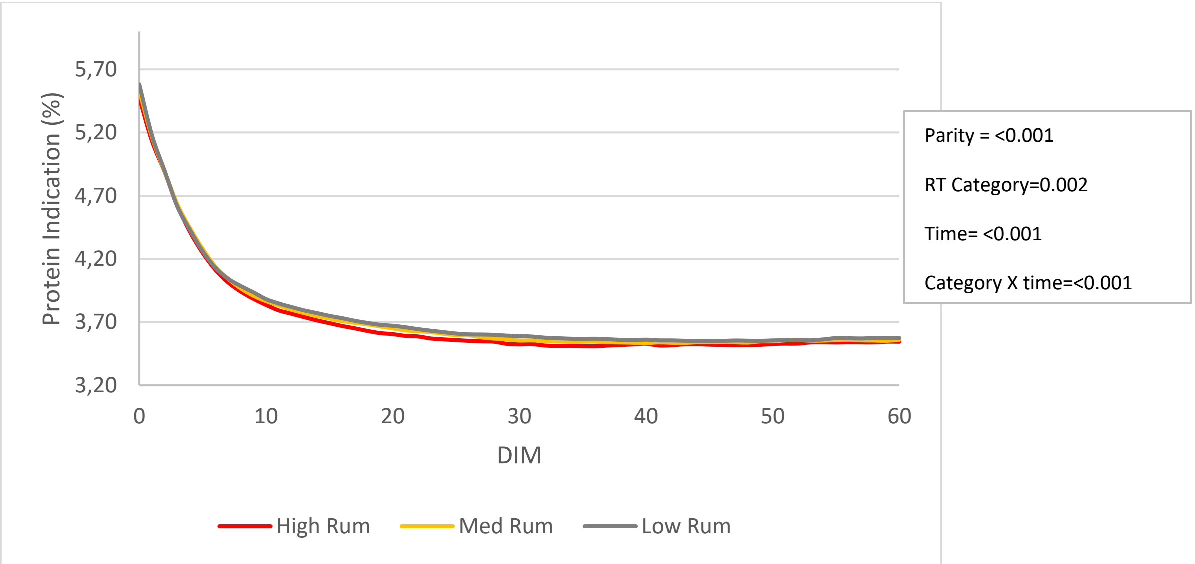
Medium RT (n=287)	426.42 ± 1.59	28.13 ± 0.31	4.16 ± 0.01	3.75 ± 0.01	3.56 ± 0.01	5.80 ± 0.01
Low RT (n=308)	378.45 ± 1.54	27.47 ± 0.31	4.24 ± 0.01	3.76 ± 0.02	3.52 ± 0.01	5.81 ± 0.01

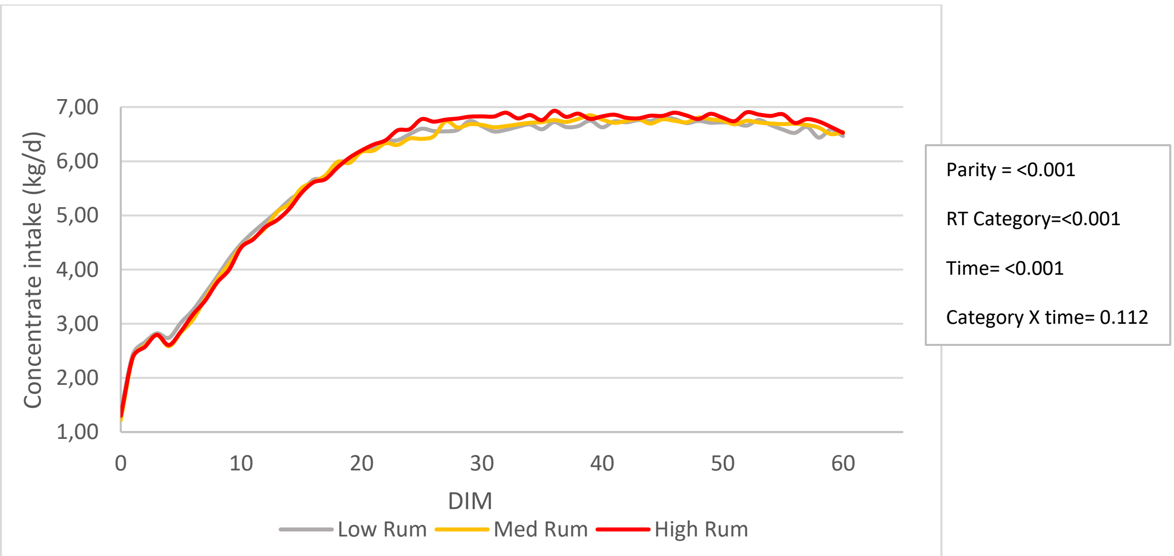
* All variables had *P*-values < 0.01; Prot. Ind. *P* < 0.05.
[1]RT = rumination time (min/d); [2]MY = milk yield (kg/d); [3]Fat Ind = milk fat indication (%); [4] Prot. Ind = milk protein indication (%); [5]Milkings = number of milkings on AMS (n/d); [6] Conc Intake = concentrate intake on AMS (kg/d).






Fat Indication (%)
6,80
6,30
5,80
5,30
4,80
4,30
3,80
3,30
0 10 20 30 40 50 60
DIM
High Rum
Med Rum
Low Rum
Parity = <0.001
RT Category=<0.001
Time= <0.001
Category X time=<0.001


Protein Indication (%)
5,70
5,20
4,70
4,20
3,70
3,20
0 10 20 30 40 50 60
DIM
High Rum
Med Rum
Low Rum
Parity = <0.001
RT Category=0.002
Time= <0.001
Category X time=<0.001


Concentrate intake (kg/d)
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0 10 20 30 40 50 60
DIM
Low Rum
Med Rum
High Rum
Parity = <0.001
RT Category=<0.001
Time= <0.001
Category X time= 0.112

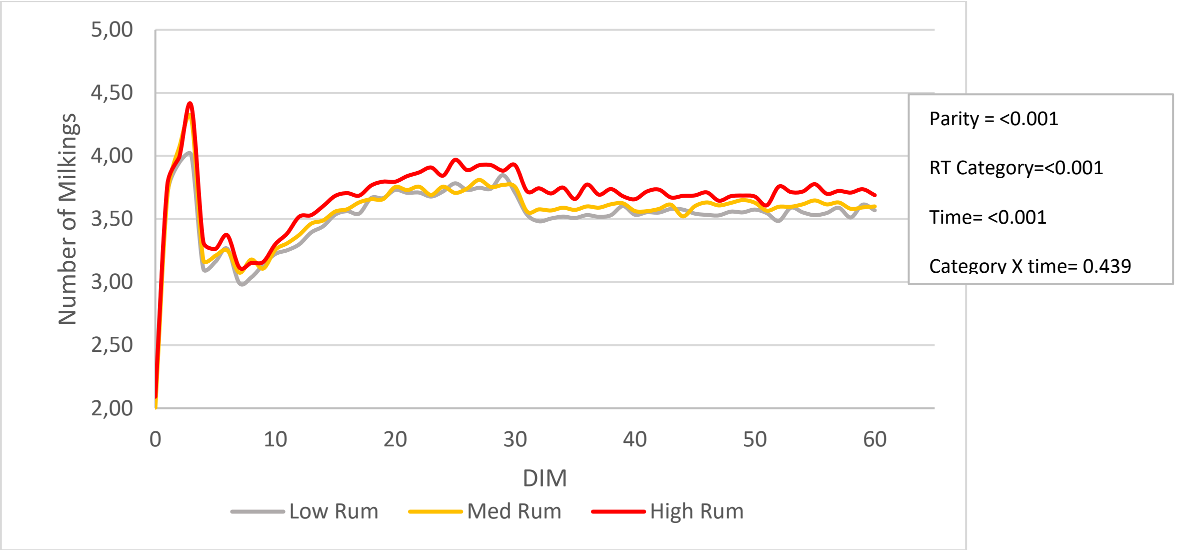


FIGURE 2-1. Adjusted means up to 60 DIM for rumination time, milk yield, milk fat indication, milk protein indication, number of milkings and concentrate intake of Jersey cows according to rumination time category. Red line: high rumination; yellow line: medium rumination; grey line: low rumination.

2.5 DISCUSSION

Concerning rumination time after calving, we can notice that primiparous cows had lower RT behavior than multiparous cows. According to Soriani et al. (2012), primiparous cows have lower RT up to 10 DIM than multiparous ones, probably due to the greater stress of environmental changes in early lactation. In an experiment conducted by Kaufman et al. (2018), with a monitoring period of 4 weeks, the RT was 442, 488, and 488 min/d for P1, P2 and P3 cows, respectively. Primiparous normally ruminate less probably because they have lower DMI compared to multiparous cows (Janovick and Drackey, 2010), which is expected since ruminal capacity is positively correlated with body size (De Boever et al., 199). In our study, interestingly, P1 cows ruminated the same time than P3 cows, whereas secundiparous cows had higher RT than both P1 and P3 cows. This intermediate RT found for older cows is probably because the multiparous cows were more challenged in the postpartum period as they are more prone to developing diseases, but more information is needed to state this.

The traffic of cows adopted by the AMS must consider a dominance between cows, as it can aggravate a competition for food and also the number of milking throughout the day, allowing the more submissive animals remain idle (Olofsson, 1999). Despite not having measured the social hierarchy in our study, according to the author of the previous statement, the highest rate of rumination was of dominant cows that tend to spend more time chewing instead of waiting for their time to feed or to be milked in the box.

Calamari et al. (2014) found an association between RT and milk yield for P2 and P3 cows and identified that cows with higher RT during 3 to 6 DIM had a higher milk yield during the first month of lactation, resulting in almost 8 kg of milk extra per day. In another study, it was reported that milk yield between 4 and 28 DIM increased 0.18 kg/d and 0.45 kg/d for P1 and P2 cows, respectively, for each additional 30 minutes in daily RT (Kaufman et al., 2018). These studies are in according with our findings, in which higher RT cows in the first 15 DIM produced more milk up to 60 DIM.

According to Wagnerstorch and Palmer (2003) and Svennersten-Sjaunja et al. (2000), the increase in the milking frequency from 2 times to 2.5 times/d showed a tendency to increase milk yield by 2 to 7%. Our results corroborate this; P1 cows were milked 2.96 times/day and they produced less milk yield compared to P2 and P3 cows, which were milked 3.92 and 3.86 times/d, respectively, and consequently they produced more milk (29.80 and 31.67 kg/d, respectively) up to 60 DIM. However, this date requires cautiously interpretation since the number of milkings in the AMS is defined through milking permission by the system that is influenced by milk yield, calving order and DIM. Therefore, the highest permission for the number of milking is given to cows that produced more milk, and physiologically we understand that P1 still less milk, because they have not reached their full productive potential.

Milking systems that adopt free traffic AMS had higher forage intake and higher RT compared to guided flow AMS (Melin et al., 2007). Cows of higher social dominance, typically older and heavier cows, enter the milking unit more frequently without spending time in a line, whereas submissive cows remain a longer total waiting time in front of the AMS. In addition, these submissive cows spent less time in the rest area (Bach et al., 2009). In this particular herd, this effect is minimized because primiparous cows are kept separated from the older cows, so they have their specific AMS and they compete only with other first lactation cows.

The average number of daily milkings per cow in the AMS over the entire lactation period is generally in the range of 2.5 to 3.0 times/day, but large differences in milking intervals are reported by commercial farms (De Konning, 2011). In our study, because we analyzed only the milking frequency up to 60 DIM, the number of milkings was even greater and was distinct for the RT groups; 3.66, 3.56, and 3.52, respectively for HRT, MRT and LRT. Another factor that may impact the number of milkings in AMS is the composition and palatability of the concentrate fed in the milking unit, since cows tend to go to milking unit more often when the concentrate is more attractive (Madsen et al., 2010). In Figure 2.1, the graphs for concentrate intake and number of milkings follow the same trend. The AMS management software defines the increase in concentrate supplied gradually, as the days in milk progress and the milk yield change. In our study, the HRT cows were milked more often and they had a greater concentrate intake.

After calving, the RT quickly return to the values observed at the final days of pregnancy; according to Calimari (2014), the RT values stabilize between 5 and 15 days of lactation. In another study (Bar and Solomon, 2011), the RT stabilized by 5 DIM for health cows. In Figure 2.1, the graph for rumination shows that the RT reached stability around 15 DIM, similar that mentioned by these authors. Ideally, RT should be monitored continuously, not just in critical moments as postpartum, since RT can be used as an index of the welfare of the cows (Calimari, 2014).

In this work, fat and protein indications showed a negative correlation with RT and evidence indicates that greater milk yield has a dilution effect on milk components (Chalupa and Sniffen, 2000). Thus, for the cows that ruminated more, the milk fat content may have been lower because they were also the cows with the highest milk production. Johnson and DeVries (2018) also observed this behavior trend for the milk components associated with RT, in which RT > 450 min made the correlation with fat even more negative.

Unlike these previous citations, DeVries et al. (2009) stated that high-producing cows are HRT cows, as they spend more time ruminating to compensate for the lower rumen pH, and that they may be associated with milk fat depression.

There are several factors related to the animal or to the environment that impact the rumination. The drop in RT culminates in a worsening of several factors demonstrated in our study, as an RT relation with all variables was found. The sooner this difference in RT pattern is noticed, the smaller are the losses in milk yield and health.

2.6 CONCLUSIONS

Cows that ruminated more during the first 15 DIM had higher milk yield, visited the milking unit more frequently, had greater intake of concentrate, and had lower estimated fat and protein contents.

2.7 ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the farmer who let this trial be carried out in his farm.

2.8 REFERENCES

Aikman, P. C., C. K. Reynolds, and D. E. Beever. 2008. Diet digestibility, rate of passage, and eating and rumination behavior of Jersey and Holstein cows. J. Dairy Sci. 91:1103–1114. https://doi .org/10.3168/jds.2007-0724.

Andersson, B., 1958. Some observations on the neuro-hormonal regulation of milk ejection. Acta Physiol. Scand. 23:1–7.

Bach, A., Devant M., Igleasias C., Ferrer A. 2009. Forced traffic in automatic milking systems effectively reduces the need to get cows, but alters eating behavior and does not improve milk yield of dairy cattle, J. Dairy Sci. 92:1272–1280.

Bar, D., and R. Solomon. 2010. Rumination collars: What can they tell us? Pages 214–216 in Proc. First N. Am. Conf. Precision Dairy Management, Toronto, Canada.

Bertoni, G., and Trevisi, E. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Veterinary Clinics of North America: Food Animal Practice 29:413–431. doi: 10.1016/j.cvfa.2013.04.004

Braun, U., S. Zürcher, and M. Hässig. 2015. Evaluation of eating and rumination behavior in 300 cows of three different breeds using a noseband pressure sensor. BMC Vet. Res. 11:231. https://doi.org/ 10.1186/s12917-015-0549-8.

Calamari, L., N. Soriani, G. Panella, F. Petrera, A. Minuti, and E. Trevisi. 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. J. Dairy Sci. 97:3635–3647. https://doi.org/10.3168/jds.2013-7709.

Chalupa, W., and C. J. Sniffen. 2000. Balancing rations for milk components. Adv. Dairy Technol. 12:27–42.

De Boever, J. L., J. I. Andries, D. L. De Brabander, B. G. Cottyn, and F. X. Buysse. 1990. Chewing activity in ruminants as a measure of its physical structure—A review of factors affecting it. Anim. Feed Sci. Technol. 27:281–291. https://doi.org/10.1016/0377 - 8401(90)90143-V.

De Koning, C.J.A.M, 2010. Automatic milking - common practice on dairy farms. Proceedings of the First North American Conference on Precision Dairy Management, p.52-67.

DeVries, T. J., K. A. Beauchemin, F. Dohme, and K. S. Schwartzkopf Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. J. Dairy Sci. 92:5067–5078. https://doi.org/10.3168/jds.2009-2102.

Ingvartsen, K. L. 2006. Feeding- and management-related diseases in the transition cow. Anim. Feed Sci. and Tech. 126:175–213. doi: 10.1016/j.anifeedsci.2005.08.003

Janovick, N. A., and J. K. Drackley. 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows. J. Dairy Sci. 93:3086–3102. https://doi.org/10.3168/jds.2009-2656.

Johnston, C., & DeVries, T. J. 2018. Short communication: Associations of feeding behavior and milk production in dairy cows. J. Dairy Sci. 101:3367–3373. doi:10.3168/jds.2017-13743

Kaufman, E. I., S. J. LeBlanc, B. W. McBride, T. F. Duffield, and T. J. DeVries. 2016. Association of rumination time with subclinical ketosis in transition dairy cows. J. Dairy Sci. 99:5604–5618. https://doi.org/10.3168/jds.2015-10509.

Kaufman, E. I., V. H. Asselstine, S. J. LeBlanc, T. F. Duffield, and T. J. DeVries. 2018. Association of rumination time and health status with milk yield and composition in early-lactation dairy cows. J. Dairy Sci. 101:462–471. https://doi.org/10.3168/jds.2017-12909.

King, M. T. M., and T. J. DeVries. 2018. Graduate Student Literature Review: Detecting health disorders using data from automatic milking systems and associated technologies. J. Dairy Sci. 101:8605–8614. https://doi.org/10.3168/jds.2018-14521.

King, M.T.M., Leclanc, S.J., Pajor, E.A., And DeVries, T.J. 2017. Cow-level associations of lameness, behavior, and milk yield of cows milked in automated systems**.** J. Dairy Sci. 100:4818-4828. 2004.

Kononoff, P. J., A. J. Heinrichs, and H. A. Lehman. 2003. The effect of corn silage particle size on eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. J. Dairy Sci. 86:3343–3353. https://doi.org/10.3168/jds.S0022-0302(03)73937 -X.

Kononoff, P. J., H. Lehman, and A. Heinrichs. 2002. Technical note— A comparison of methods used to measure eating and rumination activity in confined dairy cattle. J. Dairy Sci. 85:1801–1803. https://doi.org/10.3168/jds.S0022-0302(02)74254-9.

Liboreiro, D. N., K. S. Machado, P. R. B. Silva, M. M. Maturana, T. K. Nishimura, A. P. Brandão, M. I. Endres, and R. C. Chebel. 2015. Characterization of peripartum rumination and activity of cows diagnosed with metabolic and uterine diseases. J. Dairy Sci. 98:6812–6827. https://doi.org/10.3168/jds.2014-8947.

Madsen J., Weisbjerg M.R., Hvelplund T. 2010. Concentrate composition for automatic milking systems effect on milking frequency, Livest. Sci. 127:45–50.

Melin, M., Pettersson, G., Svennersten-Sjaunja, K., & Wiktorsson, H. 2007. The effects of restricted feed access and social rank on feeding behavior, ruminating and intake for cows managed in automated milking systems. Applied Animal Behaviour Science 107:13–21. doi: 10.1016/j.applanim.2006.09.026

Moallem, U., G. Altmark, H. Lehrer, and A. Arieli. 2010. Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. J. Dairy Sci. 93:3192–3202.

Nørgaard, P., E. Nadeau, and Å. T. Randby. 2011. A new Nordic structure evaluation system for diets fed to dairy cows: A meta-analysis. Pages 127–132 in NorFor—The Nordic Feed Evaluation System. H. Volden and A. H. Gustafsson, ed. Springer, Berlin, Germany.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82:69–79.

Pahl, C., E. Hartung, K. Mahlkow-Nerge, and A. Haeussermann. 2015. Feeding characteristics and rumination time of dairy cows around estrus. J. Dairy Sci. 98:148–154. https://doi.org/10.3168/jds.2014 -8025.

Peiter, M., N. Hannah, Phillips, and M. Endres. 2021. Association between early postpartum rumination time and peak milk yield in dairy cows: Journal of Dairy Science, J. Dairy Sci. 104 https://doi.org/10.3168/jds.2020-19698

Schirmann, K., D. M. Weary, W. Heuwieser, N. Chapinal, R. L. A. Cerri, and M. A. G. von Keyserlingk. 2016. Short communication: Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. J. Dairy Sci. 99:9917–9924. https://doi.org/10.3168/jds.2015-10548.

Schirmann, K., M. A. von Keyserlingk, D. M. Weary, D. M. Veira, and W. Heuwieser. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052–6055. https://doi.org/10.3168/jds.2009-2361.

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

Stangaferro, M. L., R. Wijma, L. S. Caixeta, M. A. Al-Abri, and J. O. Giordano. 2016a. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metaóbolic and digestive disorders. J. Dairy Sci. 99:7395–7410. https:// doi.org/10.3168/jds.2016-10907.

Stangaferro, M. L., R. Wijma, L. S. Caixeta, M. A. Al-Abri, and J. O. Giordano. 2016b. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. J. Dairy Sci. 99:7411–7421. https://doi.org/10.3168/jds .2016-10908.

Stangaferro, M. L., R. Wijma, L. S. Caixeta, M. A. Al-Abri, and J. O. Giordano. 2016c. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. J. Dairy Sci. 99:7422–7433. https://doi.org/10.3168/jds .2016-11352.

Svennersten-Sjaunja, K., I. Berglund, Pettersson G. The milking process in an automatic milking system, evaluation of milk yield, teat condition and udder health. In: H, 2000.

Wagner-Storch, A. M., Palmer R. W. 2003. Feeding behavior, milking behavior, and milk yields of cows milked in a parlor versus an automatic milking system. J. Dairy Sci. 86:1494–1502.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54:885–894. https://doi.org/10.2527/jas1982 .544885x

Welch, J. G., A. M. Smith, and K. S. Gibson. 1970. Rumination time in four breeds of dairy cattle. J. Dairy Sci.

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 085/2019, referente à pesquisa **"Avaliação de dados e de hipocalcemia em rebanho Jersey, com sistema de ordenha robotizado"**, sob a responsabilidade de **Rodrigo de Almeida** – 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 – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 11/12/2019.

Finalidade	Pesquisa científica
Vigência da autorização	Janeiro/2020 até Junho/2020
Espécie/Linhagem	*Bos taurus* (bovino)/Jersey
Número de animais	100
Peso/Idade	450 kg/2 – 6 anos
Sexo	Fêmea
Origem	Chácara Lagoa Dourada, Arapoti/PR, Brasil.

CERTIFICATE

We certify that the protocol number 085/2019, regarding the research **"Database and hypocalcemia in Jersey dairy cattle in robotic system"** under **Rodrigo de Almeida** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 1 of invasiveness, in session of 11/12/2019.

Puporse	Cientific research
Validity	January/2020 until June/2020
Specie/Line	*Bos taurus* (bovine)/Jersey
Number of animals	100
Wheight/Age	450 kg/2 – 6 years
Sex	Female
Origin	Lagoa Dourada farm, Arapoti/PR, Brazil.

Curitiba, 21 de janeiro de 2020

Chayane da Rocha

Coordenadora CEUA-SCA