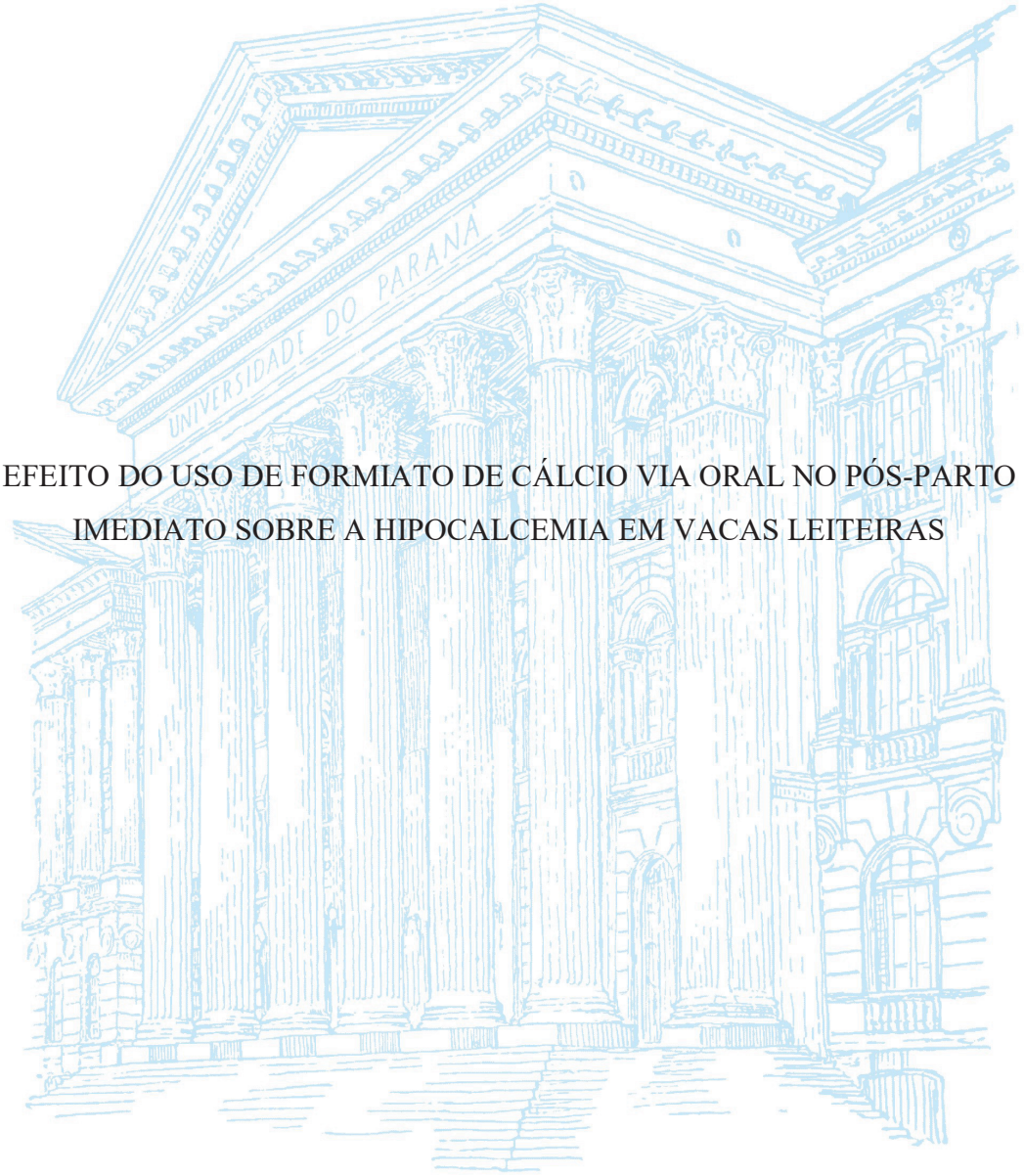
UNIVERSIDADE FEDERAL DO PARANÁ
ELINTON WEINERT CARNEIRO



EFEITO DO USO DE FORMIATO DE CÁLCIO VIA ORAL NO PÓS-PARTO IMEDIATO SOBRE A HIPOCALCEMIA EM VACAS LEITEIRAS

CURITIBA
2018

ELINTON WEINERT CARNEIRO

EFEITO DO USO DE FORMIATO DE CÁLCIO VIA ORAL NO PÓS-PARTO IMEDIATO SOBRE A HIPOCALCEMIA EM VACAS LEITEIRAS

Tese apresentada ao Programa de Pós-Graduação em Zootecnia, área de concentração Zootecnia – Nutrição e Produção de Herbívoros e Forragicultura, Departamento e Zootecnia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para a obtenção do título de Doutor em Zootecnia.
Orientador: Prof. Dr Rodrigo de Almeida

CURITIBA
2018

FICHA CATALOGRÁFICA ELABORADA PELO SISTEMA DE BIBLIOTECAS/UFPR -
BIBLIOTECA DE CIÊNCIAS AGRÁRIAS, DOUGLAS ALEX JANKOSKI CRB 9/1167
COM OS DADOS FORNECIDOS PELO(A) AUTOR(A)

C289e

Carneiro, Elinton Weinert
Efeito do uso de formiato de cálcio via oral no pós-parto imediato sobre a hipocalcemia em vacas leiteiras / Elinton Weinert Carneiro. - Curitiba, 2018.
134 f.: grafs., tabs.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia.
Orientador: Rodrigo de Almeida

1. Hipocalcemia. 2. Cálcio no organismo. 3. Bovino de leite - Doenças. I. Almeida, Rodrigo de. II. Título. III. Universidade Federal do Paraná.

CDU 631.416.7: 636.2

MINISTÉRIO DA EDUCAÇÃO
SETOR SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ZOOTECNIA

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 tese de Doutorado de **ELINTON WEINERT CARNEIRO** intitulada: **"Efeito do uso de formiato de cálcio via oral no pós-parto imediato sobre a hipocalcemia em vacas leiteiras"**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor 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, 15 de Março de 2018.

RODRIGO DE ALMEIDA
Presidente da Banca Examinadora (UFPR)

ANDRÉ THALER NETO
Avaliador Externo (UDESC)

ROSANGELA LOCATELLI DITTRICH
Avaliador Externo (UFPR)

MARCIO NUNES CORRÊA
Avaliador Externo (UFPEL)

DEDICATÓRIA

Dedico esse sonho realizado a minha família, ao João Victor, Nicolas e a Betina.

De forma muito especial a minha esposa Deolinda por ter me possibilitado atingir mais esse objetivo. Sem Você eu não conseguiria.....

AGRADECIMENTOS

Agradeço a “DEUS”, pela vida, pela família, por me mostrar o caminho do bem e do conhecimento.

A Universidade Federal do Paraná, Curitiba/PR, por me possibilitar realizar esse estudo de aperfeiçoamento.

Ao meu orientador Prof. Dr. Rodrigo de Almeida, caráter impecável, exemplo de pesquisador, incentivador e Professor sempre presente durante todo o doutorado. Prof. Rodrigo, a convivência foi intensa, com muito aprendizado. Termina essa fase, mas com certeza fica a minha admiração e minha amizade, conte sempre comigo.

Aos colegas da Pós-graduação, “Grupo do Leite”, obrigado pelo companheirismo e amizade.

A Profa. Dra. Rosangela Locatelli Dittrich e ao Olair Beltrame, do Laboratório de Patologia Clínica Veterinária da UFPR.

Ao Prof. Dr. André Thaler Neto, grande amigo, professor e pesquisador, por sua colaboração neste trabalho.

Ao Helder Córdova, amigo de longa data, por compartilhar a sua experiência.

A Bayer Saúde Animal, nas pessoas do Roger Van Der Vinne, Eduardo Ichikawa e do Saul Haten, pelo apoio no planejamento e execução desse projeto.

A CAPES, pelo apoio financeiro através da concessão de bolsa de estudo.

RESUMO

A hipocalcemia, com maior ocorrência em fêmeas leiteiras nas primeiras 24 horas após o parto, é provocada por um súbito desequilíbrio na regulação de Ca no sangue. Nesse momento, as vacas ficam mais propensas ao desenvolvimento de outras enfermidades. Dentro deste contexto, ao longo dos anos foram propostos alguns protocolos para o tratamento e estratégias para o controle preventivo. Entre essas práticas, é crescente o uso de suplementação com cálcio oral, que tem como objetivo fornecer cálcio rapidamente absorvível e prontamente disponível para a vaca, especialmente próximo ao parto. Algumas fontes de cálcio são bem conhecidas no meio científico, como o cloreto de cálcio, sulfato de cálcio, propionato de cálcio e o carbonato de cálcio. Mais recente, uma nova fonte de cálcio oral passou a ser utilizada, o formiato de cálcio. No entanto, até o momento não há trabalhos publicados avaliando os seus efeitos sobre os parâmetros bioquímicos em vacas leiteiras. Com esse objetivo, e de forma inédita, o primeiro estudo foi realizado em dois rebanhos comerciais no Estado do Paraná. A escolha dessas fazendas foi feita pela produtividade dos animais (média de 39 kg/leite/dia), escala de produção e que apresentassem um bom controle das informações do rebanho. Neste estudo, 264 vacas recém paridas da raça holandesa (100 primíparas e 164 multíparas) foram divididas de acordo com: 1º) rebanho (A e B); 2º) ordem de parição (primípara e multíparas); 3º) pela calcemia (normo ou hipocalcêmicas). Coleta semanal de amostras das dietas foram feitas no pré e no pós-parto, como também o monitoramento do pH da urina no pré-parto. A determinação da calcemia foi no tempo médio de 6 h após o parto, através da concentração do cálcio total, analisado na fazenda. O valor de corte para a definição de hipocalcemia foi de ≤ 8,0 mg/dL (≤ 2,0 m*M*). As vacas foram divididas em grupo tratamento (T) e controle (C), sendo que as do grupo controle foram suplementadas com duas doses de formiato de cálcio (6 e 33 h do parto). Foram então coletadas 6 amostras de sangue em 6, 17,5, 30, 53, 76,5 e 100 h após o parto. Os parâmetros bioquímicos analisados foram o cálcio total (tCa) e iônico (iCa), a enzima aspartatoaminotransferase (AST), a proteína albumina (ALB), ácidos graxos não esterificados (AGNES), o colesterol total (CT) e o betahidroxibutirato (BHBA). Foram verificados a ocorrência de enfermidades, de produção de leite (30 e 100 dias de lactação) e reprodutivos. O segundo experimento teve por objetivo analisar o comportamento da curva do cálcio no sangue, com uma única dose de formiato de cálcio no tempo médio de 6 h após o parto, porém com 3 grupos de animais: controle (sem cálcio oral), tratamento 1 (50 g de cálcio oral) e tratamento 2 (100 g de cálcio oral). Este estudo foi realizado no Estado do Paraná, em uma fazenda comercial com 129 vacas da raça holandesa (45 primíparas e 84 multíparas), divididas em blocos por paridade e calcemia. A determinação da calcemia foi realizada na fazenda, como descrito no primeiro estudo. As amostras de sangue foram colhidas em 0, 0,5, 1,0, 1,5, 2,0, 4,0, 8,0 e 24 h após a suplementação com o formiato de cálcio. Nas análises bioquímicas foram determinados os valores para o tCa, iCa, AGNES, BHBA, CT, AST, ALB, os minerais fósforo e magnésio. Os resultados observados nos estudos demostram uma rápida absorção de cálcio suplementado como formiato de cálcio, identificado através do rápido incremento do cálcio no sangue. Essa condição, foi benéfica para as vacas leiteiras no início da lactação, as quais tiveram melhor desempenho no metabolismo energético (<BHBA), menor incidência de enfermidades, e uma tendência de maior média diária na produção de leite em vacas multíparas nos primeiros 100 dias de lactação. Dessa forma, a suplementação oral com formiato de cálcio mostrou ser benéfica como estratégia preventiva em vacas leiteiras no início de lactação.

Palavras-chave: Cálcio oral. Formiato de cálcio. Hipocalcemia.

ABSTRACT

Hypocalcemia, which occurs most frequently in dairy cows in the first 24 hours postpartum, is caused by a sudden imbalance in the regulation of Ca in the blood. At the time the cows prone to the development of other diseases. Within this context, some protocols for treatment and strategies for preventive control have been proposed over the years. Among these practices, the use of oral calcium supplementation is increasing, which aims to provide calcium readily absorbable and readily available to the cow, especially near calving. Some sources of calcium are well known in the scientific environment, such as calcium chloride, calcium sulfate, calcium propionate and calcium carbonate. More recently, a new source of oral calcium has been used, calcium formate. However, to date there are no published papers evaluating their effects on biochemical parameters in dairy cows. With this aim, and in an unprecedented way, the first study was conducted in two commercial farms in the State of Paraná. The choice of these farms was made by the productivity of the animals (average of 39 kg/milk/day), production scale and that they had a good control of the information of the herd. In this study, 264 Holsteins cows (100 primiparous and 164 multiparous) were divided according to: 1) herd (A and B); 2) parturition order (primiparous and multiparous); 3) by calcemia (normal or hypocalcemic). Weekly sampling of the diets was done in the pre and postpartum, as well as the monitoring of the pH of the urine in the prepartum. Calcemia was determined in the mean time of 6 h after calving, through the concentration of total calcium, analyzed on-farm. The cutoff value for the definition of hypocalcemia was ≤ 8.0 mg/dL (2.0 m*M*). The cows were divided into treatment (T) and control (C) groups, and those in the control group were supplemented with two doses of calcium formate (6 and 33 h of calving). Six blood samples were collected at 6, 17.5, 30, 53, 76.5 and 100 h postpartum. The biochemical parameters analyzed were total calcium (tCa) and ionic (iCa), the enzyme aspartate aminotransferase (AST), protein albumin (ALB), non-esterified fatty acids (NEFA), total cholesterol (TC) and betahydroxybutyrate (BHBA). The occurrence of diseases, of milk production (30 and 100 days of lactation) and of reproductive activities, were recorded. The second experiment had as objective to analyze the behavior of the calcium curve in the blood, with a single dose of calcium formate in the mean time of 6 h after calving, but with 3 groups of animals: control (without oral calcium), treatment 1 (50 g of oral calcium) and treatment 2 (100 g of oral calcium). This study was carried out in the State of Paraná, in a commercial farm with 129 Holsteins cows (45 primiparous and 84 multiparous), divided into blocks by parity and calcemia. Calcemia determination on-farm, as described in the first study. Blood samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 4.0, 8.0 and 24 h after supplementation with calcium formate. In the biochemical analyzes the values for tCa, iCa, NEFA, BHBA, TC, AST, ALB, the phosphorus and magnesium minerals were searched. The results observed in the studies show a rapid absorption of calcium supplemented as calcium formate, identified through the rapid increase of calcium in the blood. This condition was benefic for dairy cows at the beginning of lactation, which had a better performance in the energy metabolism (<BHBA), a lower incidence of diseases, and a trend of higher average daily milk production in multiparous cows in the first 100 days of lactation. Thus, oral calcium formate supplementation was shown to be beneficial as a preventive strategy in dairy cows at the beginning of lactation.

Key words: Oral calcium. Calcium formate. Hypocalcemia.

LISTA DE FIGURAS

FIGURE 3.1 AVERAGE VALUES OF THE pH URINE, WEEKLY COLLECT OF 10 FEMALES IN FARMS A AND FARM B, DURING THE 9 WEEKS OF STUDY. IN THE TWO FARMS THE DIETS WHERE ANIONIC 54

FIGURE 3.2 SERUM CONCENTRATIONS (mM) IN THE FIRST 100h AFTER CALVING ACCORDING TO (A) iCa AND (B) tCa. PANEL A, THERE HAS BEEN A TREND WHILE PANEL B, HAVE EFFECT OF FORMATE CALCIUM IN TIME 30 h POSTPARTUM. AN ASTERISK (*) INDICATE MEANS DIFFER (P < 0.05)..... 56

FIGURE 3.3 THE VALUES FOR MULTIPAROUS AND PRIMIPAROUS COWS IN THE FIRST 100 h AFTER CALVING. PANEL A – PRESENTS THE VALUES OF iCa, WHILE THE B – tCa. THE LOWEST MEAN CONCENTRATION AT TIME 17.5 h. AN ASTERISK (*) INDICATES MEANS DIFFER (P < 0.01). AN SIMBOL INDICATES MEANS DIFFER (P < 0.05) 57

FIGURE 3.4 EFFECT THE USE OF Ca FORMATE IN PRIMIPAROUS AND MULTIPAROUS COWS. IN THE PAINEL A (iCa) AND B (tCa) PRIMIPAROUS COWS, AND C (iCa) AND D (tCa) MULTIPAROUS COWS FOR THE PRIMIPAROUS THE MAIN INTERACTION BETWEEN THE USE OF Ca ORAL AND THE PLASMATIC Ca WAS WITH THE tCa, HOWEVER, THE MULTIPAROUS HAPPENED TO THE iCa (*) INDICATE TIME POINTS AT WHICH TREATMENT MEANS SIGNIFICANTLY DIFFER (P ≤ 0.05)..... 59

FIGURE 3.5 KAPLAN-MEIER PLOT FOR THE EFFECT OF ORAL Ca SUPPLEMENTATION ON PREGNANCY TO 365 DIM 64

FIGURE 3.6 MILK YIELD (kg/d) DURING THE FIRST 100 DIM. PRODUCTION OF THE CONTROL GROUP (N = 134) 38.9 kg/d AND TREATMENT (N = 130) WAS AND 39.1 kg/d. THERE WERE NO ORAL INTERACTION WITH MILK PRODUCTION (P = 0.64). HOWEVER, IN THE GROUP OF MULTIPAROUS COWS PRODUCTION SHOWED A TREND (P = 0.08) OF HIGHER T

PRODUCTION (T vs. C) 44.8 vs. 44.1 ± 0.3 WHEN COMPARED TO GROUP C 65
FIGURE 3. 7 THE VALUES OF tCa IN COWS NC AND WITH SCH IN THE FIRST 100 h AFTER CALVING. THE SOLID LINE IDENTIFIES THE CUTOFF POINT FOR CALCEMIA STATUS CLASSIFICATION OF COWS. AN ASTERISK (*) INDICATES MEANS DIFFER (P < 0.01)..... 66
FIGURE 4.1 CHARACTERIZATION AT THE TOTAL Ca (A) AND IONIZED Ca (B) IN SERUM, AFTER SUPPLEMENTATION WITH ORAL Ca. *WITHIN HOUR, ADMINISTRATION OF 50 OR 100 g OF ORAL Ca DIFFERS FROM CONTROL GROUP (P<0.05). ǂ WITHIN HOUR, 100 g OF ORAL CALCIUM DIFFER FROM CONTROL GROUP (P<0.05). ◊ WITHIN HOUR, 100 g DIFFER FROM 50 g OF ORAL CALCIUM AND CONTROL GROUP (P<0.01). WITHIN HOUR, CONTROL GROUP DIFFER FROM 50 AND 100 g OF ORAL CALCIUM (P<0.01). ERROR BARS DEPICT SEM. 94
FIGURE 4.2 CHARACTERIZATION OF BLOOD SERUM iCa (A) AND tCa (B) IN PRIMIPAROUS AND MULTIPAROUS COWS. WHEN ANALYZED FOR EFFECT OF ORAL Ca FORMATE (CALFON ORAL®, BAYER ANIMAL HEALTH) ON PARITY WHEN COMPARING THE TREATMENT WITH 100 g OF Ca AND THE CONTROL GROUP. * EFFECT OF PARITY IN CONCENTRATION OF Ca (P < 0.01). ◊ EFFECT OF PARITY IN CONCENTRATION OF Ca (P < 0.05). 97
FIGURE 4. 3 - EFFECT OF Ca FORMATE ON AST ENZYME. *WITHIN HOUR, 100 g ORAL Ca DIFFER FROM CONTROL GROUP (P<0.01). ◊ WITHIN HOUR, 50 g DIFFER FROM 100 g GROUP (P<0.01). ERROR BARS DEPICT SEM. 99
FIGURE 4. 4 CHARACTERIZATION OF BLOOD SERUM ALBUMIN. (A) EFFECT PARITY, THE MULTIPAROUS COWS HAD A HIGHER MEAN CONCENTRATION WHEN COMPARED TO THE PRIMIPAROUS (P<0.01). (B) SERUM ALB CONCENTRATIONS AFTER ORAL Ca SUPPLEMENTATION

WITH 0, 50 AND 100 g (CALFON ORAL, BAYER ANIMAL HEALTH). EFFECT OF Ca FORMATE ON ALBUMIN PROTEIN AND INTERACTION BETWEEN TREATMENT AND HOUR (P<0.05). *WITHIN HOUR, 100 g ORAL Ca DIFFER FROM CONTROL GROUP. ◊ WITHIN HOUR, 50 g ORAL DIFFER FROM 100 g ORAL GROUP. ERROR BARS DEPICT SEM..... 100

FIGURE 4. 5 CHARACTERIZATION OF BLOOD SERUM AST. EFFECT OF HYPO + NEFA VS. HYPO IN COWS. *WITHIN HOUR, DIFFER (P < 0.01). ◊ WITHIN HOUR (P < 0.05) 102

LISTA DE TABELAS

TABLE 3.1 INGREDIENT COMPOSITION OF PREPARTUM AND POSTPARTUM DIET IN TWO HERDS 55
TABLE 3.2 MEAN VALUES AND THE EFFECT OF TREATMENT VS. CONTROL GROUPS, PARITY ORDER AND CALCEMIA ON THE CONCENTRATION OF Ca IN THE PLASMA OF AT THE BEGINNING OF LACTATION 61
TABLE 4.1 INGREDIENT COMPOSITION OF PREPARTUM AND POSTPARTUM DIET. 92
TABLE 4.2 EFFECT OF CALCIUM FORMATE ON SERUM CALCEMIA OF POSTPARTUM DAIRY COW 95
TABLE 4.3 EFFECT OF CALCIUM FORMATE ON SERUM MINERALS P AND Mg IN POSTPARTUM DAIRY COWS 98

LISTA DE ABREVIATURAS

AD - Deslocamento de Abomaso

AI - Inseminação Artificial

ALB - Albumina

AST - Aspartato aminotransferase

BHBA - Beta-hidroxibutirato

C - Controle

CH - Hipocalcemia Clínica

CK - Cetose clínica

Cl - Cloro

CORT - Cortisol

DCAD - Diferença Cátion-Aniônica da Dieta

DIM - Dias em lactação

DM - Matéria Seca

HYPO - Hipocalcemia

iCa - Cálcio iônico

K - Potássio

KET - Cetose

MAS - Mastite

ME - Metrite

Mg - Magnésio

mM - Milimol

Na - Sódio

NC - Normocalcemia

NEFA - Ácidos Graxos Não Esterificados

OC - Cálcio Oral

P - Fósforo

PTH - Paratormônio

ROC - Reciever Operator Characterstic

RP - Retenção de placenta

S - Enxofre

SCH - Hipocalcemia Subclínica

SK - Cetose subclínica

T - Tratamento

T1 - Tratamento 1

T2 - Tratamento 2

TC - Colesterol total

tCa - Cálcio total

TMR - Dieta totalmente misturada

VFA - Ácidos Graxos Voláteis

SUMÁRIO

1. INTRODUÇÃO GERAL 18

1.1 HIPÓTESE CIENTÍFICA 20

1.2 OBJETIVO GERAL 20

1.3 OBJETIVOS ESPECÍFICOS 20

REFERÊNCIAS 21

2 CHAPTER 1 – LITERATURE REVIEW 23

2.1 PREVENTIVE CONTROL OF HYPOCALCEMIA IN DAIRY COWS 23

ABSTRACT 23

RESUMO 23

2.2 INTRODUCTION 24

2.2.1. Calcium supplementation 30

2.2.2 Vitamin D administration 34

2.3. CONCLUSION 36

REFERENCES 37

3 CHAPTER 2 – EFFECTS OF ORAL CALCIUM FORMATE SUPPLEMENTATION IN PERIPARTUM DAIRY COWS 43

ABSTRACT 43

RESUMO 44

3.1 INTRODUCTION 45

3.2 MATERIALS AND METHODS 46

3.2.1 Animals, Dry Cows and Postpartum Management 46

3.2.2 Diet Formulation and Composition 47

3.2.3 Experimental Design and Treatments 48

3.2.4 Blood and Urine Sampling 49

3.2.5 Milk Production and Composition 49

3.2.6 Fertility Responses and Culling 50

3.2.7 Concentration of iCa and tCa 50

3.2.8 Concentration of CORT and TC 50

3.2.9 Concentrations of NEFA and BHB 51

3.2.10 Concentrations of ALB and AST 51

3.2.11 Diseases Characterization 51

3.2.12 Statistical Analysis 52

3.3 RESULTS 53

3.3.1 Diet Composition, Urinary pH in Prepartum 53

3.3.2 Concentration of minerals iCa and tCa Measures in Blood 56
3.3.3 Concentrations of ALB, AST, NEFA, BHB, TC and CORT in Blood..... 60
3.3.4 Fertility Responses, Milk Yield and Culling..... 64
3.3.5 Prevalence and Health Disorders, Cutoff tCa for Predict Diseases..... 65
3.4 DISCUSSION..... 67
3.5 CONCLUSIONS 75
REFERENCES 76
4 CHAPTER 3 - INCREASED SERUM CALCIUM IN DAIRY COWS WITH ORAL CALCIUM FORMATE SUPPLEMENTATION IN THE POSTPARTUM PERIOD..... 82
ABSTRACT.....82
RESUMO.....83
4.1 INTRODUCTION..... 84
4.2 MATERIALS AND METHODS 86
4.2.1 Animals, Management of Dry Cows and Postpartum 86
4.2.2 Diet Formulation and Composition 86
4.2.3 Experimental Design and Treatments..... 87
4.2.4 Blood and Urine Sampling 88
4.2.5 Milk Production and Composition 88
4.2.6 Fertility Rensponses and Culling..... 89
4.2.7 Concentration of minerals iCa, tCa, Mg and P..... 89
4.2.8 Concentration of NEFA, BHBA, TC, AST and ALB 89
4.2.9 Characterization of Diseases 90
4.2.10 Statistical Analysis 91
4.3 RESULTS..... 91
4.3.1 Diet Composition in Pre- and Postpartum, Urinary pH in Prepartum..... 91
4.3.2 Concentration of Minerals iCa, tCa, P and Mg in Blood 93
4.3.3 Concentrations of NEFA, BHBA, TC, ALB and AST in Blood..... 98
4.3.4 Fertility Responses, Milk Yield and Culling..... 101
4.3.5 Prevalence and Health Disorders..... 101
4.4 DISCUSSION..... 103
4.5 CONCLUSIONS 109
REFERENCES.....110
5. CONSIDERAÇÕES FINAIS..... 119
REFERÊNCIAS GERAIS 121

6. **APÊNDICES** 133
6.1 **CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS CEUA)-2015** 133
6.2 **CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) -2016** 134

1. **INTRODUÇÃO GERAL**

A intensificação da atividade leiteira, principalmente através da seleção genética e de melhorias no manejo (nutricional, bem-estar animal, etc.) promoveu ganhos em produtividade, mas um aumento no desafio da capacidade fisiológica da vaca, principalmente no período próximo ao parto e início de lactação. Nesse período, ocorrem grandes alterações hormonais, com uma queda sensível de ingestão de alimentos e um abrupto aumento na demanda de nutrientes para a produção do colostro e do leite.

De fato, aproximadamente 75% das doenças ocorrem no primeiro mês após o parto (LeBLANC et al., 2006), com efeitos sobre a produção de leite na lactação, retorno à atividade reprodutiva e resultados econômicos das fazendas leiteiras. As desordens mais comuns neste período são retenção de membranas fetais, hipocalcemia, cetose, esteatose hepática, deslocamento do abomaso, mastite, metrite e endometrite (LeBLANC et al., 2005; RIBEIRO et al., 2013) .

Dentre os distúrbios metabólicos de ordem nutricional, a hipocalcemia afeta fêmeas leiteiras entre -48 a +72 horas do parto, com maior ocorrência nas primeiras 24 horas, provocada por um súbito desequilíbrio na regulação de Ca no sangue. Pode apresentar-se sob duas formas, a clínica e a subclínica (ORTOLANI, 1995). A hipocalcemia apresenta um incremento com a idade das vacas, onde as vacas com 2 ou mais lactações têm maior risco de desenvolver esse distúrbio metabólico (REINHARDT et al., 2011)

A hipocalcemia subclínica tem uma prevalência maior que a clínica. Na forma subclínica, a prevalência descrita nos Estados Unidos é de 50% (GOFF, 2008; SANTOS, 2006) a 70% (MOORE et al., 2000) em rebanhos com sistemas mais intensivos de produção de leite, e 30 a 40% em sistema a pasto na Nova Zelândia (ROCHE et al., 2003). No Brasil, Carneiro et al. (2016) descrevem uma prevalência de 78% de hipocalcemia subclínica em rebanhos em sistema intensivo no Paraná. Nessa forma, os sintomas são menos evidentes e passam desapercebidos, no entanto, com a queda dos níveis de cálcio há um efeito cascata pela diminuição da contração muscular e a resposta imune. Dessa forma, as vacas ficam propensas ao desenvolvimento de outras doenças tais como síndrome da vaca caída, retenção de placenta, prolapso de útero, deslocamento de abomaso, pobre desempenho reprodutivo e mastite (MARTINEZ et al., 2012; CHAMBERLIN et al., 2013b).

Nos Estados Unidos, os prejuízos causados por esta enfermidade ultrapassam a casa dos 20 milhões de dólares anuais, e estima-se que sejam gastos acima de 10 milhões de dólares somente com medicamentos (HORST, R.L.; GOFF, J.P.; MCCLUSKEY, 2003). Esses custos foram provocados por perdas diretas, como perda em produção de leite, problemas reprodutivos, descartes e morte. Em Oetzel (2013), os valores estimados para cada evento de hipocalcemia clínica é de US$ 300 (perda em produção de leite e tratamento), enquanto que para a hipocalcemia subclínica é de US$ 125 (redução de produção de leite, incremento de cetose e deslocamento de abomaso). Já em Mcdowell (2002) a hipocalcemia reduziu a vida produtiva da vaca em 3,4 anos. Já Block (1984) relatou que os animais com HC reduzem a produção em 14%, comparando-se com vacas NC.

Dentro deste contexto, ao longo dos anos foram propostos alguns protocolos para o tratamento e estratégias para o controle preventivo. Uma sucessão de trabalhos foi realizada associando o conhecimento científico com técnicas econômicas e viáveis para o emprego nos rebanhos. Entre as práticas de controle preventivo efetivo, destacam-se o controle cátion-aniônico da dieta no período pré-parto (seleção de alimentos com menor poder catiônico), o uso de sais aniônicos e uso de cálcio oral.

Como forma preventiva estratégica, é crescente o uso de suplementação com cálcio oral. As fontes utilizadas até então estão bem descritas na literatura, no entanto, mais recentemente uma nova fonte vem sendo utilizada em fazendas leiteiras, o formiato de cálcio. Com isso, é importante a mensuração do efeito da sua suplementação sobre o metabolismo do cálcio e outros parâmetros bioquímicos das vacas no início da lactação.

A fim de atingir os objetivos propostos, esta tese foi organizada em capítulos baseando-se na normatização de trabalhos acadêmicos da Universidade Federal do Paraná, atualizada em 2017.

O **capítulo I: Preventive control of hypocalcemia in dairy cows.** Esse capítulo corresponde a revisão de literatura, com uma abordagem sobre as principais medidas preventivas até então conhecidas. Esse capítulo trata de informações que servirão de base para os demais.

O **capítulo II: Effects of oral calcium formate supplementation in peripartum dairy cows**. Esse capítulo teve como objetivo avaliar os parâmetros bioquímicos e o efeito sobre a sanidade, o desempenho produtivo e reprodutivo em fêmeas leiteiras.

O **capítulo III: Increased serum calcium in dairy cows with oral calcium formate supplementation in the postpartum period.** O objetivo foi avaliar a magnitude de resposta no tempo, da concentração do cálcio no sangue em vacas recém paridas após a suplementação com o formiato de cálcio.

1.1 HIPÓTESE CIENTÍFICA

A suplementação preventiva com formiato de cálcio em vacas leiteiras no início de lactação pode incrementar a concentração do cálcio no plasma, reduzindo os efeitos negativos da hipocalcemia nesse período. A hipótese é que o formiato de cálcio forneça um aporte de cálcio prontamente disponível, contribuindo para melhora da resposta metabólica da vaca, principalmente frente aos desafios nutricionais e na redução de enfermidades, pontos relevantes em um programa preventivo de rebanhos.

1.2 OBJETIVO GERAL

O objetivo desse estudo é demonstrar os efeitos da administração de formiato de cálcio via oral sobre os parâmetros bioquímicos e a resposta no desempenho de vacas leiteiras no início da lactação.

1.3 OBJETIVOS ESPECÍFICOS

- Analisar os metabólicos determinantes do metabolismo energético: beta hidroxibutirato, ácidos graxos não esterificados, colesterol total;
- Analisar o hormônio cortisol, como indicador de estresse;
- Analisar os minerais cálcio total, cálcio iônico, fósforo e magnésio;
- Analisar a função hepática através da enzima aspartato aminotransferase (AST) e da proteína albumina;
- Demonstrar o efeito do formiato de cálcio oral em três tratamentos (0, 50 e 100 g de cálcio), sobre a intensidade e a persistência do incremento da concentração do cálcio plasmático;
- Analisar a interação entre os efeitos do formiato de cálcio com a saúde da vaca (incidência de enfermidades, descarte e mortes) com a produção e composição do leite, e por fim, o desempenho reprodutivo dos animais.

REFERÊNCIAS

BLOCK, E. Manipulating Dietary Anions and Cations for Prepartum Dairy Cows to Reduce Incidence of Milk Fever. **Journal of Dairy Science**, v. 67, n. 12, p. 2939–2948, 1984. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030284816574>. .

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. **Journal of dairy science**, v. 94, n. suppl_5, p. 589–590, 2016. Disponível em: <https://academic.oup.com/jas/article/94/suppl_5/589-590/4767055>. .

CHAMBERLIN, W. G.; MIDDLETON, J. R.; SPAIN, J. N.; et al. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. **Journal of Dairy Science**, v. 96, n. 11, p. 7001–7013, 2013. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030213006474>. .

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008.

HORST, R.L.; GOFF, J.P.; MCCLUSKEY, B. J. Prevalence of subclinical hypocalcemia in US dairy operations. **Journal of Dairy Science**, v. 86, n. Suppl. 1, p. 247, 2003.

LEBLANC, S. J.; LESLIE, K. E.; DUFFIELD, T. F. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. **Journal of Dairy Science**, v. 88, n. 1, p. 159–170, 2005. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030205726746>. .

LEBLANC, S. J.; LISSEMORE, K. D.; KELTON, D. F.; DUFFIELD, T. F.; LESLIE, K. E. Major Advances in Disease Prevention in Dairy Cattle. **Journal of Dairy Science**, v. 89, n. 4, p. 1267–1279, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206721956>. .

MARTINEZ, N.; RISCO, C. A.; LIMA, F. S.; et al. Evaluation of peripartal calcium status , energetic profile , and neutrophil function in dairy cows at low or high risk of developing uterine disease. , p. 7158–7172, 2012.

MCDOWELL, L. R. Recent Advances in Minerals and Vitamins on Nutrition of Lactating Cows. **Pakistan Journal of Nutrition**, v. 1, n. 1, p. 8–19, 2002.

MOORE, S. J.; VANDEHAAR, M. J.; SHARMA, B. K.; et al. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. **Journal of dairy science**, v. 83, n. 9, p. 2095–2104, 2000.

OETZEL, G. R. Oral calcium supplementation in peripartum dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 447–455, 2013. Elsevier Inc.

ORTOLANI, E. . Aspectos clínicos, epidemiológicos e terapêuticos da hipocalcemia de vacas leiteiras. **Arquivos Brasileiros de Medicina Veterinária e Zootecnia**, v. 47, p. 799–808, 1995.

RIBEIRO, E. S.; LIMA, F. S.; GRECO, L. F.; et al. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. **Journal of Dairy Science**, v. 96, n. 9, p. 5682–5697, 2013. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021300475X>. .

ROCHE, J. R.; DALLEY, D.; MOATE, P.; et al. Dietary Cation-Anion Difference and the Health and Production of Pasture-Fed Dairy Cows 2. Nonlactating Periparturient Cows. **Journal of Dairy Science**, v. 86, n. 3, p. 979–987, 2003. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030203736819>. .

SANTOS, J. E. . Distúrbios Metabólicos. **Nutrição de Ruminantes**. p.439–461, 2006. Jaboticabal:Funep.

2 **CHAPTER 1 – LITERATURE REVIEW**

2.1 PREVENTIVE CONTROL OF HYPOCALCEMIA IN DAIRY COWS

ABSTRACT

Hypocalcemia is a metabolic disease with direct and indirect effects on the health of dairy cows. Preventive strategies have been studied and implemented since the 1930s to control or reduce its prevalence on dairy herds. These are procedures adopted could be adopted in the last 3 weeks before calving and in the first days of lactation. These measures are implemented through: 1) control of the pre-partum diet through the manipulation of four minerals; cations sodium (Na) and potassium (K) and anions chloride (Cl) and sulfur (S), which are included in the Dietary Cation-Anion Difference (DCAD) equation, as well as the supplementation with anionic salts; 2) through exogenous supplementation with calcitriol, vitamin D active form; and 3) supply of Ca in oral form. These actions aim to provide maintenance or even the addition of ionic calcium (iCa) in the blood of these animals at the end of gestation and in the first days of lactation, a critical period for the calcemia of dairy cows.

Key words: calcitriol, hypocalcemia, milk fever, prepartum diet, oral calcium.

RESUMO

A hipocalcemia é uma doença metabólica com efeitos diretos e indiretos sobre a saúde de vacas leiteiras. Para controlar ou reduzir a sua prevalência nos rebanhos, desde a década de 30, estratégias preventivas vêm sendo estudadas e implementadas. São procedimentos adotados nas últimas 3 semanas antes do parto e nos primeiros dias da lactação. Essas medidas são implementadas através de: 1) controle da dieta do pré-parto através da manipulação de quatro minerais; cátions sódio (Ca) e potássio (K) e ânions cloro (Cl) e enxofre (S), os quais são incluídos na equação de cálculo da Diferença Catiônica-Aniônica da Dieta (DCAD), bem como a suplementação com sais aniônicos; 2) através da suplementação exógena com calcitriol, forma ativa da vitamina D; e 3) fornecimento de Ca na forma oral. Estas ações visam proporcionar a mantença ou até mesmo o acréscimo de cálcio iônico (iCa) no sangue de fêmeas bovinas no final da gestação e nos primeiros dias de lactação, período crítico para a calcemia das vacas leiteiras.

Palavras-chave: calcitriol, hipocalcemia, febre do leite, dieta pré-parto, cálcio oral.

2.2 INTRODUCTION

Calcium (Ca) is the main mineral of the skeleton and one of the most abundant cations in the body, accounting for about 2% of body weight. Approximately 99% of the body's Ca is inorganic in the skeleton, mainly in the form of hydroxyapatite crystals HENRY (1995). The remaining (1%) is found in vascular space (3 g in dairy cows) and intracellular (membrane and endoplasmic reticulum) (SANTOS, 2006). Henry (1995) also stated that about 1% of the bone is freely interchangeable with the calcium of the extracellular fluid.

Of the total serum Ca, about 50 to 55% is in the ionizable form (iCa), 40 to 45% bound to albumin and the remaining 5% complexed in the form of salts. Calcium bound to albumin is variable according to the blood pH, and a more acidic pH reduces the affinity of Ca to albumin, since the binding sites in the albumin molecule are then occupied by H^+ (SANTOS, 2006).

The concentration of Ca in colostrum is 2.3 g Ca/kg, and in regular milk, 1.2 g/kg. Thus, assuming that a 600 kg cow has about 3 g of iCa throughout its plasma, to produce 8.0 kg of colostrum, 18.4 g of iCa will be drained, which is about six times the concentration of calcium readily available. This abrupt drainage of Ca is the triggering factor for hypocalcemia in dairy cows (GOFF; SÁNCHEZ; HORST, 2005).

In order to avoid an acute drop in Ca blood concentration at the beginning of lactation, the cow must replace the secreted Ca in the colostrum. This compensation is achieved by mobilizing Ca from the bone matrix, increasing renal reabsorption and improving the efficiency of Ca absorption in the intestine, through vitamin D in its active form (GOFF, 2008).

Bone mobilization is regulated by the parathyroid hormone (PTH) which is produced whenever there is a drop in calcemia. Active vitamin D (1,25-dihydroxy vitamin D or calcitriol) is required to stimulate the intestine to efficiently absorb Ca, and is activated by the kidneys, but only in response to an increase in PTH in the blood. This hormone activates the renal enzyme 1α-hydroxylase, which catalyzes the transformation of 25-hydroxy-cholecalciferol into 1,25-dihydroxy vitamin D, a much more potent metabolite than the previous one (GOFF, 2008). Vitamin D can be ingested in the vegetable form, ergocalciferol (D_2), or in the animal form, cholecalciferol (D_3), or it can be synthesized in the epidermis from 7-dehydro-cholesterol (DUKES, 1996).

Calving order is an important factor in the occurrence of hypocalcemia, explained by several factors such as the decline in the active transport of Ca in the intestine, inadequate production of 1,25 dihydroxy vitamin D and decrease in the number of receptors for calcitriol (HORST et al., 1994). Another important factor highlighted by Santos (2006) is the decrease in the number of active osteoclasts in the bone tissue of older cows, which reduces the reabsorption of Ca from the bones.

The Ca value in the blood of a periparturient adult cow ideally should be kept at around 8.5-10 mg/dL, which characterizes normocalcemic (NC) or healthy cows. When that concentration drops to ≤ 8.0 mg/dL the female is categorized as subclinical hypocalcemia (SCH) and ≤ 5.0 mg/dL as clinical hypocalcemia (CH) (GOFF, 2008; REINHARDT et al., 2011; BLANC et al., 2014). But Martinez et al. (2012) have been shown that increased incidence of metritis has already occurred with Ca levels below 8.6 mg/dL. Thereafter several dairy researchers have adopted this value as a reference or threshold to differentiate hypocalcemic from normocalcemic cows. According to Ortolani (1995), CH is a metabolic disease of acute course, which occurs most commonly at the time of calving in adult bovine females, with the onset of lactation. It is characterized by general muscle weakness, sagging skeletal muscles and decreased smooth muscle tonus, circulatory collapse with altered blood pressure and depression of consciousness.

On the other hand, SCH cows show low levels of calcium in the blood, but without clinical signs. These animals have higher serum concentrations of non-esterified fatty acids (NEFA) in the prepartum and higher concentrations of betahydroxybutyrate (BHB) in the postpartum period, which promotes a fatty liver accumulation in the first weeks after parturition, indicating an increase in the mobilization of body fat reserves (MARTINEZ et al., 2012; CHAMBERLIN et al., 2013). Higher concentrations of prepartum NEFA and postpartum BHB were also associated with losses in milk production (OSPINA et al., 2010).

Another important fact described by Martinez et al. (2012) is the association between SCH and the reduction of neutrophil function, increasing the risk of uterine diseases. These data indicate that SCH exacerbates the negative energy balance and immunosuppression, increasing the susceptibility of the periparturient animal to retained placenta, metritis, mastitis and displaced abomasum, with significantly losses of milk production and lower reproductive performance.

Calcium concentrations are typically lower in multiparous cows, with nadir (the lowest Ca concentrations in the blood) occurring between 12 and 24 h after calving in cows

not supplemented with anionic diets in pre-calving or between 24 and 36 h after calving in cows supplemented with anionic diet in the prepartum (GOFF; SÁNCHEZ; HORST, 2005; KIMURA; REINHARDT; GOFF, 2006). Thus, from the animals not supplemented with anionic diet, approximately 75% of all hypocalcemia events occur 24 h after birth, 18% between 24 and 48 h and about 7% of all cases of hypocalcemia are observed outside the peripartum (OETZEL, 2011).

Prevalence rates of 1 and 7% for CH, and 25 and 47% for SCH, respectively in primiparous and multiparous cows, were observed in the first 48 h postpartum (REINHARDT et al., 2011). Wilhelm et al. (2017) have described SCH prevalence of 2.7% in primiparous and 30.8% in multiparous cows at 2 h postpartum. Martinez et al. (2016) have found much greater rates; 69% of cows with SCH. Clinical hypocalcemia in dairy herds has an incidence of 3-7% (DEGARIS; LEAN, 2008; GOFF, 2008; RODRÍGUEZ et al., 2017).

As far goes our knowledgement, there are no published data on the prevalence of hypocalcemia in Brazil. However, Ortolani (1995) in the first national epidemiological survey carried out, have mentioned that 4.25% of the cows may present CH disease, also described as milk fever. Factors that may explain incidence variations are dietary management in the prepartum (cationic or anionic diets), age (higher incidence in older cows), breed (Jersey incidence greater than Holstein), and milk production (higher milk yields, higher risk).

Jawor et al. (2012) have observed that SCH cows produced more milk at weeks 2, 3 and 4 postpartum, with a difference of 6.0 kg/d when compared to NC cows. This difference was not observed between SCH and NC groups when milk yield was measured until 305 days of lactation (10,999 vs. 11,005 kg of milk). By other hand, Block (1984) reported that CH cows have their production decreased by 14% when compared to NC ones.

The costs of each CH and SCH event are US$300 and US$125, respectively. However, if we assume broadly observed CH and SCH incidences of 2% and 50%, SCH have a much higher cost than CH in a dairy herd. These estimates account for milk yield losses and direct costs associated with increased ketosis and displaced abomasum (OETZEL, 2013). In McDowell (2002) hypocalcemia also have reduced the cow's productive life by 3.4 years.

Within this context, some protocols for the treatment and strategies for the preventive control of hypocalcemia have been proposed over the years. Several studies were carried out associating scientific knowledge with economic viability of their adoption

in commercial dairy herds. Effective preventive control practices include monitoring minerals Ca and K, as well as the inclusion of anionic salts (manipulating dietary cation-anion balance) in the prepartum diet, the administration of calcitriol (vitamin D_3) and oral calcium supplementation.

As a preventive measure, an initial strategy is to reduce dietary Ca concentrations in the prepartum period. The dietary Ca intake should be lower than 20 g/day to be effective in the activation of the mechanisms of homeostasis before calving, allowing the cow to increase its capacity for intestinal absorption and bone resorption (GOFF et al., 2014).

Today, this practice is considered ineffective due mainly to the Ca composition typically found both in forage and concentrate feeds. Forages with low Ca usually have poor quality and may limit intake, which is not desirable for pre-calving animals. Kichura et al. (1982) suggested a method to sequester Ca and to restrict its absorption in the small intestine through the use of zeolite (aluminum silicate) in the diet. By chemically modifying the zeolito, its affinity and specificity by Ca were improved so that an effective dose of the commercial preparation is 0.5 kg/day.

Effects of dietary Ca concentrations have been demonstrated, both in low (WIGGERS et al., 1975) as in high (LOMBA et al., 1978; OETZEL et al., 1988) concentrations in the prepartum, reducing the risk of milk fever. Indeed, LEAN and co-workers (2006), in a well-known meta-analysis study, stated that the effect of dietary Ca content on the risk of milk fever is a quadratic one. These authors have suggested that extremely low (< 0.5%) or high (>1.5%) dietary Ca concentrations reduce milk fever incidence, whereas intermediate Ca concentrations contributes to increase clinical hypocalcemia.

Milk fever risk was highest with a prepartum dietary concentration of 1.35% calcium. In pre-calving diets with a high dietary calcium content (1.1 to 1.5% in DM), the cows are at the highest risk, while low levels of dietary calcium (< 0.5% in DM) predict a low incidence of hypocalcemia (LEAN et al., 2006).

Oppositely, Goff et al. (2014) stated that the dietary Ca levels do not influence the incidence of hypocalcemia in the peripartum. This conclusion is based on a trial where late-pregnant cows were fed diets with high chloride content as a preventive tool to control hypocalcemia and different levels of Ca; 0.47%, 0.98%, 1.52%, and 1.95% in DM. As a main result, dietary Ca did not influence the degree of hypocalcemia experienced or the milk produced at subsequent lactation. However, cows fed diets with the higher dietary Ca levels (1.52 and 1.95% in DM) had a reduction in DM intake when compared to cows in

the control group. In view of these results, the authors recommended the dietary Ca concentration of 1.0% as the most appropriate.

The reduction in the dietary calcium and phosphorus concentrations in the prepartum period was used as a tool to prevent hypocalcemia, aiming to increase the blood concentrations of vitamin D in its active form (calcitriol) and PTH. The problem is that this strategy did not always result in increased levels of circulating PTH with the goal of a greater Ca active absorption in the intestine (CAVALIERI; SANTOS, 2008).

The ability to reduce the incidence of hypocalcemia with the addition of anionic diets was first described by ENDER e DISHINGTON (1962). These European researchers observed a reduction in the incidence rate of clinical hypocalcemia after the use of acid-treated grass silages. Shortly thereafter, they showed that various dietary anionic salts were effective in stimulating the increase of Ca in the blood (BEEDE; SHIRE, 2013).

Block (1984) introduced the theory of the equilibrium of cations and anions in the diet for cows in the transition period in North America. Today, this strategy is commonly known as dietary cationic-anionic difference (DCAD), which more specifically addresses the calculation of DCAD in the formulation of pre-calving cow diets.

The effect of metabolic acidosis or alkalosis results from the electrical charge of biological fluids due to the predominance of anions (acidosis) or cations (alkalosis) entering the blood. The change in the electric charge of the blood results in transfer of the H^+ ions and alteration in the blood pH, that is, acid-base balance (GOFF, 2008). The blood pH is strictly regulated within a narrow physiological range, maintaining the arterial blood pH between 7.35 and 7.45, while the pH of the venous blood is slightly lower than the values above mentioned (NAGY et al., 2001).

The relevant dietary cations are sodium, potassium, calcium and magnesium; and the most important anions are chloride, sulfur and phosphorus. Among these, the sodium and potassium cations and the sulfur and chloride anions (all monovalent ions, with the exception of the divalent sulfur) exert a strong ionic effect on the acid-base balance and are denominated as "strong ions" (OETZEL, 2015).

NRC (2001) calculates the dietary cation-anion balance, but it includes only the minerals that present a higher rate of absorption in the digestive tract and higher concentration of charges in milliequivalent. Sodium, chlorine and potassium are absorbed with more than 90% efficiency, while sulfur is 60%. Other minerals such as calcium, magnesium and phosphorus may also influence the cation-anion balance, but due to their

lower absorption rate, they are generally not considered in the equations for estimating the dietary cation-anion difference.

The goal of implementing negative DCAD in the prepartum is to reduce the intensity and duration of hypocalcemia as the result of the induction of some degree of metabolic acidosis. This strategy aims to restore tissue sensitivity to PTH stimulation, reduced during the alkalosis state (GOFF et al., 2014).

Thus, anionic diets promote bone mobilization (osteoclastic reabsorption), since the bones (along with the kidneys) act as a buffer against excessive systemic acidosis. In addition, diets with low DCAD increase the amount of 1,25-dihydroxy vitamin D_3 produced per unit increase in PTH. This increases osteoclastic bone resorption, which is probably the most important calciotropic effect of acidic diets (GOFF et al., 2014).

Although the reduction of DCAD through the removal of cations is effective in the prevention of hypocalcemia, it is difficult to achieve diets with low potassium, mainly due to the agronomic use of this element in the cultivation of large-scale forage crops. The alternative to lower DCAD in pre-calving diets is to provide anionic salts as a feed supplement [$MgSO_4$, $MgCl_2$, NH_4Cl, $(NH_4)_2SO_4$, $CaCl_2$ and $CaSO_4$] or products specifically formulated to provide low or negative DCAD (DeGROOT et al., 2010).

The manipulation of the difference between cations and anions in the diet in ruminants does not result in visible changes in blood pH because there is a compensation for both the kidneys and the bones in maintaining the pH normality. For example, strongly anionic diets are acidogenic, but the blood pH remains constant because the urinary pH is reduced from 8.0-8.5 to about 6.0-6.5. Likewise, cationic diets are alkaline but have little effect on blood pH because urine becomes more alkaline (OETZEL, 2015).

Several equations were suggested for the calculation of DCAD, being the most commonly adopted: DCAD = (Na + K) − (Cl + S), cited by Ender et al. (1962) and used by Block (1984).

According to Lean et al. (2006), although simple, the equation above is the one that best reflects the association between low DCAD in the prepartum and a reduced incidence of clinical hypocalcemia (CH). These authors further point out that the inclusion of calcium, magnesium and phosphorus in DCAD equation to predict CH is questionable and does not bring relevant benefits. They exemplify this statement with the element magnesium, which despite being a cation when its dietary concentration is increased, it reduces the risk of CH. Calculated DCAD is typically expressed in mEq/100g DM; if necessary to express the value in mEq/kg DM, simply multiply the value found by 10.

The dietary cationic-anionic balance between -5 to -15 mEq/100g DM is correlated with a better response in cow health both in the prepartum as in the postpartum periods (MOORE et al., 2000; RAMOS-NIEVES et al., 2009; DEGROOT; BLOCK; FRENCH, 2010b; LENO et al., 2017). Prepartum diets even more anionic, with DCAD lower than -15 mEq/100g DM, can compromise DM intake (CHARBONNEAU et al, 2006) and because of that, they are not recommended.

Anionic diets can promote the increase of DM intake in the postpartum period in multiparous cows, but no positive (neither negative) effects on postpartum DM consumption were observed in primiparous (DeGARIS; LEAN, 2006; DeGROOT et al., 2010; LENO et al., 2017). Probably because of this positive effect on DM intake, anionic diets also increase milk production in multiparous cows (DEGROOT et al., 2010; LENO et al., 2017).

The manipulation of the minerals in the prepartum, particularly the ones included in the DCAD equation, can result in the effective reduction of hypocalcemia, both clinical (CH) and subclinical (SCH). Comparing cows that received an anionic diet in the prepartum, DeGroot et al. (2010) observed that those who did not consumed this diet showed a decrease of the serum calcium earlier than the group that consumed the anionic diet. Low DCAD is associated with low risk of CH (LEAN et al., 2006; DeGROOT et al., 2010; LENO et al., 2017).

Although all four minerals included in the DCAD equation are relevant, it seems that the cation potassium is even more important. There are several methods to manage K concentrations in the prepartum diets, thus controlling the DCAD (GOFF; SÁNCHEZ; HORST, 2005; DeGARIS; LEAN, 2008). McFadden (2008) suggested that the inclusion of significant amounts of corn silage, low-quality grass hay, straw, and their combination, could decrease K concentration in the prepartum diet. Low K grains or other feeds may also be employed to dilute and reduce potassium concentrations in transitional diets. Finally, a third method is the production of low K forage, specifically for pre-calving cows.

2.2.1. Calcium supplementation

Another preventive way to control hypocalcemia is through the use of Ca salts, in the form of gel, bolus or aqueous. The oral Ca solution should preferably provide Ca in the free (ionized) form, because this form is more readily absorbed from the rumen and abomasum. Normally, cows absorb Ca by two mechanisms; active transport through

intestinal epithelial cells and passive transport between the same intestinal epithelial cells (GOFF; HORST, 1993).

The active process of intestinal absorption and bone resorption of Ca is under the influence of the regulating hormones of Ca, the parathyroid hormone (PTH), which is secreted by the parathyroid glands and 1,25-dihydroxy vitamin D [1,25- $(OH)_2D$], which is produced in the kidney (HORST, et al., 1994). In hypocalcemic cows, secreted PTH may not stimulate the production of 1.25 (OH) as rapidly as in cows with less severe hypocalcemia, suggesting that tissues may be temporarily refractory to PTH. This occurs in alkaline conditions, such as those induced by elevated potassium mineral diets that induce a change in the shape of the PTH receptor protein, so that it is less able to recognize and bind PTH, resulting in failure of cell activation by the production of Cyclic AMP (GOFF, 2008).

In the passive form, the transport is dependent on the diffusion through a concentration gradient and the passive diffusion of Ca from the lumen of the intestine to the extracellular fluids occurs when the luminal concentration of ionized Ca exceeds 1 m*M* (BRONNER, 1987). Oral Ca treatment presumably increases the luminal concentration of Ca above 1 m*M*, favoring the passive transport of Ca to extracellular fluids (GOFF; HORST, 1993).

According to Martinez et al. (2016) in the first days postpartum a dairy cow has about 60 L of liquid in the rumen, and the supply of a dose close to 100 g of oral Ca, if all solubilized, can promote a Ca concentration greater than 10 m*M*. This concentration is greater than that required to cross the epithelium, which facilitates tissue diffusion and provides a rapid increase of Ca in the blood (SCHRODER et al., 2015). Indeed Carneiro et al. (2017) observed a significantly higher calcemia after 30 minutes of the oral Ca supplementation.

The ability of passive Ca transport is, in principle, unlimited and independent of stimulation by 1,25-dihydroxy vitamin D. Thus, the net absorption of free Ca increases linearly with greater concentrations of luminal Ca (BREVES; SCHRÖDER, 1991). The greater number of treatments and the amount administered by treatment through drench with a source of Ca that provide free Ca ions, it rapidly increases the calcemia.

As a preventive use program up to 4 doses can be used, based on the calcemia curve elaborated by Kimura et al. (2006). There are variations in the oral Ca administration protocols, such as the one described by Radostits et al. (2002) with oral Ca supplements 12 h prior to calving, immediately after calving and 24 h postpartum, which raises serum Ca

levels on the first and second postpartum days, especially in multiparous cows with three or more lactations. A practical difficulty in the above protocols is to set the schedule for the first application, which should occur 12 hours before calving. Therefore, in Carneiro et al. (2016) only two applications of oral Ca were implemented, on the first and second day postpartum.

The main sources of these calcium supplements are Ca chloride, Ca propionate, tricalcium phosphate and more recently, Ca formate. Typically, these sources are quite soluble and rapidly absorbed in the gastrointestinal tract.

Ca chloride ($CaCl_2$) is the most studied oral Ca source. All products with Ca chloride reduce blood pH. To a certain extent, this reduction is beneficial in the periparturient cow, reducing blood alkalinity and increasing tissue sensitivity to parathyroid hormone. However, excessive use of oral $CaCl_2$ may induce more severe metabolic acidosis, which may cause inappetence at a time when feed intake is already compromised (GOFF et al., 1996).

In order to reduce mucosal effects and eliminate the risk of aspiration pneumonia, Thilsing-Hansen et al. (2002) reported the use of a commercial product with a combination of $CaCl_2$ with $CaSO_4$, formulated in a solid fat-coated bolus. Generally speaking, using $CaCl_2$ as an oral Ca source has been shown to increase Ca blood concentrations, reduce the risk of clinical and subclinical hypocalcemia and decrease the risk of displaced abomasum (OETZEL, 1996).

Calcium propionate supplementation through an oral thick paste can raise calcemia when administered to cows, although its effect on serum Ca is not as fast as the one observed with Ca chloride. Ca propionate does not have an acidifying effect on blood pH, but by another hand can be of use as a gluconeogenic precursor at a time when the cow is in negative energy balance. It has the disadvantage of presenting only 21.5% Ca, which requires larger volumes of preparation to be administered orally (GOFF et al., 1996; PEHRSON et al., 1998).

Another source of oral Ca, Ca formate ($Ca(HCOO)_2$), is a molecule that comes from formic acid, presenting 36.5% Ca and has its use indicated as a food preservative and as an additive for animals. It is produced by chemical synthesis as a co-product during the manufacture of trimethylolpropane (TMP). Hydrated lime is used as the source of Ca. Calcium formate is then separated from the solution by heat treatment to remove formaldehyde (EFSA, 2015). According to Ulfabele et al. (1997) the absorption of Ca from Ca formate in the digestive tract is effective compared to the absorption of the other Ca

supplements. However, unlike Ca chloride, Ca formate does not cause corrosion or irritation of the mucous membranes of the digestive tract.

Carneiro et al. (2016) evaluated the effect of Ca formate supplementation on 264 cows (100 primiparous and 164 multiparous) at the beginning of lactation on total Ca and ionized Ca concentrations, as well as on other serum metabolites. The experimental animals were divided in 2 groups; the treatment group (T) received 2 doses of 50 g of oral Ca (Ca formate) in the mean time of 6 and 31 h postpartum, and the control group (C) did not receive any dose of oral Ca. The treatment group showed higher serum ionized Ca concentration and lower BHB concentration at day 5 postpartum (0.86 vs. 0.97 m*M*). Oral Ca supplementation also had a favorable effect on the milk yield of multiparous cows, where oral Ca-supplemented cows produced +0.7 kg/d in the first 100 days of lactation; 45.6 vs. 44.9 kg/d on T and C cows, respectively.

Carneiro et al. (2017) supplemented Ca formate on another group of 129 cows (45 primiparous and 84 multiparous) evaluating serum total Ca, ionized Ca and other metabolites eight times, from 30 minutes to 24 hours after oral Ca administration. Calcium formate supplementation showed an effect on serum Ca concentration from 30 minutes (time point of greatest increase) and lasted up to 8 h after its administration, when it returned to the basal concentration. Thus, supplementation with oral Ca after calving has been shown to be beneficial because it increases serum ionized calcium soon after calving, at a metabolic moment very critical to the dairy cow.

Mcart and Oetzel (2015) developed a stochastic model to estimate the economic impact of oral Ca supplementation in postparturient multiparous cows. Four different strategies were evaluated: 1) supplementation of cows with a high milk yield in the previous lactation; 2) supplementation of lame cows; 3) supplementation of both cows that have a high previous lactation mature-equivalent milk yield and cows that are lame; and 4) supplementation of all multiparous cows of the herd. All fixed and variable costs were calculated, in addition to the price paid per liter of milk, risk of death, cost of replacement, risk of diseases, risk of claudication and increase in milk production.

As general conclusions, Mcart and Oetzel (2015) found a greater net impact of oral Ca supplementation of high milk yield and lame cows. When all postpartum multiparous cows were supplemented, there was a positive net herd impact approximately 80% of the time. A herd's average milk yield at first test had the highest influence on the net impact of oral Ca supplementation to all multiparous milk yield, followed by the

decrease in risk of health events in lame cows given oral calcium, a herd's prevalence of lameness at calving, and the price of milk

2.2.2 Vitamin D administration

Another preventive strategy to control hypocalcemia is vitamin D administration in its active form 1,25-dihydroxy vitamin D_3 (or calcitriol). Studies at the beginning of the last century have shown that vitamin D was important in the absorption of Ca from the intestine. One of the first published reports suggested the administration of large oral doses of vitamin D_2 in prepartum. At the time, the hypothesized mechanism of action suggested that vitamin D acted directly on target tissues (bones and gut), increasing the movement of Ca from these tissues into the bloodstream. However, it is now known that the true mechanism of action is associated with the production of the active form of vitamin D.

For approximately 30 years, considerable interest and resources were directed to this form of treatment (HORST et al., 1997). Blunt et al. (1968) found a more active metabolite of vitamin D, identified as 25-hydroxy vitamin D_3 (or calcidiol). Later, however, it was discovered that in fact the active form of vitamin D is 1,25-dihydroxy vitamin D_3 (or calcitriol), which is produced from 25-hydroxy vitamin D_3 in the kidneys by the enzyme 1α-hydroxylase (HORST et al., 1997).

The literature recommended feeding or injecting massive doses (up to 10 million units) of vitamin D within 10 to 14 days before calving to prevent milk fever. Unfortunately, this dose of vitamin D that was considered effective in controlling milk fever is very close to the level that causes irreversible metastatic soft tissue calcification (NRC, 2001). Today it is considered a reasonable supplementary practice to dry cows with 20-30,000 IU/day of vitamin D in the diet (GOFF, 2006) or 30 IU of supplemental vitamin D per kg of BW (NRC, 2001).

Another potential problem with the use of vitamin D and its metabolites is an inhibitory effect on renal 1α-hydroxylase. Littledike and Horst (1982) demonstrated that many cows treated with vitamin D3 or 1α-hydroxylated vitamin D metabolites showed clinical signs of hypocalcemia 10 to 14 days postpartum. These researchers demonstrated that cows treated with vitamin D compounds did not produce endogenous 1,25 $(OH)_2D_3$ and therefore could not recover from hypocalcemic episodes. In Goff and Horst (1990) the problem of suppression of renal production of 1,25-dihydroxy vitamin D_3 was minimized by the slow withdrawal of the exogenous hormone in the days following calving.

In cows with hypocalcemia, the suggested hypothesis was initially of primary shortage of PTH and/or vitamin D_3 secretions. However, this hypothesis was refuted when the researchers found that both PTH and vitamin D_3 actually are in higher concentration in the blood of hypocalcemic cows (HORST et al., 1994).

Horst et al. (1994) have reported that there is a subtype of hypocalcemia (which may affect 20% of the cows with hypocalcemia) where the production of vitamin D_3 is non-existent or delayed. This syndrome was observed in cows who relapsed from milk fever, requiring intravenous Ca on more than one occasion. In these relapsing cows, plasma vitamin D_3 concentrations did not increase as the cows became hypocalcemic. After 24 to 48 h of severe hypocalcemia, these cows began to produce vitamin D_3. Plasma PTH concentrations were as high or even higher in these cows with recurrent clinical hypocalcemia than in those that had not relapsed. As PTH should have stimulated the renal production of vitamin D_3, the data suggest that the kidneys of these cows with clinical hypocalcemia are temporarily refractory to PTH stimulation.

Vitamin D_3 is the form of vitamin D that is synthesized by vertebrates and vitamin D_2 is the main natural form of the vitamin in plants (HORST et al., 1994). Supplementing dairy cows in the peripartum with 25-(OH)D_3 when fed an anionic diet increases the plasma concentration of Ca (WILKENS et al., 2012), but had no effect when supplemented with a diet containing positive DCAD (TAYLOR et al., 2008). Anionic diets increase the conversion from 25-(OH)D_3 to 1,25 $(OH)_2D_3$ (GOFF et al., 1991).

Weiss et al. (2015) conducted an experiment to verify the effect of vitamin D administration by associating its effect with DCAD in prepartum diets. Multiparous Holstein cows were used, which were divided into 3 treatments for the last 13 days of gestation (ranged from 7 to 22 days). The control diet was formulated to provide 18,000 IU/day of vitamin D_3 and DCAD of 165 mEq/kg of DM; the second group received the same amount of vitamin D_3, but had a DCAD of -139 mEq/kg DM; and the third group received 6 mg/day of vitamin D_3 with DCAD of -138 mEq/kg DM. When supplementing cows with vitamin D_3 in combination with a negative DCAD in the last 13 days of gestation, serum vitamin D from cows increased. This has led to increased concentrations of vitamin D in colostrum, milk and calf serum. However, despite having observed a short duration increase in serum Ca before calving in the treatment groups, no effect on serum Ca was observed at calving and postpartum.

Recommendation of injectable vitamin D_3 administration is on days 1 to 3 before calving, however, a problem with these prepartum strategies is in predicting the exact day

of calving so that the vitamin D_3 metabolites are administered at the right time. It then appears as an option to supplement it immediately after calving in an attempt to prevent any further decline in blood Ca postpartum.

Vieira-Neto et al. (2017) conducted an experiment to evaluate the effect of injectable vitamin D_3. Cows within 6 h of calving received 300 μg of calcitriol, while in the control group vitamin D_3 free solution was used. As main results, injectable administration of a single dose of vitamin D_3 immediately after calving showed: increased plasma calcitriol concentrations in the cows for approximately 3 days, increased ionized Ca and total Ca concentrations in the blood of dairy cows in the immediate postpartum, reduction in the prevalence of subclinical hypocalcemia, and improvement in innate immune function measurements. Finally, neutrophil phagocytosis and oxidative burst were improved by treating cows with calcitriol.

Treatment with vitamin D_3 in the peripartum may interact positively with other nutritional management strategies in the transition period, but this potential still needs to be further studied.

2.3. CONCLUSION

Hypocalcemia is a important metabolic disease of dairy cows in early lactation. Recent research has clearly demonstrated negative effects from subclinical hypocalcemia that start whenever blood total calcium concentrations drop below 8.5 mg/dL. The effects include lower milk yield, increased risk for displaced abomasum, metritis, and ketosis and impaired reproductive performance. Strategies to prevent as much hypocalcemia as possible can be divided into dietary practices in prepartum such as lower dietary K, control of dietary Ca and cation-anion balance manipulation, and individual cow supplementation with oral calcium and calcitriol. From the strategies mentioned above, the control of DCAD in pre-partum is a more consolidated practice in commercial dairy herds. However, the other techniques mentioned, individually or synergistically, have been studied and improved, demonstrating positive results in the control of hypocalcemia.

REFERENCES

BEEDE, D.K.; SHIRE, J. . DCAD revisited: Prepartum use to optimize health and lactational performance. In: Proc. Southwest Dairy Nutrition and Management Conf. **Anais**... . p.1–11, 2013. AZ, EUA. Disponível em: <https://www.researchgate.net/publication/237144494_DCAD_revisited_Prepartum_use_to_optimize_health_and_lactational_performance>. .

BLANC, C. D.; VAN DER LIST, M.; ALY, S. S.; ROSSOW, H. A.; SILVA-DEL-RÍO, N. Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium. **Journal of Dairy Science**, v. 97, n. 11, p. 6901–6906, 2014. Elsevier.

BLOCK, E. Manipulating Dietary Anions and Cations for Prepartum Dairy Cows to Reduce Incidence of Milk Fever. **Journal of Dairy Science**, v. 67, n. 12, p. 2939–2948, 1984. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030284816574>. .

BLUNT, J. W.; DELUCA, H. F.; SCHNOES, H. K. 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D3. **Biochemistry**, v. 7, n. 10, p. 3317–3322, 1968.

BREVES, G.; SCHRÖDER, B. Comparative aspects of gastrointestinal phosphorus metabolism. **Nutrition research reviews**, v. 4, n. 1991, p. 125–140, 1991.

BRONNER, F. Intestinal calcium absorption: mechanisms and applications. **The Journal of nutrition**, v. 117, n. 8, p. 1347–1352, 1987.

CARNEIRO, E. W; HONORATO, S. H.; ICHIKAWA E. E.; ALMEIDA, R. Increased serum calcium in dairy cows with oral calcium formate supplementation in the postpartum period. **Journal of dairy science**, v. 100, n. American Dairy Science Association – ADSA Annual Meeting, June 25-28, 2017 ., p. p.87-88, 2017. Pittsburgh, Pennsylvania, EUA. Disponível em: <http://m.adsa.org/2017/abs/t/70424>. .

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. **Journal of dairy science**, v. 94, n. suppl_5, p. 589–590, 2016. Disponível em: <https://academic.oup.com/jas/article/94/suppl_5/589-590/4767055>. .

CAVALIERI, F.L.B.; SANTOS, G. . Balanço catiônico-aniônico em vacas leiteiras no pré-parto. Disponível em: <http://www.nupel.uem.br/balanco.pdf.>. .

CHAMBERLIN, W. G.; MIDDLETON, J. R.; SPAIN, J. N.; et al. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. **Journal of Dairy Science**, v. 96, n. 11, p. 7001–7013, 2013. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030213006474>. .

CHARBONNEAU, E.; PELLERIN, D.; OETZEL, G. R. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. **Journal of Dairy Science**, v. 89, n. 2, p. 537–548, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206721166>. .

DEGARIS, P. J.; LEAN, I. J. Milk fever in dairy cows: A review of pathophysiology and control principles. **Veterinary Journal,** v. 176, n. 1, p. 58–69, 2008. Elsevier Ltd.

DEGROOT, M. A.; BLOCK, E.; FRENCH, P. D. Effect of prepartum anionic supplementation on periparturient feed intake, health, and milk production. **Journal of Dairy Science**, v. 93, n. 11, p. 5268–5279, 2010. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030210005692>. .

DUKES, H. H. **Fisiologia dos Animais Domésticos**. 11 Ed ed. Rio de Janeiro, 1996.

ENDER, F.; DISHINGTON, I.W.; HELGEBOSTAD, A. Parturient paresis and related forms of hypocalcaemic disorders induced experimentally in dairy cows. **Acta Vet. Scand**, v. 3, n. Suppll.1, p. 5–52, 1962.

GOFF, JESSE P., JORGE ML SÁNCHEZ, AND R. L. H. Mineral Nutrition Conference. University of Tennessee. Hypocalcemia biological effects and strategies for prevention. **Anais**... . p.5–10, 2005. Disponível em: <ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/TNC2005/Goff.pdf>. .

GOFF, P. J.; SÁNCHEZ, J. M.; HORST, R. L. Hypocalcemia: Biological Effects and Strategies for Prevention. **Nutrition Conference**, Departament of Animal Science, University of Tennessee, p. 5–10, 2005. Disponível em: <ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/TNC2005/Goff.pdf>. .

GOFF, J. P. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. **Animal Feed Science and Technology**, v. 126, n. 3–4, p. 237–257, 2006.

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008a. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2007.12.020>. .

GOFF, J. P. Transition period management and nutrition problems - **A few solutions. High Plains Dairy Confernce**, p. 123–138, 2008. Disponível em: <http://www.highplainsdairy.org/2008/21 Goff.pdf>. .

GOFF, J. P.; HORST, R. L. Effect of Subcutaneously Released 24F-1,25-Dihydroxyvitamin D3 on Incidence of Parturient Paresis in Dairy Cows. **Journal of Dairy Science**, v. 73, n. 2, p. 406–412, 1990. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030290786870>. .

GOFF, J. P.; HORST, R. L. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. **Journal of Dairy Science**, v. 76, n. 1, p. 101–108, 1993. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030293773282>. .

GOFF, J. P.; HORST, R. L.; JARDON, P. W.; BORELLI, C.; WEDAM, J. Field trials of an oral calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. **Journal of dairy science**, v. 79, n. 3, p. 378–383, 1996.

GOFF, J. P.; HORST, R. L.; MUELLER, F. J.; et al. Addition of Chloride to a Prepartal Diet High in Cations Increases 1,25-Dihydroxyvitamin D Response to Hypocalcemia Preventing Milk Fever. **Journal of Dairy Science**, v. 74, n. 11, p. 3863–3871, 1991. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030291785792>. .

GOFF, J. P.; LIESEGANG, A.; HORST, R. L. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. **Journal of Dairy Science**, v. 97, n. 3, p. 1520–1528, 2014. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030214000228>. .

HENRY, J. B. **Diagnósticos clínicos e tratamento por métodos laboratoriais**. 1995.

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A. Calcium and Vitamin D Metabolism in the Dairy Cow. **Journal of Dairy Science**, v. 77, n. 7, p. 1936–1951, 1994. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203029477140X>. .

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A; BUXTON, D. R. Strategies for preventing milk fever in dairy cattle. **Journal of dairy science**, v. 80, n. 7, p. 1269–1280, 1997. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(97)76056-9>.

JAWOR, P. E.; HUZZEY, J. M.; LEBLANC, S. J.; VON KEYSERLINGK, M. A G. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. **Journal of dairy science,** v. 95, n. 3, p. 1240–8, 2012. Elsevier. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22365207>. .

KICHURA, T. S.; HORST, R. L.; BEITZ, D. C.; LITTLEDIKE, E. T. Relationships between prepartal dietary calcium and phosphorus, vitamin D metabolism, and parturient paresis in dairy cows. **The Journal of nutrition**, v. 112, n. 3, p. 480–7, 1982.

KIMURA, K.; REINHARDT, T. A.; GOFF, J. P. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. **Journal of dairy science**, v. 89, n. 7, p. 2588–2595, 2006. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(06)72335-9>. .

LEAN, I. J.; DEGARIS, P. J.; MCNEIL, D. M.; BLOCK, E. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. **Journal of dairy science**, v. 89, n. 2, p. 669–684, 2006.

LENO, B. M.; NEVES, R. C.; CURLER, M. D.; et al. Association of immediate postpartum plasma Ca concentration with early- lactation clinical diseases , culling , reproduction , and milk production. **Journal of Dairy Science** Vol. 100, Suppl. 2, v. 100, p. 39, 2017. Disponível em: <https://blogs.cornell.edu/jessmcartlab/files/2017/06/ADSA-Poster_Neves-2017_Final_06.23.2017-129b0t3.pdf>. .

LITTLEDIKE, E. T.; HORST, R. L. Vitamin D3Toxicity in Dairy Cows,. **Journal of Dairy Science**, v. 65, n. 5, p. 749–759, 1982. Disponível em:

<http://linkinghub.elsevier.com/retrieve/pii/S0022030282822637>. .

LOMBA, F.; CHAUVAUX, G.; TELLER, E.; LENGELE, L.; BIENFET, V. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. **The British journal of nutrition**, v. 39, n. 3, p. 425–9, 1978.

MARTINEZ, N.; RISCO, C. A.; LIMA, F. S.; et al. Evaluation of peripartal calcium status , energetic profile , and neutrophil function in dairy cows at low or high risk of developing uterine disease. **Journal of Dairy Science** , p. 7158–7172, 2012.

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; RISCO, C. A.; et al. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8417–8430, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304519>. .

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; LOPERA, C.; et al. Effects of oral calcium supplementation on mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8397–8416, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304520>. .

MCART, J. A. A.; OETZEL, G. R. A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. **Journal of Dairy Science**, v. 98, n. 10, p. 7408–7418, 2015. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030215005354>. .

MCDOWELL, L. R. Recent Advances in Minerals and Vitamins on Nutrition of Lactating Cows. **Pakistan Journal of Nutrition**, v. 1, n. 1, p. 8–19, 2002.

MCFADDEN, M. Growing low potassium grass hay for close-up dry cows. , 2008. Michigan: **Michigan Dairy Review**. Disponível em: <www.msu.edu/~mdr/vol13no2/mcfadden.html>. .

MOORE, S. J.; VANDEHAAR, M. J.; SHARMA, B. K.; et al. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. **Journal of dairy science**, v. 83, n. 9, p. 2095–2104, 2000.

NAGY, O.; KOVÁC, G.; SEIDEL, H.; WEISSOVÁ, T. The effect of arterial blood sampling sites on blood gases and acid-base balance parameters in calves. **Acta. Vet. Hung,** v. 49, p. 331–340, 2001.

NRC. NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. **Acad. Press** , Washington, DC, p. 1–333, 2001.

OETZEL, G. R. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. **J. Am. Vet. Med. Assoc**., v. 209, p. 958–961, 1996.

OETZEL, G. R. Non-Infectious Diseases : Milk Fever. In J. W. Fuquay & P. L. H. McSweeney (Eds.), **Encyclopedia of Dairy Sciences**, v. 2, p. 239–245, 2011. Disponível em: <https://www.academia.edu/11909930/Non-Infectious_Diseases_Milk_Fever>. .

OETZEL, G. R. Minimizing hypocalcemia during early lactation. **Proceedings of the Tri-State Dairy Nutrition Conference**, , n. 608, p. 23–34, 2013. Disponível em: <https://www.cabdirect.org/cabdirect/abstract/20133232153>. .

OETZEL, G. R. Use of Acidifying Diets for Prevention of Milk Fever in Dairy Cattle. Disponível em: <http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=9FAD99E5D0D2D760EBB915CEACD7B0C5?doi=10.1.1.538.9658&rep=rep1&type=pdf>. Acesso em: 1/2/2018.

OETZEL, G. R.; OLSON, J. D.; CURTIS, C. R.; FETTMAN, M. J. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. **Journal of Dairy Science,** v. 71, n. 12, p. 3302–3309, 1988.

ORTOLANI, E. . Aspectos clínicos, epidemiológicos e terapêuticos da hipocalcemia de vacas leiteiras. **Arquivos Brasileiros de Medicina Veterinária e Zootecnia**, v. 47, p. 799–808, 1995.

OSPINA, P. A; NYDAM, D. V; STOKOL, T.; OVERTON, T. R. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. **Journal of dairy science**, v. 93, n. 2, p. 546–554, 2010. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2009-2277>. .

PEHRSON, B.; SVENSSON, C.; JONSSON, M. A comparative study of the effectiveness of calcium propionate and calcium chloride for the prevention of parturient paresis in dairy cows. **Journal of dairy science**, v. 81, n. 7, p. 2011–6, 1998. Elsevier. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0022030298757753>. .

RADOSTITS, O. M. Clinica veterinária: um tratado de doenças de bovinos, ovinos, suínos, caprinos e equinos. In: G. Koogan (Org.); **Clínica Veterinária**. 9 Ed ed., p.1278–1291, 2002. Rio de Janeiro.

RAMOS-NIEVES, J. M.; THERING, B. J.; WALDRON, M. R.; JARDON, P. W.; OVERTON, T. R. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. **Journal of dairy science**, v. 92, n. 11, p. 5677–5691, 2009.

REINHARDT, T. A.; LIPPOLIS, J. D.; MCCLUSKEY, B. J.; GOFF, J. P.; HORST, R. L. Prevalence of subclinical hypocalcemia in dairy herds. **Veterinary Journal**, v. 188, n. 1, p. 122–124, 2011. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2010.03.025>. .

RODRÍGUEZ, E. M.; ARÍS, A.; BACH, A. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. **American Dairy Science Association**, n. 2012, p. 1–8, 2017.

SANTOS, J. E. . **Distúrbios Metabólicos. Nutrição de Ruminantes**. p.439–461, 2006. Jaboticabal:Funep.

SCHRO DER, B.; WILKENS, M. R.; RICKEN, G. E.; et al. Calcium transport in bovine rumen epithelium as affected by luminal Ca concentrations and Ca sources. **Physiological Reports,** v. 3, n. 11, p. e12615–e12615, 2015. Disponível em: <http://physreports.physiology.org/cgi/doi/10.14814/phy2.12615>. .

TAYLOR, M. S.; KNOWLTON, K. F.; MCGILLIARD, M. L.; SEYMOUR, W. M.; HERBEIN, J. H. Blood Mineral, Hormone, and Osteocalcin Responses of Multiparous Jersey Cows to an Oral Dose of 25-Hydroxyvitamin D3 or Vitamin D3 Before Parturition. **Journal of Dairy Science**, v. 91, n. 6, p. 2408–2416, 2008. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030208711913>. .

THILSING-HANSEN, T., JORGENSEN, R.J., OSTERGAARD, S. Milk Fever Control Principles : A Review. **Acta Veterinária Scandinavica**, v. 43, n. 1, p. 1–19, 2002.

ULFABELE, I.; EXAMINER, P.; HENLEY, R. United States Patent [191 Date of Patent : , p. 4–6, 1997. Disponível em: <https://patents.google.com/patent/US5631289A/en>. .

VIEIRA-NETO, A.; LIMA, I. R. P.; JR, F. L.; et al. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2016-12506>. .

WEISS, W. P.; AZEM, E.; STEINBERG, W.; REINHARDT, T. A. Effect of feeding 25-hydroxyvitamin D3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. **Journal of Dairy Science**, v. 98, n. 8, p. 5588–5600, 2015. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021500377X>. .

WIGGERS, K. D.; NELSON, D. K.; JACOBSON, N. L. Prevention of Parturient Paresis by a Low-Calcium Diet Prepartum : A Field Study. **Journal of Dairy Science**, v. 58, n. 3, p. 430–431. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(75)84584-X>. .

WILHELM, A. L.; MAQUIVAR, M. G.; BAS, S.; et al. Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. J**ournal of Dairy Science**, p. 1–9, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217301224>. .

WILKENS, M. R.; OBERHEIDE, I.; SCHRÖDER, B.; et al. Influence of the combination of 25-hydroxyvitamin D3 and a diet negative in cation-anion difference on peripartal calcium homeostasis of dairy cows. **Journal of Dairy Science**, v. 95, n. 1, p. 151–164, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030211006862>. .

3. CHAPTER 2 - EFFECTS OF ORAL CALCIUM FORMATE SUPPLEMENTATION IN PERIPARTUM DAIRY COWS

ABSTRACT

The objective of this study was to evaluate the effects of oral calcium formate administration on biochemical parameters and on the productive and reproductive performance of early-lactation dairy cows. In two commercial dairy farms in Castro county, Paraná State, Southern Brazil, 264 Holstein cows (164 multiparous and 100 primiparous) were blocked by herd, parity, and calcemia (tCa) status 6 h after calving. Blood samples were analyzed for group allocation (normal and hypocalcemic groups) using 8.7 mg/dL (2.175 m*M*) as the cutoff (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME). Within each block, fresh cows were randomly allocated to treatment (T) and control (C) groups, with treated-cows being supplemented with two dosis, 6 and 31 hours after parturition, with 350 ml of 14.3% (w/w) calcium as a 48.6% aqueous suspension of calcium formate (Calfon Oral®, Bayer HealthCare). Six blood samples were collected from each animal (6, 12, 31, 54, 78 and 102 h after calving) for the determination of tCa and ionized calcium (iCa), aspartate aminotransferase (AST), albumin (ALB), cortisol (CORT), total cholesterol (TC), betahydroxybutyrate (BHB), and non-esterified fatty acids (NEFA). Information on the incidence of diseases, culling and deaths, milk production and composition, and reproductive performance of cows were recorded. Data was analyzed using MIXED procedure of SAS with a model containing the effects of block, treatment, time, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Subclinical hypocalcemia incidence rates were 39% using on-farm tCa from VetTest (≤ 2.175 m*M*), 64% using tCa (≤ 2.0 m*M*) and 76% using iCa (concentration ≤ 1.0 m*M*). For clinical hypocalcemia, the incidence was 2.2%. Nadir for plasmatic Ca was observed at the adjusted mean time of 17.5 h after calving. It was observed an increase of the concentration in the plasma values of iCa ($P < 0.05$) and tCa ($P = 0.08$) in Ca formate-treated cows when compared with control group, 30 h postpartum. Subclinical ketosis (serum BHB ≥1.2 mmol/L) incidence rate was 24%. Estimates of BHB on day 5 were lower for treated cows ($P < 0.01$) compared with the non-treated ones. No differences were detected ($P > 0.05$) for NEFA, CORT, AST, ALB and TC concentrations between T and C animals. Cows from the control group showed a higher ($P < 0.05$) incidence of displaced abomasum. In the evaluation of the cow performance, there was a trend ($P = 0.08$) for greater milk yield in the treated group in multiparous cows in the first 100 DIM. No effect on reproductive parameters was observed. During the experimental period, both farms had shown very high levels of subclinical hypocalcemia. The oral calcium formate supplementation had shown modest, but beneficial effects in early-lactation dairy cows, increasing ionized Ca, reducing BHB concentrations and decreasing the incidence of some diseases, important goals to control metabolic disorders in dairy farms.

Keywords: milk fever, ketosis, subclinical hypocalcemia

RESUMO

O objetivo deste estudo foi avaliar os efeitos da administração oral de formiato de cálcio nos parâmetros bioquímicos e no desempenho produtivo e reprodutivo das vacas leiteiras no início da lactação. Em duas fazendas leiteiras comerciais no município de Castro, Estado do Paraná, Sul do Brasil, 264 vacas da raça Holandesa (164 multíparas e 100 primíparas) foram divididas em blocos por rebanho, paridade e calcemia através do cálcio total (tCa) 6 h após o parto. As amostras de sangue foram analisadas para alocação de grupo (grupos normo e hipocalcêmico) usando 8,7 mg/dL (2.175 m*M*) como ponto de corte (IDEXX VetTest® Chemistry Analyzer, Inc., Westbrook, ME). Dentro de cada bloco, as vacas recém paridas foram alocadas aleatoriamente para os grupos de tratamento (T) e controle (C), sendo as vacas tratadas duas doses, 6 e 31 horas após o parto, com 350 ml de cálcio a 14,3% (p/p) com uma suspensão aquosa com 48,6% de formiato de cálcio (Calfon Oral®, Bayer Saúde Animal). Foram coletadas seis amostras de sangue de cada animal (6, 12, 31, 54, 78 e 102 h após o parto) para a determinação de tCa e cálcio ionizado (iCa), aspartatoaminotransferase (AST), da albumina (ALB), cortisol (CORT), colesterol total (TC), betahidroxibutirato (BHBA) e ácidos graxos não esterificados (NEFA). Foram registradas informações sobre a incidência de doenças, descartes e mortes, produção e composição do leite e desempenho reprodutivo das vacas. Os dados foram analisados usando o procedimento MIXED do SAS com um modelo contendo os efeitos do bloco, tratamento, tempo e interação do tratamento*tempo como efeitos fixos e a vaca dentro do tratamento como efeito aleatório. As taxas de incidência de hipocalcemia subclínica foram 39% usando tCa na fazenda com VetTest (≤ 2,125 m*M*), 64% usando tCa (≤ 2,0 m*M*) e 76% usando iCa (concentração ≤ 1,0 m*M*). Para hipocalcemia clínica, a incidência foi de 2,2%. O Nadir para o Ca plasmático foi observado no tempo médio ajustado de 17,5 h após o parto. Observou-se aumento da concentração nos valores plasmáticos de iCa ($P < 0,05$) e tCa ($P = 0,08$) em vacas tratadas com formiato de Ca quando comparadas com o grupo controle, 30 h pós-parto. A taxa de incidência de cetose subclínica (BHB sérica ≥1,2 m*M*) foi de 24%. As estimativas de BHBA no dia 5 foram menores para as vacas tratadas ($P < 0,01$) em comparação com as não tratadas. Não foram detectadas diferenças ($P > 0,05$) para as concentrações de NEFA, CORT, ALB, AST e TC entre animais dos grupos T e C. As vacas do grupo C apresentaram maior incidência ($P < 0,05$) de deslocamento de abomaso. Na avaliação do desempenho das vacas, houve uma tendência ($P = 0,08$) para maior produção de leite no grupo de vacas multíparas tratado nos primeiros 100 dias em lactação (DEL). Não foi observado efeito nos parâmetros reprodutivos. Durante o período experimental, ambas as fazendas mostraram níveis muito altos de hipocalcemia subclínica. A suplementação oral de formiato de cálcio mostrou efeitos modestos, mas benéficos em vacas leiteiras no início da lactação, aumentando o Ca ionizado, reduzindo as concentrações de BHBA e a incidência de algumas doenças, metas importantes para controlar distúrbios metabólicos nas fazendas leiteiras.

Palavras-chave: febre do leite, cetose, hipocalcemia subclínica

3.1 INTRODUCTION

The transition period has a relatively short duration in the productive cycle of the dairy cow, but it is where most of the infectious diseases and metabolic disorders occur, such as retained placenta, metritis, hypocalcemia, ketosis, fatty liver and displaced abomasum (DRACKLEY, 1999). In fact, approximately 75% of diseases occur in the first month after calving (LeBLANC et al., 2006), with consequences on the expected lactation milk yield, reproduction performance and the economic results of dairy farms.

Hypocalcemia occurs near calving and very early lactation, due to an abrupt demand for Ca for the colostrum synthesis in the mammary gland. Calcium concentration in the colostrum is estimated to be 7 to 10 times higher than the Ca blood concentration, resulting in a drop in its concentration by a temporary incapacity of adaptation of the cow to this drainage (HORST; GOFF; REINHARDT, 2005). This metabolic imbalance is more common in multiparous and older cows, and the nadir of Ca blood concentrations occurs between 12 and 36 h postpartum (OETZEL, 2004; GOFF, 2008; BLANC et al., 2014).

Normal limits for calcium concentration (**Ca**) in the blood from an adult cow were set at 2.43 to 3.10m*M* (KANEKO; H HARVEY; BRUSSBRUSS, 1997), however in more recent studies the values were adjusted for 2.125 a 2.375 m*M* (KIMURA REINHARDT; REINHARDT; GOFF, 2006) and 2.2 to 2.7 m*M* (VIEIRA NETO et al., 2017).

Reinhardt et al. (2011) using total calcium (**tCa**) and thresholds between 1.4 and 2.0 m*M*, found a prevalence of hypocalcemia in the first 48 h postpartum of 1% for clinical hypocalcemia (**CH)** and 25% for subclinical hypocalcemia (**SCH)** in primiparous cows, and 7% for **CH** and 47% for **SCH** in multiparous cows. Martinez et al. (2016) found an even higher SCH prevalence; 69% of cows categorized as SCH, with cutoff ≤ 2.125 m*M*. Wilhelm et al. (2017) with 2 h after calving reported hypocalcemia incidences of 2.7% for primiparous and 30.8% for multiparous, with the cutoff for tCa ≤ 2.0 m*M*. Thus, the proportion of cows categorized as SCH may depend on the cutoff point and sampling time in relation to calving.

In order to study the negative effects of hypocalcemia, it is not required the cow to show obvious clinical signs. Subclinical hypocalcemia (SCH) is more expensive than CH because it affects a much higher percentage of lactating cows in the herd (OETZEL, 2013). Clinical hypocalcemia (CH) is associated with uterine prolapse, retained placenta, endometritis, impaired fertility, mastitis and decreased motility of the rumen and abomasum (GOFF, 2008; JAWOR et al., 2012). Likewise, SCH is associated with metritis

(MARTINEZ et al. 2012), ketosis (CURTIS et al., 1985), displaced abomasum (CHAPINAL et al., 2011), reduced responsiveness of immune cells (KIMURA; REINHARDT; GOFF, 2006; MARTINEZ et al., 2012) and the increase of involuntary culling (ROBERTS et al., 2012).

Effective reduction of CH incidence (or milk fever) in commercial dairy herds was possible through management and nutrition practices in the prepartum through the use of acidogenic salts, limited calcium intake, and vitamin D supplementation, however, the prevalence of SCH remains high (REINHARDT et al., 2011; NEVES et al., 2017). Oral Ca solutions initially were developed for CH cows (THILSING-HANSEN et al., 2002), but later it was discovered its use as a prophylactic alternative in the critical peripartum period, by the rapid absorption of Ca through passive diffusion, raising its concentration in the blood (GOFF et al., 1996).

The evaluated commercial herds present the management and technologies for high productivity of the animals, such as the use of acidogenic diet as a preventive protocol in the transition period. Like other farms in Brazil, there is no established protocol for the use of oral calcium and monitoring the prevalence of hypocalcemia in animals. Several studies on the effect of oral Ca were performed, mainly with proprionate and Ca chloride, with the initial idea of CH control. This study analyzed the effect of the use of Ca formate (Calfon oral®, Bayer Animal Health), a product that is already commercialized in Brazil, however, with a shortage of scientific work evaluating its preventive use on hypocalcemia in dairy cows.

The objectives of this study were to evaluate the prevalence of HYPO, the effects of oral calcium formate supplementation on blood Ca concentrations, biochemical parameters, postpartum diseases occurrence, milk yield, and fertility in early-lactation Holstein cows.

3.2 MATERIALS AND METHODS

3.2.1 Animals, Dry Cows and Postpartum Management

The experiment was conducted between June, 2015 through June, 2016 in two commercial dairy farms in Castro county, Paraná State, Southern Brazil (24º 47' 28" S, 50º 00' 43" W, with an altitude of 3278 feet). All procedures involving cows in the experiment

were approved by the Federal University of Paraná State Animal Ethics Committee. The choice of these farms was due to the proximity between them, production system employed, enrollment in an official milk recording production, production scale and similar animal productivities (average herd size of 800 cows and 39 kg of milk/day). Both herds were 100% Holsteins, and the cows were kept confined in a free-stall. The total number of females used in the experiment was 264 cows distributed among control (**C**) and treatment (**T**) groups. Means (±SD) were lactation number 2.54 ± 1,54 (3,2 ± 1,42 for multiparous), and BW at calving of 692 ± 59.3 kg and 575 ± 49,5 kg for multiparous and primiparous, respectively. Pregnant cows were dried 58 ± 3 d before the expected calving day and All cows were moved to the close-up group on average 20 ± 5 d before the day of calving. The diet was common to all prepartum cows and it was formulated to achieve a negative DCAD, offered for ad libitum intake and distributed twice a day as TMR.

3.2.2 Diet Formulation and Composition

Close-up and fresh cows were fed twice and three times daily, respectively. Representative TMR samples were collected weekly (a total of nine weeks) just before feeding and forage samples were collected monthly, frozen until they were transported to the laboratory for DM content analysis (dried at 55º C for 72h in a forced-air oven), and milled in a Willey type mill with 1 mm sieves. The determination of the dry matter content (DM) was completed in an oven at 105ºC for 24 h and the ash content was obtained by incineration in muffle at 600ºC for 3 h (AOAC International, 1990). Measurements of neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (ADF) were performed according to Van Soest (1994) methodology adapted to the sequential method using ANKOM Fiber Analyzer (ANKOM® Technology Corp.) according to the technique described by (HOLDEN, 1999). Crude Protein (CP) was executed by the method of Dumas (WILES; GRAY; KISSLING, 1998), in autoanalyzer of nitrogen (ELEMENTAR® rapid N cube). Analysis of the ether extract was done in the ANKOM® XT 10 extractor, using petroleum ether solvent, with extraction time of 6 hours (SILVA; QUEIROZ, 2002). Levels of K and Na minerals were determined by atomic absorption spectrophotometry, at the Animal Nutrition Laboratory at Federal University of Paraná State (**UFPR**; Curitiba, Paraná, Brazil). Chlorine was determined by aqueous shaking Malavolta, E.; Vitti, G. C.; Oliveira (1989) and sulfur by turbidimetry (MIYAZAWA et al., 1999) at the ABC Foundation Laboratory in the city of Castro, Paraná, Brazil. With the results from the above

analysis, the dietary cationic-anionic difference (DCAD) was calculated [(K + Na) - (Cl + S)], using the software NRC (2001).

3.2.3 Experimental Design and Treatments

In this study, the blood sampling to determine the status of calcemia were in the winter months, a fact that according to Østergaard et al. (2003) has no effect on the incidence of hypocalcemia. Initially, 270 cows were enrolled, however, data from 6 animals were discarded from the study because they presented CH and have been treated with an injected Ca supplement. For the formation of the groups the 264 animals were divided according to the following criteria: first, herd (**A** =160 and **B** = 104 cows); second, calving order in primiparous (n = 100) and multiparous (n = 164) cows; and third, calcemia status in normocalcemic (**NC**; n = 96) and SCH (n = 168). For the on-farm measurement of calcemia, blood samples (plasma) were taken at the average time of 6 h after calving, tCa measured by an automated equipment (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME) to be then categorized. Blood samples were measured on-farm to define which cows had SCH after calving using as the cut-point at ≤ 2.225 m*M* for tCa concentration in IDEXX VetTest®, value with optimal sensitivity and specificity with the laboratory results (cut-point ≤ 2.0 m*M*), established by using the receiver operator characteristic (**ROC** curve). Within each block, fresh cows were randomly allocated to T (n = 130) and C (n = 134) groups, with treated-cows being supplemented twice, in day 0 (6 h postpartum) and day 1 (33 hours after parturition), with 350 ml of 14.3% (w/w) calcium (50 g of Ca supplementation) as a 48.6% aqueous suspension of calcium formate (Calfon Oral®, Bayer Animal Health. Six blood samples from each animal were collected; 6, 17.5, 30, 53, 76.5, and 100 h after calving (**t1** to **t6**) for determination of tCa and iCa, aspartate aminotransferase (**AST**) enzyme and albumin (**ALB**). Non-esterified fatty acids (**NEFA**) concentration was determined only in the samples taken 6 h after calving. On samples collected 6 h and 5 d after calving, cortisol (**CORT**) and total cholesterol (**TC**) were analysed; while for beta-hydroxybutyrate (**BHB**) analysis, blood samples were collected on days 3, 5 and 7.

3.2.4 Blood and Urine Sampling

In the prepartum period, the urinary pH was measured weekly from the sampling of 10 close-up animals in each farm, using a portable pH meter with electrode (Model HI 98108 – Hanna Instruments, Inc.). The urine was collected through manual massage in the subvulvar region. In order to have its urine samples collected, the females had to be consuming the anionic diet provided in the close-up group for at least 5 days. The targeted urine pH at these farms was between 5.5 and 6.5.

In the postpartum period, for the evaluation of tCa on the farm, 1.5 mL of blood was collected immediately before treatment in Eppendorf tube with lithium heparin, which sample was centrifuged (Eppendorf Minispin® Plus) at 12.100 × g for 5 minutes. Before performing the tCa test, the equipment was calibrated with standard solutions. After the calibration, the test was started with a 30µL aliquot of plasma obtained through the equipment pipettor (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME), which uses dry biochemistry slides with the processing and result in mg/dL is available in 8 minutes. The resulting value was used for the categorization of the animals (NC or SCH) and its subsequent blocking.

For the plasma metabolites measurements, blood samples (9.0 mL) of the coccygeal vein or artery were collected in tubes with sodium heparin (vacutainer). The samples were centrifuged (CENTRIBIO®, model 80-2B) at 1.800 x g for 10 minutes to obtain the plasma. The samples were then identified and stored in Eppendorf tubes (in duplicate) at -20ºC until they were transported to the Laboratory of Veterinary Clinical Pathology at Federal University of Paraná State (UFPR) where the samples were kept frozen at -80°C and analyzed until 80 days after collection.

3.2.5 Milk Production and Composition

Experimental cows were milked 3 times a day, with daily milk production recorded through herd management software Delpro® (DeLaval). The effects on the accumulated milk production during the first 30 and 100 days of lactation were analyzed. The individual milk composition (fat, protein, lactose, and total solids contents) was obtained from the first four test-days after calving because both herds are enrolled in an official milk recording program with Holstein Association of Paraná State, Brazil.

3.2.6 Fertility Responses and Culling

Reproductive management on farms was similar, with cows being released to the artificial inseminations (**AI**) with 60 DIM, after evaluation by a veterinarian. Estrus monitoring was done by trained employees 3 times a day. After the 30-day AI period, pregnancy diagnosis by imaging is performed by ultrasonography. After diagnosis, animals with negative pregnancy are directed to one of the farm's reproductive protocols for re-AI. The reproductive data were obtained through herd management software Delpro® (DeLaval), and the effects of treatment on pregnancy, days open and number of AI for pregnancy, during the period 150 and 365 days after calving, were analyzed. Involuntary culling was monitored during the 365 days postpartum.

3.2.7 Concentration of iCa and tCa

For analysis of total Ca (tCa) concentrations six samples were collected at 6, 17.5, 30, 53, 76.5, and 100 h after calving; they were determined in the plasma through an automated biochemical analyzer (BS-200, MINDRAY® Chemistry Analyzer), using the colorimetric method (DIALAB®), (ARZENAZO III), at average time of 80 d after collecting samples. For analysis of ionized Ca (iCa) concentrations, samples taken at the same hours described above, the method used was gasometry by selective ion electrode (RAPIDLab® 348 EX Blood Gas Analyzer, Siemens Healthineers). The cut point to categorize animals as SCH was tCa ≤ 2.0 m*M*, (GOFF, 2008; REINHARDT et al., 2011; WILHELM et al., 2017).

3.2.8 Concentration of CORT and TC

Cortisol determination (6 h and 5 d postpartum) was done by the enzyme immunoassay method (ELISA - Enzyme Linked Immunosorbent Assay). It was used anti-cortisol antibody (Polyclonal R4866) and cortisol-HRP conjugated hormone (Coralie Munro - University of California, Davis, CA, USA) diluted 1:8,500 and 1:20,000 respectively. Cortisol levels were obtained in ng/ml by the validated ELISA kit for bovine serum samples, according to the method described by Brown et al. (2004). Dosages were performed in duplicate. The inter-assay coefficient of variation was less than 10%. The

laboratory analyzes were done in the Laboratory of Endocrine Physiology and Animal Reproduction at the Federal University of Paraná State.

For the analysis of TC, Dialab® reagents (CHOD-PAP Cholesterol) through the colorimetric enzymatic method were used for their determination after hydrolysis and enzymatic oxidation. As normal parameters were used for CORT 13 to 29.3 ng/mL and CT of 80 to 120 mg/dL (KANEKO et al., 2008)

3.2.9 Concentrations of NEFA and BHB

Determinations of NEFA (6 h postpartum) and BHB (3, 5, and 7 d postpartum) were quantified by colorimetric enzymatic methodology using standard commercial Randox® reagents (Kit NEFA FA115 and Kit Ranbut D-3 Hydroxybutyrate RB 1007). Analyzes were performed in an automatic biochemical analyzer (BS-200, MINDRAY® Chemistry Analyzer). Hemolyzed samples were discarded (STOKOL; NYDAM, 2006). As cut point parameters were used for NEFA < 0.7 m*M,* and BHB < 1.2 m*M* for healthy animals, 1.2 to 2.9 m*M* for subclinical ketosis and > 2.9 m*M* for clinical ketosis (OETZEL, 2004; OSPINA et al., 2010; CHAPINAL et al., 2011).

3.2.10 Concentrations of ALB and AST

For the ALB protein and the AST enzyme, aliquots of the six blood samples collected were used. Reagents were from Dialab® reagents (Albumin-BCG), by the bromocresol green method (BCG), and the results were expressed as mg/dL and later converted to g/dL.

The AST assays were performed using the UV kinetic method, optimized according to the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) using the TGO kit (ASAT), brand Dialab® reagents, and the results expressed as U/L. As normal concentrations for ALB, values between 3.03 and 3.55 g/dL were used, and for AST enzyme, values between 78 a 132 U/L.

3.2.11 Diseases Characterization

Definitions of the diseases were already standardized and implemented by farms in accordance with veterinarian guidelines. The standard definitions of diseases were as

follows: (1) clinical hypocalcemia (**CH**); apathy, lack of coordination, muscle tremors, cold extremities, prostration and decubitus; (2) displaced abomasum (**DA**) – off-feed, decreased milk production, movement of the abomasum to the left side of the cow, which was detected by listening to a metallic sound of "ping" on the left flank through the percussion with the finger; (3) ketosis (**KET**) – clinical ketosis (**CK**) and subclinical (**SK**) – for CK reduced feed intake, decreased milk production, without other detectable signs of disease and with positive ketosis result through test strips ≥ 2.9 m*M* (FreeStyle® Optium Neo, Abbott), at 3, 5, 7 or 11 days postpartum. For SK with positive test strips between 1.2 to 2.9 m*M*; (4) retained placenta (**RP**) – it was diagnosed when fetal membranes were not completely expelled from the birth canal within 24 h of labor (5) metritis (**ME**) - sick cow who showed signs of apathy, inappetence, a body temperature > 39.5°C (at 3, 7 and 11 days postpartum) with a foul discharge from the vulva (purulent or red to brown or both) LeBlanc (2010), diagnosed during the daily checks of cows between days 3 and 11 of lactation; (6) clinical mastitis (**MAS**) – through the presence of udder edema, decreased milk production, as well as alteration of color and presence of flakes, clots or seruous milk.

3.2.12 Statistical Analysis

The experiment was completely randomized, with cow being the experimental unit. Statistical models included the effects by herd (A and B), parity (primiparous and multiparous), and tCa (NC and SHC) status 6 h after calving. For definition of NC cow the concentration of tCa in blood > 2.0 mM and SCH when tCa in blood was ≤ 2.0 mM. The animals were randomly assigned to the T and C groups. Blood samples were analyzed for group allocation using the 8.7 mg/dL as the cutoff (IDEXX VetTest® Chemistry Analyzer, Inc., Westbrook, ME). The cutoff was established through the optimum sensitivity (54.8) and specificity (86.8) with the tCa from the laboratory, using receiver operator characteristic (ROC), with MedCalc software (version 14.8.1). Statistical analyzes were conducted using SAS software (version 9.4; SAS/STAT®, SAS Inst. Inc., Cary, NC). All measurements repeated over time were subjected to repeated measures ANOVA using the MIXED procedure, with an autoregressive order 1 covariance structure, based on the Akaike information criterion (AIC). The data were previously tested for normality of residues by the Shapiro-Wilk test. The model contains the effects of treatment, time, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Binary traits such as the estimate of the number of AI to conceive and the risk of pregnancy

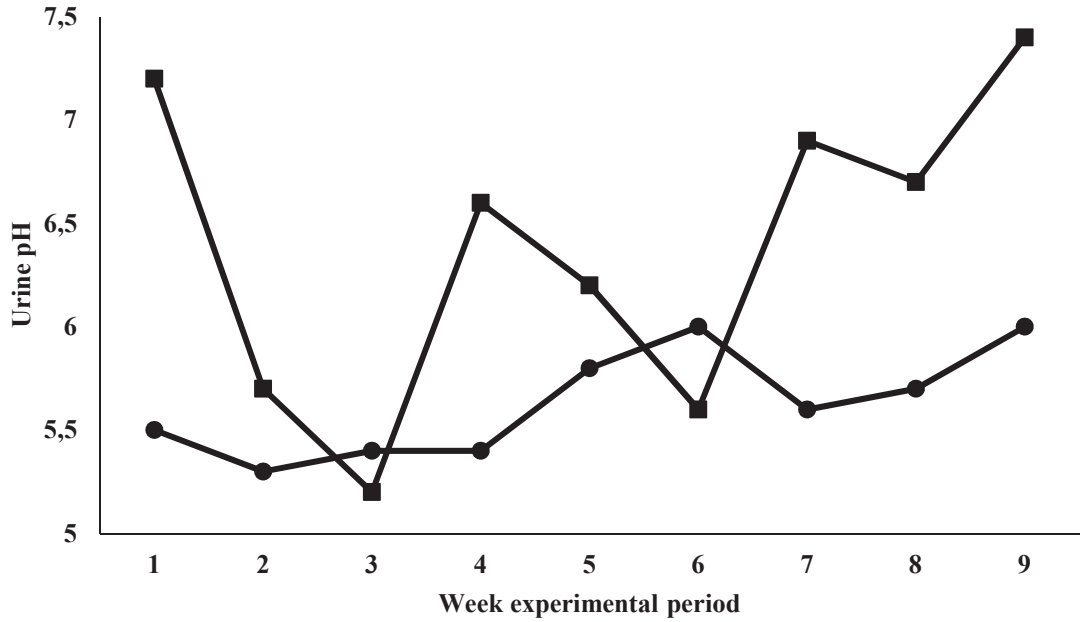
at 150 and 365 days were analyzed by log-Poisson regression using the GENMOD procedure. Kaplan-Meyer survival analysis (MedCalc, v. 14.8.1) was used to compare the interval from enrollment to removal from the herd and from calving to establishment of pregnancy between treatments. For the association of hypocalcemia incidence and disease prevalence, discards were analyzed by logistic regression using the GLIMMIX procedure with treatment as a binary variable (T or C) and also parity as a fixed effect (primiparous or multiparous cows). A receiver operator characteristic (ROC) analysis was performed (MedCalc, v. 14.8.1) to determine the plasma tCa concentration capable of predicting the incidence of diseases assessed (displaced abomasum, ketosis, metritis, retained placenta and mastitis). The Youden index (YOUDEN, 1950) was used to select the cutoff point at plasma tCa concentration (the point with the smallest difference between sensitivity and specificity) for each pathology evaluated. The CORR procedure was used to determine Pearson correlation coefficients between Ca concentrations and the analytes NEFA, BHB, TC, AST, ALB, and COR. Differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered as a tendency.

3.3 RESULTS

3.3.1 Diet Composition, Urinary pH in Prepartum

Analyzed of diet concentration used in prepartum are on shown in TABLE 3.1. The intake of the pre-calf diet by cows was 19.6 days, the main difference being between the farms the type of feed provided, the farm A used the wheat straw while in the hay *Cynodon*. Thus, there was an effect of the composition of the minerals of these fodder on the anionic cation balance of the diets. The composition of minerals K and Na was 1.19 and 0.03, 1.88 and 0.15% in DM for wheat straw (A) and hay *Cynodon* (B), with a cation-anion difference of 23 and 44.6 mEq/100 g of DM consecutively. With the use of anionic concentrate in the same inclusion per animal formulated by the same supplier, the average DCAD was -20.6 (A) and -12.9 (B) mEq/100 g of DM. The difference in the composition of the cations (K and Na) of the diets of experimental farms can be observed through the weekly monitoring (9 weeks) of urine pH (FIGURE 3.1).

FIGURE 3. 1 AVERAGE VALUES OF THE pH URINE, WEEKLY COLLECT OF 10 FEMALES IN FARMS A AND FARM B, DURING THE 9 WEEKS OF STUDY. IN THE TWO FARMS THE DIETS WHERE ANIONIC



FONTE: O autor (2018).

The mean pH values of urine were 5.6 and 6.4 for farm A and B, consecutively. Among the herds of this study, the different diets in prepartum resulted in an effect on the plasma Ca concentration of cows. Differences were observed for iCa ($P < 0.01$) at t4 (0.99 vs. 0.95 ± 0.01 m*M*) and t5 (0.94 vs. 0.91 ± 0.01 m*M*) and for tCa ($P < 0.05$) in t3 (1.91 vs. 1.87 ± 0.01 m*M*) between herds A and B consecutively.

The mean Ca content in farm A was 0.6% in DM (0.3 to 0.9%) and in B 1.1% in DM (0.3 to 3.1%).

TABLE 3.1 INGREDIENT COMPOSITION OF PREPARTUM AND POSTPARTUM DIET IN TWO HERDS

	Prepartum		Postpartum	
	Farm A	Farm B	Farm A	Farm B
Ingredient- kg/DM/d[1]				
Corn silage	6.0	5.4	8.4	8.22
Wheat straw	1.8	1.8	0.45	-
Brewers grain wet	0.5	0.5	0.63	0.84
Prepatum concentrated[2]	2,9	2.7	-	-
Concentrated feed[3]	-	-	-	2.7
Ryegrass silage	-	-	3.04	3.70
Corn high-moisture	-	-	1.00	-
Soybean meal	-	-	1.76	1.50
Soybean roasted	-	-	0.91	-
Corn glúten meal	-	-	0.43	-
Protected fat[4]	-	0.3	-	-
Cottonseed	-	-	-	0.88
Corn ground dry	-	-	2.11	0.44
Bicox[5]	-	-	-	0.30
Mineral and vitamin mix[6]	-	-	0.89	0.20
Chemical composition[7]				
DM, % (±SD)	37.8 ± 3.5	39.6 ± 0.4	44.5 ± 2.8	48.9 ± 2.0
NEL, Mcal/kg of DM	1.43	1.37	1.65	1.48
CP, % of DM	13.0 ± 0.6	12.2 ± 1.4	15.7 ± 1.3	17.1 ± 0.4
NDF, % of DM	43.1 ± 1.8	35.5 ± 2.5	35.9 ± 3.2	33.2 ± 1.2
ADF, % of DM	21.1 ± 1.1	17.4 ± 1.9	17.9 ± 1.4	17.5 ± 1.7
Ether extract, % of DM	4.3 ± 0.6	4.2 ± 0.9	4.7 ± 0.9	5.2 ± 1.0
Ca, % of DM	0.6 ± 0.2	1.1 ± 0.2	1.1 ± 0.8	0.9 ± 0.2
P, % of DM	0,3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0,1
Mg, % of DM	0.41	0.41	0.36	0.29
K, % of DM	0.77	0.89	1.13	1.10
Na, % of DM	0.04	0.03	0.06	0.05
S, % of DM	0.37	0.33	0.29	0.18
Cl, % of DM	0.57	0.46	0.14	0.84
DCAD[8] (mEq/100 g of DM)	-20.6	-12.9	-	-

[1]Composição das dietas para o pré e pós-parto das duas fazendas experimentais, com o pré-parto consumindo pelo tempo médio de 19,6 d.

[2]Composto de 24% PB, 4.01% extrato etéreo, 1.60 Mcal/kg ELL, 3.14% fibra bruta, 4.05% FDA, 3.49% Ca, 0.74% P total, 0.78% K, 1.19% Mg, 1.13% S, 0.026% Na, 2.5% Cl, 196 mg/kg Mn, 243 mg/kg Zn, 54 mg/kg Cu, 2.1 mg/kg Co, 2.3 mg/kg Se, 5 mg/kg biotina, 1.1 mg/kg colina, 64.0 KIU/kg vitamina A, 14.3 KIU/kg vitamina D3, 333.4 KIU/kg vitamina E, 41 mg/kg monensina (Elanco Animal Health, Greenfield, IN). O DCAD dessa ração é -12 mEq/100g de MS (Ração pré-parto, Coop Castrolanda, Castro-Paraná/Brasil).

[3]Comercial anionic concentrade, composed of 18% PB, 3.0 EE, 5.0% FDA, 1.1% Ca, 0.5% P, 0.48% Mg, 0.14% S, 0.04% Na, 62.5 mg/kg Mn, 137.5 mg/kg Zn, 31.25 mg/kg Cu, 0.62 mg Co, 0.75 mg/kg Se, 2.5 mg/kg biotina, 10.0 KIU vitamina A, 3.0 KIU vitamina D3, 55.5 UI vitamina E, 30.0 mg/kg monensin (Elanco Animal Health, Greenfield, IN) (Ração B3B ELITE, Coop Castrolanda, Castro-Paraná/Brasil).

[4]Royal Energy, energetic nucleus, composed of 12.0% PB, 12.0% EE, 9.0% FDA, 14.0% MM and 3.0% Ca (De Heus®, company of Royal de Heus, Campinas-SP/Brasil);

[5]Commercial Buffer (sodium bicarbonate and magnesium oxide) composed of 2.0% Ca, 1,85% Na, 1,25% Mg.

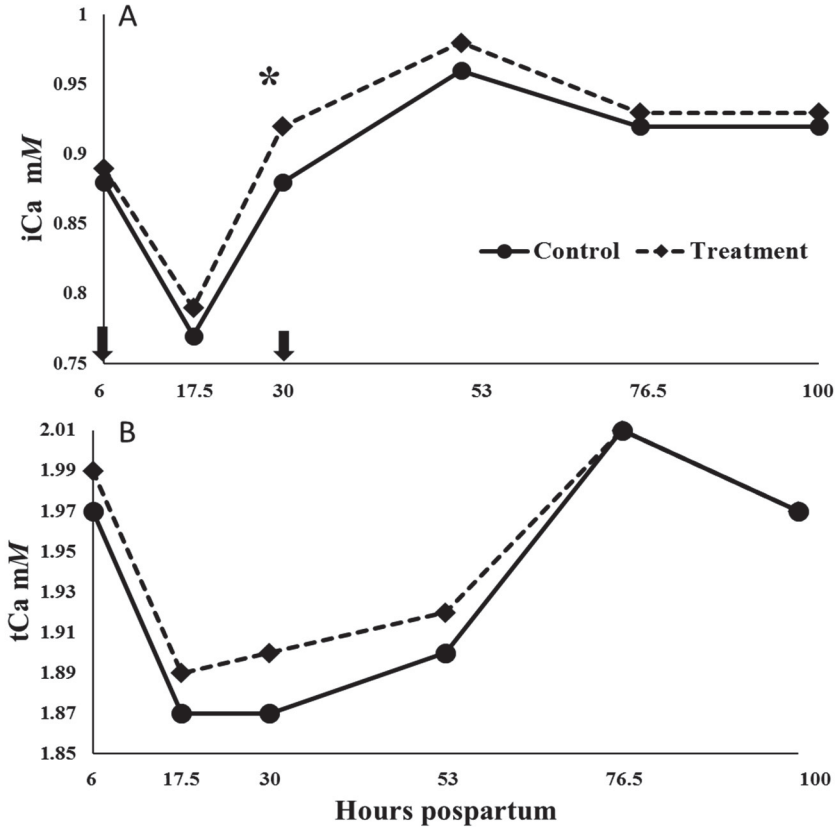
[6]Mineral and vitamin premix contained 22% Ca, 4.8% P, 4.3% Mg, 2.3% S, 5.8% Na, 1.25 mg/kg Mn, 2.75 mg/kg Zn, 625 mg/kg Cu, 12.5 mg Co, 15 mg/kg Se, 50 mg/kg biotina, 200 KIU vitamina A, 60 KIU vitamina D3, 1.1 UI vitamina E, 600 mg/kg monensin (Elanco Animal Health, Greenfield, IN) (Núcleo MELK, Coop Castrolanda, Castro-Paraná/Brasil).

[7]Os valores químicos das dietas reflete a média das análises das 9 amostras semanais colhidas após a mistura no totalmix.
[8]A DCAD foi determinada [$(Na^+ + K^+) - (Cl^- + S^{2-})$] pela média das análises químicas das dietas na primeira e 9ª semana experimental, com o programa NRC 2001.
FONTE: o autor (2018)

3.3.2 Concentration of minerals iCa and tCa Measures in Blood

The Ca concentration in plasma did not differ between groups (FIGURE 3.2 A and 3.2 B) prior to administration of oral calcium. Drainage of Ca through colostrum resulted in a drop in blood Ca in both groups, with less intensity in the group that received Ca formate.

FIGURE 3. 2 SERUM CONCENTRATIONS (mM) IN THE FIRST 100h AFTER CALVING ACCORDING TO (A) iCa AND (B) tCa. PANEL A, THERE HAS BEEN A TREND WHILE PANEL B, HAVE EFFECT OF FORMATE CALCIUM IN TIME 30 h POSTPARTUM. AN ASTERISK (*) INDICATE MEANS DIFFER (P < 0.05)

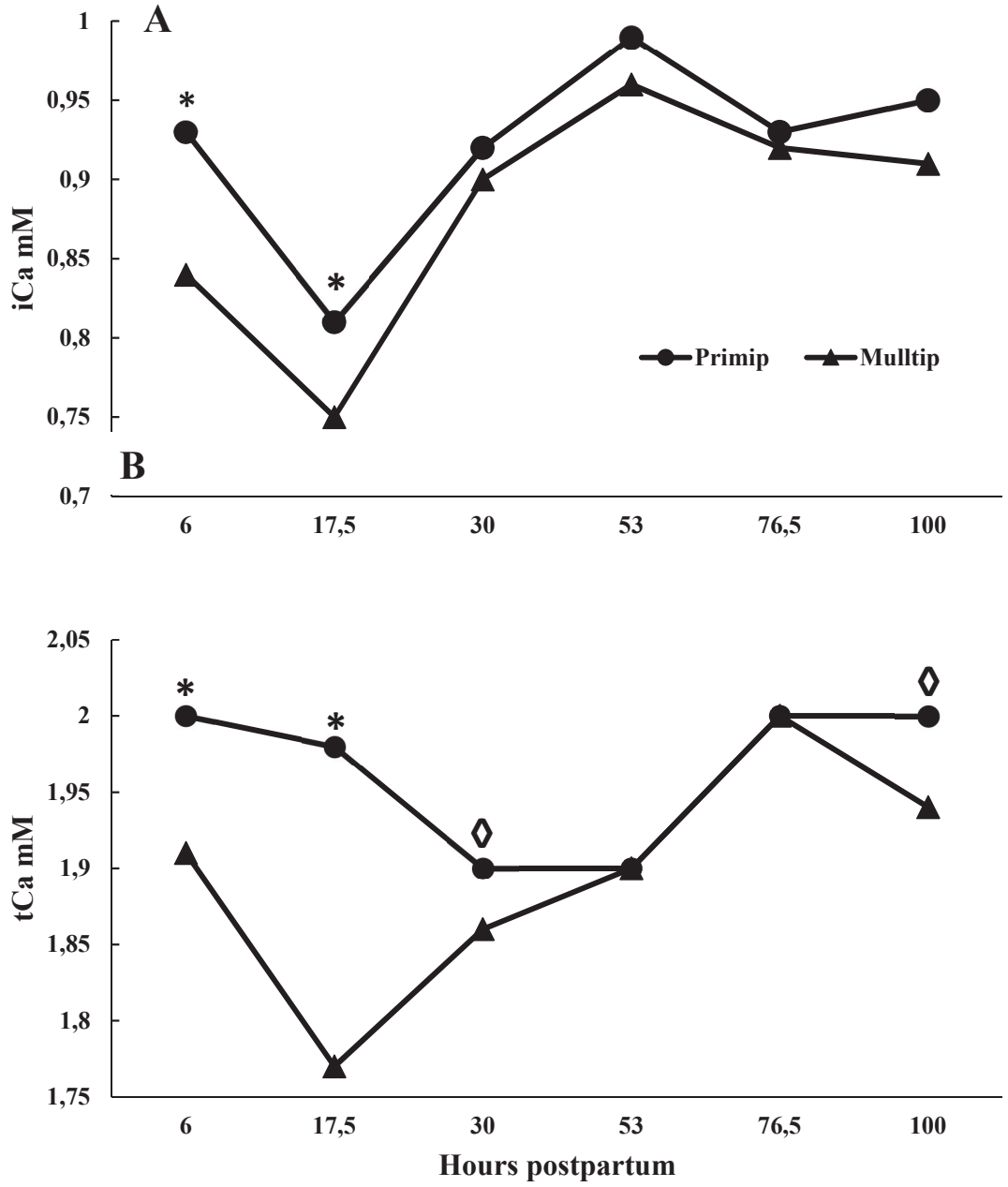


FONTE: O autor (2018)

The nadir was observed in the adjusted mean time of 17.5 h (tCa=1,85±0,22 e iCa= 0,77±0,17 m*M*). When analyzed separately, the nadir in the pluriparous cows (FIGURE

3.3) was in the mean time of 17.5 (tCa = 1.77 ± 0.22 m*M*, iCa = 0.75 ± 0.17 m*M*), while the primiparous in 30 h (tCa = 1.90 ± 0.13 m*M*, ICa = 0.92 ± 0.13 m*M*.

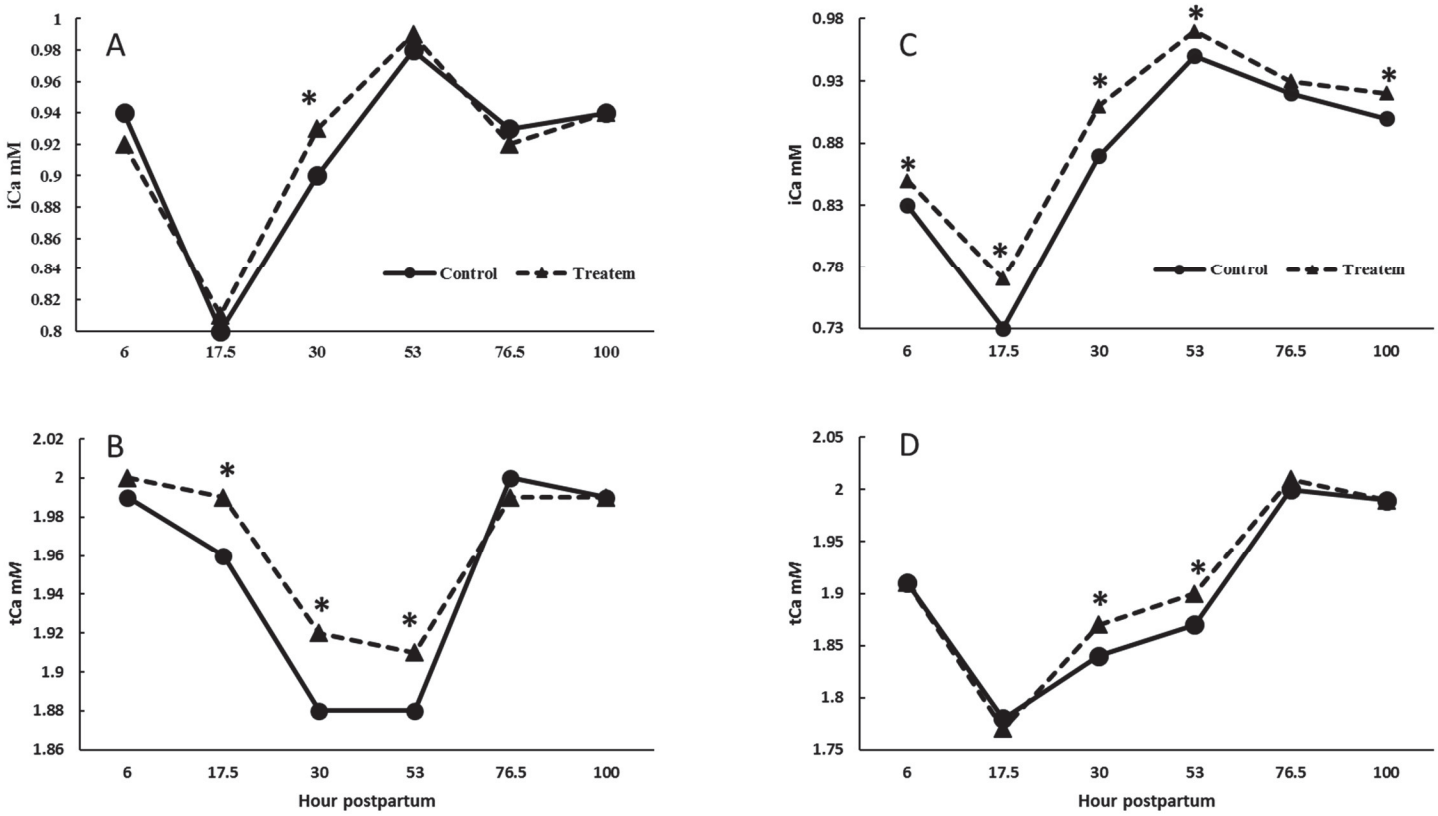
FIGURE 3. 3 THE VALUES FOR MULTIPAROUS AND PRIMIPAROUS COWS IN THE FIRST 100 h AFTER CALVING. PANEL A – PRESENTS THE VALUES OF iCa, WHILE THE B – tCa. THE LOWEST MEAN CONCENTRATION AT TIME 17.5 h. AN ASTERISK (*) INDICATES MEANS DIFFER (P < 0.01). AN SIMBOL INDICATES MEANS DIFFER (P < 0.05)



The percentage of tCa in the ionized form had the average value of 46,8%, its lowest value 42.1% and its highest value 51.4% at 17.5 h and 53 h consecutively, with no significant difference between T and C groups.

After supplementation with oral Ca, there was an increase in the plasma concentration of iCa values in the T group (0.92 vs. 0.89 ± 0.01 m*M*; $P = 0.02$), and there was a trend for tCa (1, 90 vs. 1.87 ± 0.01 m*M*, $P = 0.08$) in time 30 h postpartum compared with C group (FIGURE 3.4).

FIGURE 3. 4 EFFECT THE USE OF Ca FORMATE IN PRIMIPAROUS AND MULTIPAROUS COWS. IN THE PAINEL A (iCa) AND B (tCa) PRIMIPAROUS COWS, AND C (iCa) AND D (tCa) MULTIPAROUS COWS FOR THE PRIMIPAROUS THE MAIN INTERACTION BETWEEN THE USE OF Ca ORAL AND THE PLASMATIC Ca WAS WITH THE tCa, HOWEVER, THE MULTIPAROUS HAPPENED TO THE iCa (*) INDICATE TIME POINTS AT WHICH TREATMENT MEANS SIGNIFICANTLY DIFFER ($P \leq 0.05$)



FONTE: o autor (2018)

3.3.3 Concentrations of ALB, AST, NEFA, BHB, TC and CORT in Blood

The average concentration of the protein, enzymes and metabolites analyzed is shown in TABLE 3.2. For animals supplemented with calcium formate, higher concentrations of the AST enzyme were observed in T3 (T vs. C; 84.2 vs. 74.3 ± 2.7 U/L, $P < 0.01$) and lower concentration of BHBA at T5 (0.7 vs. 0.9 ± 0.06 m*M*; $P < 0.01$).

A tendency of higher value for the T group was observed for the ALB in the T2 (T vs. C; 3.54 vs. 3.43 ± 0.04 g/dL, $P = 0.06$) and for the T4 (3.51 vs. 3.46 ± 0.02 g/dL, $P = 0.07$). No differences were detected ($P > 0.05$) for NEFA, CORT and TC concentrations between T and C animals. On the other hand, for the parturition order, the multiparous presented highest NEFA concentration (0.51 vs. 0.75 ± 0.4 m*M*, $P < 0.01$) and BHBA ($P < 0.05$) on days 3, 5 and 7 postpartum; the ALB protein ($P < 0.05$) in all collections; a lowest TC concentration in day of calving (88.2 vs. 73.3 ± 2.1 mg/dL, $P < 0.01$) and on day 5 postpartum (88.5 vs. 81.6 ± 2.1 mg/dL, $P < 0.05$). The primiparous cows presented higher average values of AST during the experiment when compared to cows, but within the physiological values. In 30% of the primiparous cows the mean plasma concentration of AST was 127 U/L (100.6 to 203.1U/L) at day 3 postpartum.

Regarding calcemia, NC cows (NC vs. SCH) presented lower values for BHBA in the blood on day 3 (0.6 vs. 0.8 ± 0.04 m*M;* ($P < 0.01$) and day 5 (0.67 vs. 0.92 ± 0.06 m*M*; ($P < 0.01$) postpartum. On day 5 for CORT (4.5 vs. 5.9 ± 0.4, $P < 0.01$), however, it was higher for TC (88.2 vs. 82.0 ± 2.1 mg/dL; $P < 0,05$). A moderate correlation between tCa vs. BHBA was observed at T3 time ($r = -0.32$, $P < 0.01$), as well as between CORT vs. BHBA ($r = 0.38$, $P < 0.01$).

TABLE 3.2 MEAN VALUES AND THE EFFECT OF TREATMENT VS. CONTROL GROUPS, PARITY ORDER AND CALCEMIA ON THE CONCENTRATION OF Ca IN THE PLASMA OF COWS AT THE BEGINNING OF LACTATION

		Groups[2]		Parity[3]		Calcemia[4]			*P*-value[5]			Reference values
Item	Average[1]	Control	Treatment	Multip.	Primip.	HYPO	NC	EM	C vs. T	M vs. P	H vs. N	
tCa t1* (m*M*)	1.94	1.97	1.99	2.00	1.91	1.85	2.10	0.01	0.30	<0.01	<0.01	> 2.0 m*M*
tCa t2 (m*M*)	1.85	1.87	1.89	2.00	1.77	1.80	1.96	0.02	0.47	<0.01	<0.01	
tCa t3 (m*M*)	1.88	1.87	1.90	1.90	1.86	1.83	1.95	0.01	0.08	<0.05	<0.01	
tCa t4 (m*M*)	1.89	1.90	1.9	1.90	1.90	1.86	1.93	0.01	0.16	0.72	<0.01	
tCa t5 (m*M*)	2.00	2.01	2.01	2.00	2.00	1.97	2.05	0.01	0.84	0.53	<0.01	
tCa t6 (m*M*)	1.96	1.97	1.97	2.00	1.94	1.95	1.99	0.01	0.94	<0.05	0.07	
iCa t1 (m*M*)	0.88	0.88	0.89	0.93	0.84	0.85	0.92	0.01	0.48	<0.01	<0.01	> 1.0 m*M*
iCa t2 (m*M*)	0.77	0.77	0.79	0.81	0.75	0.75	0.81	0.01	0.21	<0.01	<0.01	
iCa t3 (m*M*)	0.90	0.89	0.92	0.92	0.90	0.90	0.92	0.01	<0.05	0.11	0.11	
iCa t4 (m*M*)	0.97	0.96	0.98	0.99	0.96	0.95	0.99	0.01	0.15	0.01	<0.01	
iCa t5 (m*M*)	0.93	0.92	0.93	0.93	0.92	0.91	0.94	0.01	0.64	0.58	0.01	
iCa t6 (m*M*)	0.93	0.92	0.93	0.95	0.91	0.92	0.93	0.01	0.29	0.02	0.45	
ALB t1 (g/dL)	3.56	3.53	3.59	3.46	3.61	3.52	3.60	0.03	0.19	<0.01	0.14	3.03 to 3.55
ALB t2 (g/dL)	3.47	3.43	3.54	3.40	3.52	3.46	3.52	0.04	0.06	<0.05	0.32	
ALB t3 (g/dL)	3.51	3.50	3.55	3.46	3.55	3.48	3.56	0.03	0.13	<0.05	0.07	
ALB t4 (g/dL)	3.48	3.46	3.51	3.42	3.51	3.46	3.51	0.02	0.07	<0.01	0.17	

TABLE 3.2 - MEAN VALUES AND THE EFFECT OF TREATMENT VS. CONTROL GROUPS, PARITY ORDER AND CALCEMIA ON THE CONCENTRATION OF Ca IN THE PLASMA OF COWS AT THE BEGINNING OF LACTATION. Contin…

Item	Average[1]	Groups[2]		Parity[3]		Calcemia[4]		EM	*P*-value[5]			Reference Values[7]
		Control	Treatment	Multip.	Primip.	HYPO	NC		C vs. T	M vs. P	H vs. N	
ALB t5 (g/dL)	3.30	3.31	3.36	3.20	3.37	3.27	3.40	0.03	0.24	<0.01	<0.01	
ALB t6 (g/dL)	3.46	3.45	3.47	3.40	3.50	3.44	3.48	0.03	0.67	<0.01	0.26	
AST t1 (U/L)	76.80	76.10	77.50	83.90	72.50	76.90	76.70	2.70	0.72	<0.01	0.97	78 to 132
AST t2 (U/L)	78.90	76.22	85.10	88.80	72.80	72.90	88.40	2.80	0.09	<0.01	0.98	
AST t3 (U/L)	77.80	74.30	84.20	86.90	72.12	78.70	79.90	2.70	<0.01	<0.01	0.77	
AST t4 (U/L)	86.40	86.44	89.57	93.20	82.40	86.81	89.18	3.00	0.44	<0.05	0.57	
AST t5 (U/L)	86.65	84.80	87.90	91.00	84.00	90.90	91.50	2.60	0.38	0.06	<0.05	
AST t6 (U/L)	81.00	78.90	82.40	87.40	77.30	83.39	77.90	2.80	0.37	<0.05	0.17	
BHBA 3d (m*M*)	0.70	0.73	0.67	0.58	0.77	0.80	0.60	0.04	0.33	<0.01	<0.01	< 1.2
BHBA 5d (m*M*)	0.80	0.90	0.70	0.66	0.86	0.92	0.67	0.06	<0.01	<0.05	<0.01	
BHBA 7d (m*M*)	0.90	0.96	0.85	0.75	0.96	0.96	0.85	0.07	0.29	<0.05	0.30	
NEFA t1 (m*M*)	0.66	0.70	0.60	0.51	0.75	0.80	0.50	0.04	0.19	<0.01	<0.01	< 0.7
TC[6] calving (mg/dL)	79.00	76.70	81.20	88.20	73.30	77.00	80.10	2.10	0.12	<0.01	0.18	80 to 120
TC day 5 (mg/dL)	84.20	84.20	86.00	88.50	81.60	82.00	88.20	2.10	0.55	0.02	<0.05	
CORT calving (ng/mL)	15.60	15.30	15.90	16.70	15.00	16.80	14.50	1.10	0.69	0.32	0.17	13 to 29.3
CORT day 5 (ng/mL)	5.12	5.40	4.90	4.8	5.3	5.90	4.50	0.04	0.33	0.44	0.01	

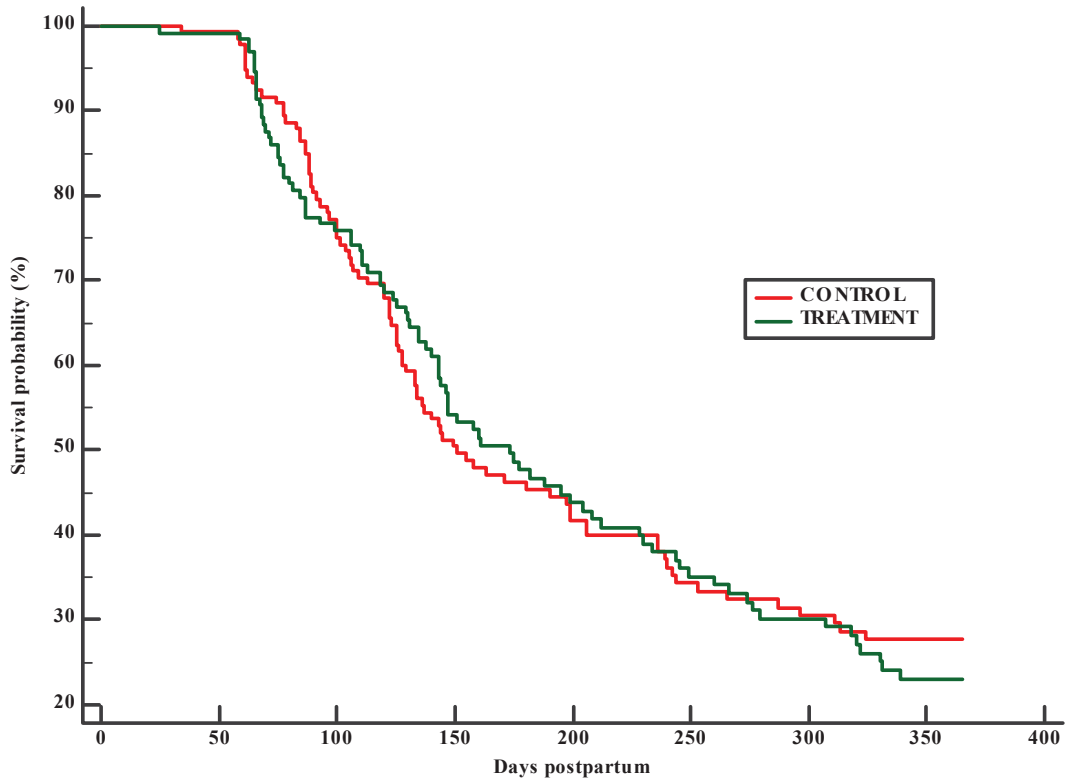
[1]Average value of the mineral or metabolite; t1, t2, t3, t4, t5, t6 = are the mean times of collection of blood samples, 6, 17.5, 30, 53, 76.5 and 100 h after calving consecutively.

[2]Average value of the mineral or metabolite in T and C group, in which the cows received two doses of calcium formate (50 g of Ca) in the mean time of 17.5 and 30 h postpartum. Within treatment in a given row, means with distinct superscripts differ after adjustment by Tukey ($P < 0.05$);
[3]Mult. = multiparous; Prim. = primiparous. Within parity in a given row, means with distinct superscripts differ after adjustment by Tukey ($P < 0.05$).
[4]HYPO = hypocalcemic – NC = normocalcemic. Within calcemia in a given row, means with distinct superscripts differ after adjustment by Tukey ($P < 0.05$).
[5]C vs. T = control group vs. treatment group; M vs. P = multiparous vs. primiparous; H vs. N = Subclinical HYPO vs. NC.
[6]TC = total cholesterol.
[7]Reference values – iCa and tCa (GOFF, 2008; REINHARDT et al., 2011; WILLELM et al., 2017); CORT, ALB, TC and AST (KANEKO et al., 2008); NEFA and BHBA (OSPINA et al., 2004; CHAPINAL et al., 2011)
* The times t1, t2, t3, t4, t5, t6 reference at 6, 17.5, 30, 53, 76.5 and 100 h after calving consecutively
FONTE: o autor (2018)

3.3.4 Fertility Responses, Milk Yield and Culling

The mean time between birth and the first AI was 78 DMI. Twenty percent (54/264) of cows became pregnant at the first AI, 47.3% (125/264) at 150 days and 67% (177/264) at 365 DIM. The mean time to pregnancy was 141 DIM, with 2.9 AI per pregnancy. There was no effect of Ca formate on pregnancy in the first AI at 150 and 365 DIM. In FIGURE 3.5, analyzing the risk of pregnancy through the Kaplan-Mayer survival curve, there was no significant difference between the C and T groups in the 365 DIM. However, of cows with negative pregnancy at 150 and 365 DIM, 71% (98/138) and 77% (67/87) were of the SCH group; 69% (96/139) and 77% (67/87) for multiparous cows ($P < 0.01$), consecutively.

FIGURE 3. 5 KAPLAN-MEIER PLOT FOR THE EFFECT OF ORAL Ca SUPPLEMENTATION ON PREGNANCY TO 365 DIM



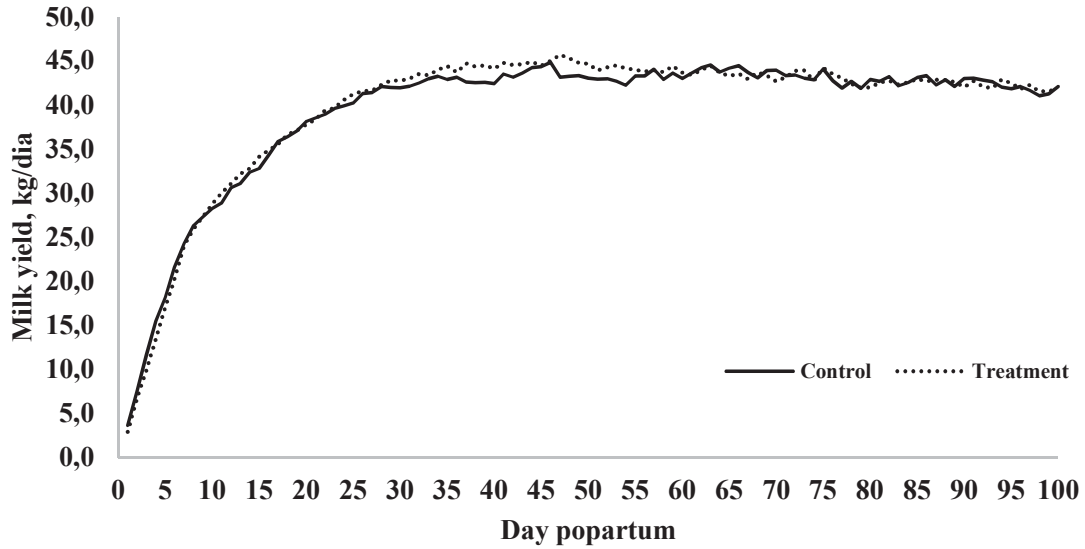
FONTE: o autor (2018)

The average milk production was 35.5 and 42 kg/milk/day in 30 and 100 DIM (FIGURE 3.6). There was a trend ($P = 0.08$) of the effect of Ca formate on the average milk yield in the 100 DIM in pluriparous cows, with an increase of 0.7 kg of milk per day (44.8 vs. 44.1 kg/day). Pluriparous cows with SCH produced more milk when compared to NC cows

(45.7 vs. 43.3 kg/milk/day, $P < 0.01$), a fact not observed in primiparous animals (34.5 vs. 33.7; $P = 0.26$).

The effect of oral Ca supplementation on discarding was not observed. However, of the 45 discarded cows, 78% (35/45) were from the multiparous group and 80% (36/45) with SCH ($P < 0.05$).

FIGURE 3. 6 MILK YIELD (kg/d) DURING THE FIRST 100 DIM. PRODUCTION OF THE CONTROL GROUP (N = 134) 38.9 kg/d AND TREATMENT (N = 130) WAS AND 39.1 kg/d. THERE WERE NO ORAL INTERACTION WITH MILK PRODUCTION (P = 0.64). HOWEVER, IN THE GROUP OF MULTIPAROUS COWS PRODUCTION SHOWED A TREND (P = 0.08) OF HIGHER T PRODUCTION (T vs. C) 44.8 vs. 44.1 ± 0.3 WHEN COMPARED TO GROUP C

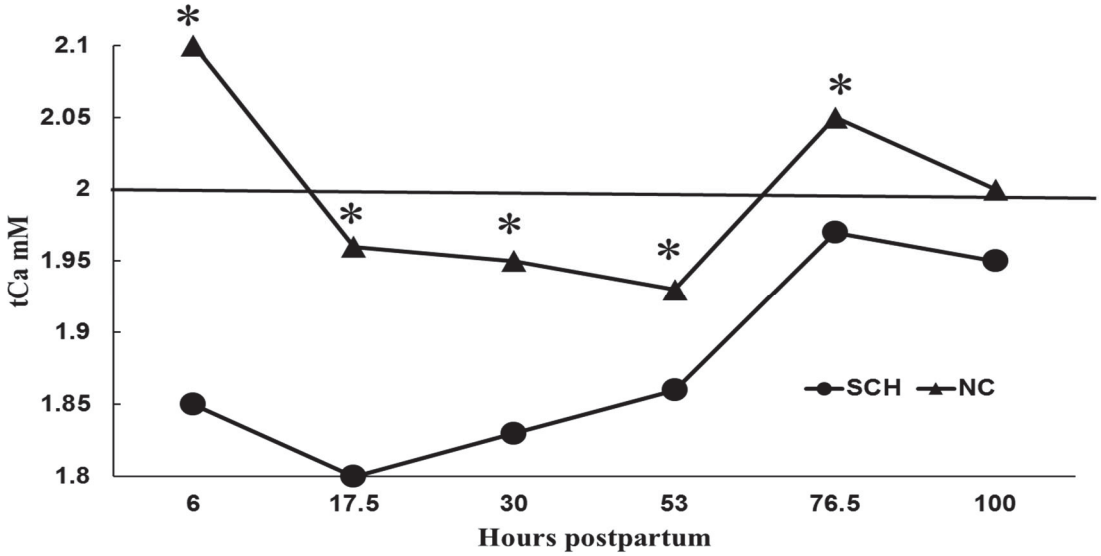


FONTE: o autor (2018)

3.3.5 Prevalence and Health Disorders, Cutoff tCa for Predict Diseases

For the present study SCH was determined in T1, with the incidence rates were 39% using on-farm tCa the VetTest (≤ 2,175 m*M*), 64% using tCa (≤ 2.0 m*M*) and 76% for iCa (concentration ≤ 1.0 m*M*) analyzed in laboratory. Through the tCa in time T1 the primiparous presented an average incidence rate of SCH of 55% for primiparous cows and 68% for multiparous (57% for second-lactation cows, 78% for third-lactation cows and 76% for cows with ≥ 4 lactations). In FIGURE 3.7 cows classified as hypocalcemic presented lower values of Ca when compared with the NC cows in T1 a T5. The lowest Ca values being observed at 17.5 h postpartum. When the categorization of calcemia is performed in T3, the SCH value increases to 80% for both multiparous and primiparous cows.

FIGURE 3. 7 THE VALUES OF tCa IN COWS NC AND WITH SCH IN THE FIRST 100 h AFTER CALVING. THE SOLID LINE IDENTIFIES THE CUTOFF POINT FOR CALCEMIA STATUS CLASSIFICATION OF COWS. AN ASTERISK (*) INDICATES MEANS DIFFER (P < 0.01).



FONTE: O autor (2018)

NEFA analysis was performed only on the day of calving (6 h postpartum). The mean observed was 0.66 m*M*, but 36% of the cows presented NEFA above 0.70 m*M* (97/270) with a mean of 1.18 m*M*. Despite a low correlation between NEFA and tCa in T1 (r = -0.35, *P* < 0.01), of the 97 animals, 79 were hypocalcemic. However, the correlation between NEFA and BHBA was moderate (day 3, r = 0.47, *P* < 0.01, day 5, r = 0.41, *P* < 0.01), but cows with hypocalcemia and NEFA above 0.70 m*M* presented mean values of BHBA higher on day 3 (1.01 vs. 0.70 m*M*, SCH vs. NC), day 5 (1.14 vs. 0.75 m*M*), day 7 (1,2 vs. 0,9 m*M*) postpartum.

The diseases related to this work had a morbidity in the 30 DIM of 36 % (95/264), with a higher occurrence in cows with SCH 72% (69/95), multiparous 61% (58/95) and group C 57 (54/95). When analyzed by parity, in the primiparous with SCH 73% (27/37) and in multiparous with SCH 72% (42/58) and group C 60% (23/58).

The incidence of other diseases such as of CH, CK, SK, MET, RP, DA and MAST in the current study was 2,2, 3,8, 23,9, 20,5, 19,1, 5,7 and 7,0%, respectively. Estimates of BHBA on day 5 were lower (*P*<0.01) for treated cows 0.70 vs. 0.90 ± 0.06 m*M* for the non-treated ones. For DA, the highest occurrence of cases (12/15) was observed in group C and with SCH (*P*<0.05). In cows with DA the mean serum composition was 1.01 m*M* for NEFA, tCa and iCa with 1.87 and 0.91 m*M*, BHBA of 1.55 ± 0.5, day, 1.93 ± 0.7, 2.36 ± 0.9 m*M* in day 3, 5 and 7 consecutively.

The occurrence of diseases was higher in the group of cows with SCH, 73% (46/63) of cows with KET, 74% (37/50) of the cows with RP, 72% (39/54) with MET, 80% (12/15) with DA, 63% (12/19) with MAST and 78% (36/46) of culling cows.

No effect of Ca formate on MET ($P = 0.56$), KET ($P = 0.15$), PR ($P = 0.61$) and MAST ($P = 0.87$) was observed, however, reduce the occurrence of DA ($P < 0.05$).

Through the ROC analysis cutoff point of plasma tCa, with 95% confidence interval of ≤ 1.9 m*M* was determined as predictor of KET (with specificity of 66.6% and a sensitivity of 54.0%); for MET of ≤ 2.0 m*M* (with specificity of 38.6% and a sensitivity of 72.2%); PR of ≤ 1.95 m*M* (with specificity of 50.9% and a sensitivity of 64.0%); DA ≤ 1.82 m*M* (with specificity of 77.9% and a sensitivity of 47.0%); MAST ≤ 1.77 m*M* (with specificity of 86.9% and a sensitivity of 26.3%).

3.4 DISCUSSION

In the present study, the feed anionic salts prepartum has been reported to significantly reduce the incidence of CH (BLANC et al., 2014). The use of anionic diet in the prepartum was effective, with a calculated average DCAD negative, which contributes to maintain a low prevalence for CH, however, for SCH maintained a high prevalence. The mean values calculated for the diets are in accordance with the recommendations for anionic diets of -5 to -15mEq/100g of DM to promote metabolic acidosis and contribute to the reduction of the incidence of hypocalcemia (MOORE et al. 2000; LENO et al., 2017). However, feeding anionic salts has decreased the incidence of SCH in some studies (MOORE et al. 2000), but failed to do so in others (RAMOS-NIEVES et al., 2009). The plasma Ca concentrations in postpartum increased linearly with decreasing prepartum DCAD and had decreased prevalence of SCH in the days immediately after parturition (LENO et al., 2017). That feeding anionic salts before calving in a large field study (n = 510 cows, all parities) reduced the subclinical hypocalcemia from 50% in the control cows to 19% in the cows receiving the anionic salts. Thus, the goal of implementing the use of negative DCAD in the prepartum diet is to reduce the intensity or duration of hypocalcemia as a result of the induction of some degree of metabolic acidosis (BEEDE et al., 1992). This strategy aims at restoring tissue sensitivity to stimulation of PTH, reduced during the alkalosis state (GOFF; LIESEGANG; HORST, 2014). However, that although the inclusion of anionic salts in the prepartum primiparous diet induced a compensated metabolic acidosis, there was no increase in calcium metabolism, and the animals had a

decrease in dry matter intake, an increase in NEFA and triglyceride accumulation in the liver (MOORE et al., 2000).

Although the reduction of DCAD through the removal of cations is effective in the prevention of HYPO, it is difficult to use diets with low K, mainly by the type of forage and the agronomic use of this element to increase the productivity of the forage crops. In farm B there was an increase of cations (Na and K) through the forage species used (*Cinodon* hay). In order to obtain benefits in the use of anionic diet in the prepartum of Holsteins cows, in the monitoring of the urine pH, this should be between 5.5 and 6.8 (GOFF, 2008; MOORE et al. 2000; BLANC et al. 2014; LENO et al. 2017). The restoration of target tissue PTH sensitivity has been demonstrated at a relatively low (< 6.0) urine pH (GOFF; LIESEGANG; HORST, 2014).

The anionic diet was effective in reducing the pH of urine, but a greater of pH amplitude was observed in farm B. The observed difference in the pH of urine between the farms is mainly related to the forage type and the constancy in the mixture of the ingredients in the TMR. The mean concentration of Ca in the prepartum diet was higher in farm B than in farm A. For animals receiving anionic diet the supply of > 100 g de Ca/day, to compensate for the elevation of its excretion via urine (HORST et al.,1997). In practice, pre-partum diet with a high dietary Ca content (1.1 to 1.5%), leads the cow to maximum risk, while low levels of Ca in the diet (< 0.5%) predict a low incidence of HYPO (LEAN et al., 2006).

For the calving order, iCa, being the most available fraction of plasma calcium, presented the largest change, with the lowest mean concentration at time 17.5 h postpartum, resulting from Ca drainage via colostrum milking. According to the study of Quiroz-Rocha et al. (2009) observed that multiparous, second lactation or greater already had a lower concentration of serum calcium in the prepartum compared to primiparous.

For the determination of hypocalcemia, the time of calving and the cutoff point are important observations. Traditionally, the cutoff used to identify SCH has been 8.0 mg/dL (2.0 m*M*) tCa, and 4.0 mg/dL (1.0 m*M*) iCa due to assumption that total blood calcium is composed of 50% ionized calcium (MARTINEZ et al., 2012).

The cows had the highest proportion of subclinical hypocalcemia about 16 h after calving, which is close to the in the current study (RAMOS-NIEVES et al., 2009). In study with multiparous cows, the tCa values was of 2.32 and 2.13 m*M* at 72 and 9 h before calving, with nadir occurring at 28 postpartum with a mean value of 1.87 m*M*. The conclusions this finding was that plasma tCa in 12-h period before parturition was negatively associated with age and colostrum volume, and 24-h after parturition with age (MEGAHED et al., 2018). The blood Ca concentrations reach a nadir at approximately 12 to 24 h after calving, with almost 25% and

50% of heifers and cows, consecutively, will have a Ca concentration in the blood < 2 m*M* (8 mg/dL) (GOFF, 2008). However, with high risk for animals to metritis having the lowest concentrations between 24 and 48 h after parturition (MARTINEZ et al., 2012).

Therefore, SCH classification might be dependent on the timing of sampling relative to parturition. Thus, as already described, through the tCa in time t1 the primiparous presented an average prevalence rate of SCH of 55%, whereas this value was 68% for the multiparous. When the categorization of calcemia is performed in t3, the SCH value increases to 80% for both multiparous and primiparous cows. For the determination of calcemia, in addition to the management factors, the breed and other particularities of the farms, the methodology of the study can be cited as an important factor.

Some relevant studies presented different methodologies both for the time of blood collection and also the cutoff value in calcemia classification postpartum. As examples of studies with different methodologies and cut-off point for definition of SCH: where the prevalence of HYPO in the first 48 h postpartum was 2.4% (<1.4 mM) for CH and 25 and 47% for SCH (1.4 to 2.0 m*M*), in primiparous and multiparous cows, respectively (REINHARDT et al., 2011). with values defined as 2.125 mM in at least 1 of the 3 DIM, the prevalence was 66-69% for SCH (MARTINEZ et al., 2012; MARTINEZ et al., 2016b); used 2.0 m*M* on the day of calving, with a prevalence of 51% SCH (CHAMBERLIN et al., 2013); for the cutoff point was 2.14 m*M* between 24-48 h of pospartum, with 78% SCH (RODRÍGUEZ; ARÍS; BACH, 2017); with 2,0 m*M* at 2 h postpartum, with 30.8% SCH (WILHELM et al., 2017). There is no consensus among researchers in the definition of reference values for the definition of hypocalcemia, mainly from the point of view of borderline values for risk of disease in cows.

The results of studies on the effect on postpartum oral Ca use have been promising. In Martinez et al. (2016b) found that serum tCa concentration was higher for cows supplemented with oral Ca (CaCl2 and CaSO4) compared to control cows as well as primiparous cows over multiparous cows. Afshar Farnia et al. (2018) compared the effect of dietary acidogenic at the end of gestation and oral Ca ($CaCl_2$) postpartum, concluding that the combination of both provided a higher concentration of Ca in the blood at 6 and 12 h postpartum when compared after calving alone.

According to Oetzel (2013), the initial idea of oral Ca supplementation for the control of hypocalcemia was 4 doses; at 12 hours before calving, at calving, 1 to 12 hours after calving and the fourth dose at 24 hours after calving. A practical difficulty in this protocol is to set the schedule for the first application, which should occur 12 hours before calving. The objective of

the protocol was to facilitate the on-farm management and to supplement the cows with oral Ca at the critical moment, where nadir of blood Ca normally occurs.

In this study, cows supplemented with oral Ca formate (T2 and T3) had higher values for plasma Ca in t3 (immediately before administration of the second dose). Drainage via the mammary gland and the low systemic supply may explain the low persistence of plasma Ca concentrations following oral administration. The cows absorb an effective amount of calcium into the bloodstream within approximately 30 minutes of supplementation keeping the concentration in the blood for 4 to 6 hours (OETZEL, 2013). If samples are taken after 8 h of treatment, the differences are minimized or even cease to exist, which may probably raise the values for the prevalence and incidence of SCH in cows receiving oral Ca (OETZEL; MILLER, 2012). This effect is due to passive transport by increasing the luminal concentration of Ca above 1 m*M*, and for ruminal absorption > 1.5 m*M* (GOFF; HORST, 1993). This form of iCa absorption is in principle unlimited and independent of stimulation by 1.25-dihydroxy vitamin D, the net absorption of free Ca increases linearly with increasing luminal Ca concentrations (THILSING-HANSEN et al., 2002).

The effect of oral Ca was higher for multiparous cows when compared to primiparous cows, with a higher response to iCa. The different responses in blood tCa between primiparous and multiparous receiving the same dose of oral calcium are explained for physiological differences and the way they cope with the increased energy and calcium demands in the transition period (CHAPINAL et al., 2012). Multiparous when compared to primiparous cows, produce a higher volume of colostrum, have a higher volume of rumen (higher rate of absorption), higher DMI, with a faster passage rate of the digestive content and that dilutes the volume of absorbed Ca (MARTINEZ et al., 2016b)

The responses for supplementation with oral Ca are likely related to susceptibility to hypocalcemia, apart from that the oral Ca administration affected acid-base status with a compensated metabolic acidosis; perhaps primiparous cows are more sensitive to the potential negative effects of metabolic acidosis during early lactation (MARTINEZ et al., 2016a). However, the benefits of the additional acidification from oral Ca administration cause more benefits via improved Ca metabolism than potentially detrimental effects from systemic acidification (OETZEL; MILLER, 2012).

The effects the Ca in blood are more likely to be obtained if oral Ca is administered before parturition (THILSING-HANSEN et al., 2002; MARTINEZ et al., 2016a; MEGAHED et al., 2018). or within 24 h of calving (HERNANDEZ et al., 1999). Further studies need to be done to clarify the best timing for the preventive use of oral Ca.

The NEFA concentrations above 0.4 m*M* in the prepartum or above 0.7 m*M* in the postpartum period are related to a higher occurrence of several diseases in the transition period (OSPINA et al., 2010; CHAPINAL et al., 2011). HYPO cows had higher plasma NEFA concentrations than NC cows at d 0, so HYPO cows also had a higher liver lipid ratio in d 7 and 35 compared to NC cows (CHAMBERLIN et al., 2013).

The SCH could be responsible for DMI fall, but compared cows with SCH and NC and demonstrated DMI in cows with SCH increased in the last 2 weeks before calving and no differences were observed in postpartum compared with NC cows (JAWOR et al., 2012). HYPO increases lipolysis, raising the concentration of NEFA. This fact can be explained by Ca being an important second messenger for energetic metabolism in hepatocytes. The increase in the concentration of cytosolic Ca and consequently in the mitochondria is an important factor for the stimulation of mitochondrial oxidative metabolism. When there is a drop in iCa concentration in plasma, and consequently in hepatocellular Ca concentrations, as a result there is a decrease in carbohydrate metabolism through the tricarboxylic acid cycle, resulting in a more severe negative energy balance (CHAMBERLIN et al., 2013).

The BHBA is one of the major ketone bodies produced by the liver and an important indicator of NEB in cows (OSPINA et al., 2010). The increased serum concentration of ketone bodies occurs when the amount of NEFA exceeds the capacity for oxidation by the liver (DRACKLEY, 1999). Plasma BHBA values were lower for T group cows, with significant ($P < 0,01$) in day 5 postpartum. The BHBA concentration increases slightly in the last three days before calving, but its increase is expressive after calving, peaking at the fifth day of lactation (LEBLANC et al., 2005).

In this study, the primiparous and HYPO cows presented higher values for BHBA. The condition of increase in the concentration of BHBA is consequence the intensity of mobilization of NEFA (DRACKLEY, 1999; OSPINA et al., 2010). Values above 1.2 m*M* are indicative of subclinical ketosis and are related to decreased herd health. Elevated serum concentrations of NEFA and BHBA in wk 1, 2 and 3 postpartum were not associated with milk loss, i.e., cows with a serum NEFA concentration between 0.5 and 0.7 mEq/L and BHBA above 0.8 m*M* produced more milk than cows with concentrations below the same thresholds (OETZEL, 2004). However, when only BHBA (≥ 1,4 m*M*) was measured in wk 1 in primiparous cows, there was an estimated loss of -2.9 kg/d, whereas for NEFA (≥ 0.7 m*M*) in multiparous cows, the estimate of daily production loss was -1.6 kg/d (CHAPINAL et al., 2012).

The liver function indicators analyzed were TC, the enzyme AST and the ALB. TC, a metabolite that represents the energetic metabolism pathway, is also an essential molecule as a precursor of steroid hormones, vitamin D, and is part of the formation of membranes and synthesis of bile acids (KANEKO et al., 2008). The primiparous cows had a highest concentration ($P < 0.05$) of TC after calving and on the day 5 postpartum, whereas cows with SCH did so on day 5 postpartum. The concentration of TC is related to food intake, so the decrease in TC synthesis occurs when there is food restriction, leading to lower insulin concentration and increased glucagon (GRUMMER, 1995). The TC concentration below 77.34 mg/dL is associated with low voluntary consumption or metabolic problems (GURETZKY et al., 2006), being recommended the limit of 80mg/dL (KANEKO et al., 2008). In cows with increased postpartum DIM, there is an increase in TC concentrations, with elevated lipid absorption by the liver and an improvement in the negative energy balance (STENGÄRDE et al., 2008).

AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows. It exists in many tissues as two isoforms, in the cytosol and in the mitochondria. For this reason, because it is a mitochondrial and cytosolic enzyme, it needs a larger lesion to be released into the bloodstream. In contrast, CK (creatine kinase) and LDH (lactate dehydrogenase), because they are cytosolic and of small size, can overcome the cell membrane, even if there is no great tissue damage. Its use is an indicator of damage in these tissues. Points out that, in the case of muscle injury, the increase in AST occurs more slowly when compared to CK, and the maximum values of this enzyme are found in the blood 24 to 36 hours after the occurrence of the injury (GONZÁLEZ; SILVA, 2006). In cattle, the sensitivity is reported to be 94% for hepatic lipidosis (KANEKO et al., 2008). This effect exudes the change in hepatic metabolism and may be related to milk production (REYNOLDS et al., 2003). In this study, higher plasma levels of AST were observed in primiparous cows, but still within physiological values. However, 30% presented AST values higher than 100 U/L. AST values above 100 U/L are indicative of hepatic lesions (OETZEL, 2004; XU; WANG, 2008). The AST enzyme is found primarily in the liver, erythrocytes and in the skeletal and cardiac muscles. It is used as an indicator of necrosis in these tissues, especially in hepatic lesions. It is a mitochondrial and cytosolic escape enzyme, requiring a larger lesion to be released into the bloodstream (HOFFMANN; SOLTER, 2008).

ALB, synthesized exclusively in the liver, determines the osmotic pressure in plasma and is one of the major proteins for transporting metabolites in plasma. ALB levels are positively related to productive and reproductive performance (PAYNE; PAYNE, 1987). In

this study, the plasma concentration in both primiparous and multiparous cows is within the reference values. However, a lower plasma ALB concentration was found in multiparous cows compared to primiparous cows during the first 4 days postpartum. The lower concentration of serum ALB in pluriparous cows may be related to the redirection of the protein to the milk protein synthesis by the mammary gland, in addition to hepatic synthesis (MOORBY et al., 2000; PARK et al., 2010). Other factors that may be involved are increased milk production and reduced post-partum food intake. There is a negative correlation between the degree of fatty changes in the liver and the concentration of serum ALB (REID et al., 1983).

As a stress indicator, plasma CORT was monitored on t1 and 5 DIM, with the highest values detected shortly after calving. This observed physiological condition when maternal plasma cortisol rises in the last 4 days of gestation, reaching the highest value with calving and declining between days 3 and 7 postpartum (TAVERNE et al., 1988). Plasma concentrations of cow cortisol may increase from 4 to 8 ng/ml in 3 d prior to calving to 15 to 30 ng/ml at calving. The answer of cortisol secretion is even more pronounced in cows developing milk fever (GOFF; HORST, 1997), observed fact in this study, at where the HYPO cows presented higher concentration of cortisol (P <0,01) only day 5 postpartum. No effect of Ca formate on the plasma concentration of this hormone was observed. In cows with hypocalcemia exhibit higher concentrations of cortisol, which exacerbates immunosuppression near labor, but also results in loss of muscle tone in the uterus and the sphincter of the teat. These combined effects may explain the increased incidence of placental retention and mastitis occurring in cows with HC. (GOFF; HORST, 1997)

In the present study, the prevalence of CH and SCH was 2.2% and 64%, consecutively. Cows with hypocalcemia were responsible for the higher occurrence of early lactation and discard diseases in the herds of this study. In this group of cows, the plasma levels of NEFA, BHBA, and CORT were higher, which increases the risk of disease occurrence. This increase in metabolites observed in cows with HYPO can be explained by DMI impairment, increased insulin resistance, which prevents glucose absorption and exacerbates the negative energy balance (MARTINEZ et al., 2016; WILHELM et al., 2017). The hitherto related events of SCH on dairy cows at early lactation, contribute to the reduction of their innate immune response. This fact compromises the ability of leukocytes to fight infections, which increases the risk for mastitis, metritis, interfering with the productive and reproductive performance of dairy cows (KIMURA et al., 2006; MARTINEZ et al., 2012).

HYPO predisposes the cow to DA, which was observed in the study, where the highest occurrence of the cases was in the cows with SCH in the C group (P < 0.05). Causes an

increased risk of ketosis, which, together with DMI fall, reduces filling and rumen mat thickness, allowing more VFA to reach the abomasum and reduce its contractility (GOFF et al., 1989). HYPO is factor that increase the risk for RP, MET, elevation of NEFA in prepartum and BHBA in the postpartum period, (LeBLANC et al., 2005). In this study, Ca formate had an important role in the control of DA, through the increase in concentration of Ca in blood, which contributed to the maintenance of muscular functions and thus the improvement of the energy metabolism of the cows of the T group. The reduction in the occurrence of AD caused by the treatment with Ca formate has an effect on treatment costs, reduction of risk of death and discard, as well as reduction of loss of milk production.

For the prediction of disease risk, through the ROC curve were determined through tCa values, with the best sensitivity and specificity. Thus, for the results obtained in this study, the value of ≤ 2.0 m*M* seems to be the cutoff point that best defines the threshold for predicting the risk of disease occurrence at the beginning of lactation. The cutoff point was through the prediction of MET, the highest value from which there is a risk of disease occurrence. This value has also been described by Wilhelm et al. (2017) in the 2 h postpartum time, however, differs from the value of ≤ 2.125 m*M* defined in the study of Martinez et al. (2012), in at least 1 d in the first 3 days postpartum. We believe that there is a need for further studies to clarify the best value of tCa as a cutoff point for disease prediction.

Several studies demonstrate the interaction of SCH on the production and reproduction of dairy cows (MARTINEZ et al., 2012). The risk of developing hypocalcemia increases by 9% with cow's age (LEAN; DEGARIS, 2010), with losses of almost 2.9 kg milk/day in the first six weeks postpartum, so an economically important disease (CHAPINAL et al., 2012).

In the present study supplementation with oral Ca did not affect milk production ($P > 0.05$) in the first 30 and 100 DIM. However, there was a tendency ($P = 0.08$) of higher milk yield in the 100 DIM in the pluriparous cows of the T group compared to those in the C group. In a similar study, it was concluded that supplementation with oral Ca did not affect milk production in the first 5 months postpartum. However, when analyzing the results with only multiparous cows, an interaction between oral Ca supplementation and milk production was found in the first 30 DIM (MARTINEZ et al., 2016b). In contrast, other studies found no effect of oral Ca supplementation on milk production or fertility (MELENDEZ et al., 2003; WILHELM et al., 2017).

In cows with SCH the mean adjusted milk yield in the 100 DIM was higher ($P < 0.01$) when compared to the NC, with the highest difference between the multiparous. This condition was also described for multiparous cows (2 or more lactation) with CH produced 1.6 kg/d more

of lactating milk compared to health cows (RAJALA-SCHULTZ et al., 1999). According to the authors, the higher milk yield is related to the cow's production potential, and the production would have been higher since the beginning of lactation if the cow had not passed the CH. A similar situation was found by Jawor et al. (2012) where cows with SCH produced, on average, 5.7 kg/d more milk during week 2, 3 and 4 compared to control cows multiparous, however, cows with 3 or more lactation with SCH had higher milk yields over 280 DIM. They describe that the mechanism between hypocalcemia and milk production has not been fully clarified, but cows with higher milk production capacity, although transient, are more prone to greater net loss of calcium soon after calving.

In this study, the use of Ca formate had no effect on the reproductive parameters analyzed.

3.5 CONCLUSIONS

The prevalence of subclinical hypocalcemia was high, but with a low incidence of clinical hypocalcemia. In this study, hypocalcemic cows produced more milk in the 100 DIM, however, they presented higher NEFA at calving, BHBA and cortisol at day 5 postpartum. This condition of metabolic challenge led this group of animals to present the highest incidence of diseases in the beginning of lactation as well as of discards. In this context, calcium formate at the early of lactation increased the iCa concentration, a desirable condition in this critical period for the cow. Cows receiving a 50 g dose of oral Ca after calving, and a second dose after 24 h, showed a lower concentration of BHBA at day 5 postpartum. This condition is positive and contributes to the metabolic energy balance at the beginning of lactation. The use of oral Ca supplementation reduced the incidence of abomasal displacement, with 80% of cases occurring in the control group. There was no effect on the reproductive parameters analyzed. Calcium formate oral supplementation showed modest but beneficial effects in early-initiating dairy cows, increasing ionized Ca, reducing BHB concentrations and decreasing the incidence of some diseases, important goals for controlling metabolic disorders in dairy farms.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Bayer Animal Health for the financial support of this project and the Brazilian government institution CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for the Ph.D. scholarship. We also thank the staff from the Fini Farm, herd where this study was conducted.

REFERENCES

AFSHAR FARNIA, S.; RASOOLI, A.; NOURI, M.; et al. Effect of postparturient oral calcium administration on serum total calcium concentration in Holstein cows fed diets of different dietary cation-anion difference in late gestation. **Research in Veterinary Science**, v. 117, n. November 2017, p. 118–124, 2018. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0034528817307324>. .

AOAC INTERNATIONAL. Official methods of analysis. **Journal of Pharmaceutical Sciences**, v. 1, n. Volume 1, p. 1230, 1990. Disponível em: <http://sutlib2.sut.ac.th/sut_contents/H125800.pdf%5Cnhttp://doi.wiley.com/10.1002/jps.2600650148>. .

BLANC, C. D.; VAN DER LIST, M.; ALY, S. S.; ROSSOW, H. A.; SILVA-DEL-RÍO, N. Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium. **Journal of Dairy Science**, v. 97, n. 11, p. 6901–6906, 2014. Elsevier.

BROWN, J. L.; PH, D.; WALKER, S.; STEINMAN, K.; ROYAL, F. Endocrine Manual for Reproductive Non-Domestic Species. **Smithsonian's National Zoological Park Conservation & Research Center Endocrine Workbook**, p. 69, 2004.

CHAMBERLIN, W. G.; MIDDLETON, J. R.; SPAIN, J. N.; et al. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. **Journal of Dairy Science**, v. 96, n. 11, p. 7001–7013, 2013. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030213006474>. .

CHAPINAL, N.; CARSON, M.; DUFFIELD, T. F.; et al. The association of serum metabolites with clinical disease during the transition period. **Journal of Dairy Science**, v. 94, n. 10, p. 4897–4903, 2011. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021100511X>. .

CHAPINAL, N.; CARSON, M. E.; LEBLANC, S. J.; et al. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. **Journal of Dairy Science**, v. 95, n. 3, p. 1301–1309, 2012. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2011-4724>. .

CURTIS, C. R.; ERB, H. N.; SNIFFEN, C. J.; SMITH, R. D.; KRONFELD, D. S. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. **Journal of Dairy Science**, v. 68, n. 9, p. 2347–2360, 1985. Disponível em: <http://jds.fass.org/cgi/content/abstract/68/9/2347>. .

DRACKLEY, J. K. Biology of dairy cows during the transition period: The final frontier? **Journal of Dairy Science**, v. 82, n. 11, p. 2259–2273, 1999. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(99)75474-3>. .

GERARDO, F. Q. R.; LEBLANC, S. J.; DUFFIELD, T. F.; et al. Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. **Canadian Veterinary Journal**, v. 50, n. 4, p. 383–388, 2009.

GOFF, J.; HORST, R. L. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. , v. 302, n. May 2016, 1993.

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2007.12.020>. .

GOFF, J. P.; HORST, R. L. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders 1 , 2. **Journal of Dairy Science**, v. 80, n. 7, p. 1260–1268, 1997. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(97)76055-7>. .

GOFF, J. P.; KEHRLI, M. E.; HORST, R. L. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. **Journal of dairy science**, v. 72, n. 5, p. 1182–7, 1989. Elsevier. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/2745825%5Cnhttp://www.journalofdairyscience.org/article/S0022-0302(89)79222-5/pdf>. .

GOFF, J. P.; LIESEGANG, A.; HORST, R. L. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. **Journal of Dairy Science**, v. 97, n. 3, p. 1520–1528, 2014. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030214000228>. .

GOFF J, HORST R, JARDON P, BORELLI C, W. J. Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows 1. **Journal of Dairy Science**, v. 79, n. 10, p. 378–383, 1996.

GONZÁLEZ, F. H. D.; SILVA, S. C. **Introdução a bioquímica clínica veterinária.** Editora da UFRGS. 2 ed. 355 p. 2006.

GRUMMER, R. R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow Impact of Changes in Organic Nutrient Metabolism Feeding the Transition Dairy Cowl. **Journal of Animal Science**, v. 73, p. 2820–2833, 1995.

GURETZKY, N. A. J.; CARLSON, D. B.; GARRETT, J. E.; DRACKLEY, J. K. Lipid Metabolite Profiles and Milk Production for Holstein and Jersey Cows Fed Rumen-Protected Choline During the Periparturient Period. **Journal of Dairy Science**, v. 89, n. 1, p. 188–200, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206720835>. .

HOFFMANN, W. E.; SOLTER, P. F. Diagnostic Enzymology of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 351–378, 2008.

HOLDEN, L. A. Comparison of Methods of In Vitro Dry Matter Digestibility for Ten Feeds. **Journal of Dairy Science**, v. 82, n. 8, p. 1791–1794, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299754093>. .

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A. Adapting to the transition between gestation and lactation: Differences between rat, human and dairy cow. **Journal of Mammary Gland Biology and Neoplasia**, v. 10, n. 2, p. 141–156, 2005.

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A.; BUXTON, D. R. Strategies for Preventing Milk Fever in Dairy Cattle. **Journal of Dairy Science**, v. 80, n. 7, p. 1269–1280, 1997. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030297760569>. .

JAWOR, P. E.; HUZZEY, J. M.; LEBLANC, S. J.; VON KEYSERLINGK, M. A. G. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. **Journal of Dairy Science**, v. 95, n. 3, p. 1240–1248, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212000823>. .

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. Clinical Biochemistry of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 661, 890–894, 2008. Disponível em: <http://medcontent.metapress.com/index/A65RM03P4874243N.pdf%5Cnhttp://books.google.com/books?hl=en&lr=&id=spsD4WQbL0QC&oi=fnd&pg=PP1&dq=Clinical+Biochemistry+of+Domestic+Animals&ots=T_f6aJml8f&sig=sKSGfll8B1Rjre6_UTJHLMZSxbI%5Cnhttp://books.google.at/books>. .

KIMURA, K.; GOFF, J. P.; KEHRLI, M. E.; REINHARDT, T. A. Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. **Journal of Dairy Science**, v. 85, n. 3, p. 544–550, 2002. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030202741076>. .

KIMURA, K.; REINHARDT, T. A.; GOFF, J. P. Parturition and Hypocalcemia Blunts Calcium Signals in Immune Cells of Dairy Cattle. **Journal of Dairy Science**, v. 89, n. 7, p. 2588–2595, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206723359>. .

LEBLANC, S. Monitoring metabolic health of dairy cattle in the transition period. **The Journal of reproduction and development**, v. 56 Suppl, p. S29-35, 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20629214>. .

LEBLANC, S. J.; LESLIE, K. E.; DUFFIELD, T. F. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. **Journal of Dairy Science**, v. 88, n. 1, p. 159–170, 2005. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030205726746>. .

LEBLANC, S. J.; LISSEMORE, K. D.; KELTON, D. F.; DUFFIELD, T. F.; LESLIE, K. E. Major Advances in Disease Prevention in Dairy Cattle. **Journal of Dairy Science**, v. 89, n. 4, p. 1267–1279, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206721956>. .

LENO, B. M.; NEVES, R. C.; CURLER, M. D.; et al. Association of immediate postpartum plasma Ca concentration with early- lactation clinical diseases , culling , reproduction , and milk production. **Journal of Dairy Science Vol. 100, Suppl. 2**, v. 100, p. 39, 2017. Disponível em: <https://blogs.cornell.edu/jessmcartlab/files/2017/06/ADSA-Poster_Neves-2017_Final_06.23.2017-129b0t3.pdf>. .

LENO, B. M.; RYAN, C. M.; STOKOL, T.; et al. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217303259>. .

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações. **Associação Brasileira para Pesquisa da Potassa e do Fosfato,**. p.139–169, 1989. Piracicaba, São Paulo, Brasil.

MARTINEZ, N.; RISCO, C. A.; LIMA, F. S.; et al. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. , p. 7158–7172, 2012.

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; et al. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8417–8430, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304519>. .

MEGAHED, A. A.; HIEW, M. W. H.; EL BADAWY, S. A.; CONSTABLE, P. D. Plasma calcium concentrations are decreased at least 9 hours before parturition in multiparous Holstein-Friesian cattle in a herd fed an acidogenic diet during late gestation. **Journal of Dairy Science**, p. 1–14, 2018. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2017-13376>. .

MELENDEZ, P.; DONOVAN, G. A.; RISCO, C. A.; LITTELL, R.; GOFF, J. P. Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets. **Theriogenology**, v. 60, n. 5, p. 843–854, 2003.

MIYAZAWA, M.; PAVAN, M. A.; MURAOKA, T. ET AL. **Manual de Análises Químicas de Solos, Plantas e Fertilizantes.** Brasília, DF, 1999.

MOORBY, J. M.; DEWHURST, R. J.; TWEED, J. K.; DHANOA, M. S.; BECK, N. F. Effects of altering the energy and protein supply to dairy cows during the dry period. 2. Metabolic and hormonal responses. **Journal of dairy science**, v. 83, n. 8, p. 1795–1805, 2000. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(00)75050-8>. .

MOORE, S. J.; VANDEHAAR, M. J.; SHARMA, B. K.; et al. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. **Journal of Dairy Science**, v. 83, n. 9, p. 2095–2104, 2000. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(00)75091-0>. .

NEVES, R. C.; LENO, B. M.; STOKOL, T.; OVERTON, T. R.; MCART, J. A. A. Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. **Journal of Dairy Science**, v. 100, n. 5, p. 3796–3804, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021730231X>. .

OETZEL, G. R. Monitoring and testing dairy herds for metabolic disease. **Veterinary Clinics of North America - Food Animal Practice**, v. 20, n. 3 SPEC. ISS., p. 651–674, 2004.

OETZEL, G. R. Oral calcium supplementation in peripartum dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 447–455, 2013. Elsevier Inc.

OETZEL, G. R.; MILLER, B. E. Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. **Journal of Dairy Science**, v. 95, n. 12, p. 7051–7065, 2012. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2012-5510>. .

OSPINA, P. A; NYDAM, D. V; STOKOL, T.; OVERTON, T. R. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. **Journal of dairy science**, v. 93, n. 8, p. 3595–3601, 2010. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2010-3074>. .

ØSTERGAARD, S.; SØRENSEN, J. T.; HOUE, H. A stochastic model simulating milk fever in a dairy herd. **Preventive Veterinary Medicine**, v. 58, n. 3–4, p. 125–143, 2003.

RAJALA-SCHULTZ, P. J.; GRÖHN, Y. T.; MCCULLOCH, C. E.; GUARD, C. L. Effects of Clinical Mastitis on Milk Yield in Dairy Cows. **Journal of Dairy Science**, v. 82, n. 6, p. 1213–1220, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299753440>. .

RAMOS-NIEVES, J. M.; THERING, B. J.; WALDRON, M. R.; JARDON, P. W.; OVERTON, T. R. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. **Journal of dairy science**, v. 92, n. 11, p. 5677–5691, 2009.

REID, I. M.; DEW, S. M.; COLLINS, R. A.; et al. The relationship between fatty liver and fertility in dairy cows: A farm investigation. **The Journal of Agricultural Science**, v. 101, n. 2, p. 499–502, 1983.

REINHARDT, T. A.; LIPPOLIS, J. D.; MCCLUSKEY, B. J.; GOFF, J. P.; HORST, R. L. Prevalence of subclinical hypocalcemia in dairy herds. **Veterinary Journal**, v. 188, n. 1, p. 122–124, 2011. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2010.03.025>. .

REYNOLDS, C. K.; AIKMAN, P. C.; LUPOLI, B.; HUMPHRIES, D. J.; BEEVER, D. E. Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. **Journal of Dairy Science**, v. 86, n. 4, p. 1201–1217, 2003. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030203737047>. .

ROBERTS, T.; CHAPINAL, N.; LEBLANC, S. J.; et al. Metabolic parameters in transition cows as indicators for early-lactation culling risk. **Journal of Dairy Science**, v. 95, n. 6, p. 3057–3063, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212002809>. .

RODRÍGUEZ, E. M.; ARÍS, A.; BACH, A. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. , , n. 2012, p. 1–8, 2017. American Dairy Science Association.

SILVA, D.J.; QUEIROZ, A. . **Análises de alimentos (métodos químicos e biológicos)**. 3º ed. Viçosa, MG, Brasil, 2002.

SOEST, P. J. V. S. Sumário. **Nutrition ecology of ruminants. Ithaca. Cornell University Press,** v. 2, p. 1–117, 1994.

STENGÄRDE, L.; TRÅVÉN, M.; EMANUELSON, U.; et al. Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. **Acta Veterinaria Scandinavica**, v. 50, n. 1, p. 1–11, 2008.

STOKOL, T.; NYDAM, D. V. Effect of Hemolysis on Nonesterified Fatty Acid and -Hydroxybutyrate Concentrations in Bovine Blood. **Journal of Veterinary Diagnostic Investigation**, v. 18, n. 5, p. 466–469, 2006. Disponível em: <http://vdi.sagepub.com/lookup/doi/10.1177/104063870601800507>. .

TAVERNE, M. A. M.; BEVERS, M. M.; VAN DER WEYDEN, G. C.; DIELEMAN, S. J.; FONTIJNE, P. Concentration of growth hormone, prolactin and cortisol in fetal and maternal blood and amniotic fluid during late pregnancy and parturition in cows with cannulated fetuses. **Animal Reproduction Science**, v. 17, n. 1–2, p. 51–59, 1988.

THILSING-HANSEN, T.; JØRGENSEN, R. J.; ØSTERGAARD, S. Milk fever control principles: a review. **Acta veterinaria Scandinavica**, v. 43, n. 1, p. 1–19, 2002. Disponível em: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1764183/>. .

VIEIRA-NETO, A.; LIMA, I. R. P.; JR, F. L.; et al. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2016-12506>. .

WILES, P. G.; GRAY, I. K.; KISSLING, R. C. Routine analysis of protein by Kjeldahl and Dumas methods: review and interlaboratory study dairy products. **. J. AOAC Int.**, v. 81, p. 620–632, 1998.

WILHELM, A. L.; MAQUIVAR, M. G.; BAS, S.; et al. Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. **Journal of Dairy Science**, p. 1–9, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217301224>. .

XU, C.; WANG, Z. Comparative proteomic analysis of livers from ketotic cows. **Veterinary Research Communications**, v. 32, n. 3, p. 263–273, 2008.

YOUDEN, W. J. Index for rating diagnostic tests. **Cancer**, v. 3, n. 1, p. 32–35, 1950.

4 CHAPTER 3 - INCREASED SERUM CALCIUM IN DAIRY COWS WITH ORAL CALCIUM FORMATE SUPPLEMENTATION IN THE POSTPARTUM PERIOD

ABSTRACT

The objective of the study was to evaluate the effects of oral calcium formate supplementation on serum total calcium (tCa), ionic calcium (iCa), non-esterified fatty acids (NEFA), ß-hydroxybutyrate (BHBA), total cholesterol (TC), AST enzyme (AST), albumin (ALB), P and Mg minerals in early lactation cows. Also, the results on diseases, milk production and reproduction. In a commercial dairy farm in Castro County, Paraná State, Southern Brazil, 129 Holstein cows (45 primiparous and 84 multiparous) were blocked by parity and by tCa status (Idexx VetTest® Chemistry Analyser, Inc., Westbrook, ME) 6 h after calving. Blood samples were analyzed for group allocation (normal and hypocalcemia groups) using 8.2 mg/dL (2.05 mM) as the cutpoint. Within each block, fresh cows were randomly allocated to three groups; 0 (C = control), 350 (T1) and 700 ml (T2) of 14.3% (w/w) calcium as a 48.6% aqueous suspension of calcium formate (Calfon Oral®, Bayer HealthCare), 6 h after parturition. Ten blood samples were collected 6(t1),6.5 (t2), 7.0 (t3), 7.5 (t4), 8.0 (t5), 10 (t6), 14 (t7), 24 (t8) h, day 5 and 10 after calving. Data was analyzed using the MIXED procedure of SAS with a model containing the effects of block, treatment, time, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Hypocalcemia incidence rates were 37% using on-farm tCa from VetTest (≤ 2.05 m*M*), 40% using tCa (≤ 2.0 m*M*) and 71% using iCa (<1.0 m*M*). The lowest tCa and iCa values were observed at 6 and 24 h postpartum for multiparous an primiparous, consecutively. It has shown an increase (P <0.05) in serum levels of iCa and tCa between t2 to t7 for T1 and T2 compared to group C. The highest increments were at t2 (0.5 h after Ca administration) with 0.98, 1.07 and 1.29 ± 0.04 m*M* iCa and 2.00, 2.18 and 2.50 ± 0.01 m*M* tCa for C, T1 and T2, respectively. Oral Ca supplemented cows have shown higher (P < 0.05) AST concentrations at t4 to t8 and lower (P < 0.05) Mg levels at t5 to t8. No differences were detected (P > 0.05) for NEFA, TC, BHBA, ALB and P concentrations. No effect was observed the use of oral Ca in the incidence of diseases between groups, and index reproductive. The oral calcium formate supplementation has shown beneficial effects on the increment of ionic and total Ca on early lactation dairy cows, important goals to control metabolic disorders in dairy farms.

Key words: milk fever, subclinical hypocalcemia, transition period

RESUMO

O objetivo do estudo foi avaliar os efeitos da suplementação oral de formiato de cálcio no cálcio total sérico (tCa), cálcio iônico (iCa), ácidos graxos não esterificados (AGNE), ß-hidroxibutirato (BHBA), colesterol total (CT), enzima AST (AST), albumina (ALB), e os minerais de P e Mg em vacas no início da lactação. Também foram analisados os dados de enfermidades, produção de leite e reprodução. Em uma fazenda comercial no município de Castro, no estado do Paraná, no sul do Brasil, 129 vacas da raça Holandesa (45 primíparas e 84 multíparas) foram blocadas por paridade e pelo status de tCa (Idexx VetTest® Chemistry Analyzer, Inc., Westbrook, ME) 6 h após parto. As amostras de sangue foram analisadas para alocação de grupo (grupos normo e hipocalcêmicos) usando 8,2 mg/dL (2,05 m*M*) como ponto de corte. Dentro de cada bloco, as vacas recém paridas foram distribuídas aleatoriamente em três grupos; 0 (C = controle), 350 (T1) e 700 ml (T2) de 14,3% (p/p) de cálcio como uma suspensão aquosa a 48,6% de formato de cálcio (Calfon Oral®, Bayer Saúde Animal), 6 h após o parto. Foram colhidas oito amostras de sangue 6 (t1), 6,5 (t2), 7,0 (t3), 7,5 (t4), 8,0 (t5), 10 (t6), 14 (t7) e 24 (t8) h, dia 5 e 10 após o parto. Os dados foram analisados usando o procedimento MIXED do SAS com um modelo contendo os efeitos do bloco, tratamento, tempo e interação do tratamento*tempo como efeitos fixos e vaca no tratamento como efeito aleatório. As taxas de incidência de hipocalcemia foram de 37% usando tCa na fazenda com o VetTest (≤ 2,05 m*M*), 40% com tCa (≤ 2,0 m*M*) e 71% utilizando iCa (<1,0 m*M*). Os valores mais baixos de tCa e iCa foram observados aos 6 e 24 h pós-parto para multíparas e primíparas, consecutivamente. Foi observado um aumento ($P < 0.05$) nos níveis séricos de iCa e tCa entre t2 a t7 para T1 e T2 em comparação com o grupo C. Os maiores incrementos foram em t2 (0,5 h após administração de Ca) com 0,98, 1,07 e 1,29 ± 0,04 m*M* para o iCa e 2,00, 2,18 e 2,50 ± 0.01 m*M* para o tCa nos grupos C, T1 e T2, respectivamente. As vacas suplementadas com Ca oral apresentaram maiores concentrações de AST ($P < 0.05$) em t4 a t8 e menores ($P < 0.05$) e para o Mg em t5 a t8. Não foram detectadas diferenças ($P > 0.05$) para as concentrações de AGNES, CT, BHBA, ALB e P. Não houve efeito da suplementação do Ca oral na incidência de doenças entre grupos, e índices reprodutivos. A suplementação oral de formiato de cálcio mostrou efeitos benéficos no incremento de Ca iônico e total em vacas leiteiras de lactação inicial, metas importantes para controle de distúrbios metabólicos nas fazendas leiteiras.

Palavras-chave: febre do leite, hipocalcemia subclínica, período de transição

4.1 INTRODUCTION

The transition period for cows is a time of significant metabolic challenge, with a several increases in cow requirement for energy, protein and minerals within days of pre and post-calving. The cow promotes homeorrheic and homeostatic adaptations in an effort to maintain a physiological mineral balance (BAUMAN; CURRIE, 1980), but they occasionally fail, occurring then milk fever or hypocalcemia (**HYPO**) then occurring in dairy cattle peripartum (HORST; GOFF; REINHARDT, 2005).

This is due to the increase in demand for Ca for the synthesis of colostrum can represent 7 to 10 times the amount of estimated of total calcium (**tCa**) in the blood, which induces a sudden drop in Ca blood concentration (HORST; GOFF; REINHARDT, 2005). In this way, some cows develop clinical (**CH**) or subclinical hypocalcemia (**SCH**) (BLOCK, 1984; REINHARDT et al., 2011; MARTINEZ et al., 2016). This metabolic imbalance is more common in cows of 2 or more births, the nadir being between 12 and 24 h (OETZEL, 2004; GOFF, 2008; BLANC et al., 2014).

Normal limits for serum calcium (**Ca**) concentration in an adult cow were set at 2.43 to 3.10 m*M* (KANEKO et al., 1997), but in more recent studies the values were adjusted to 2.125 to 2.7 m*M* (KIMURA; REINHARDT; GOFF, 2006; LIMA et al., 2017). The concentrations of tCa <1.4 mM are classified as CH (DeGARIS; LEAN, 2008; REINHARDT et al., 2011). A prevalence of 1% and 7% for CH and 25% and 47% for SCH in primiparous and multiparous cows was observed in the first 48 h postpartum, with cut point of tCa between 2,0 and 1,4 m*M* (REINHARDT et al., 2011); in Martinez et al. (2016) 69% of cows with SCH (cut point ≤ 2.125 m*M*); Wilhelm et al. (2017) at 2 h postpartum was 2.7% in primiparous cows and 30.8% in multiparous cows (tCa ≤ 2,0 m*M*). For Leno et al. (2017) SCH classification based on a single sample collection should be within 12 h of calving since the lowest amount of plasma tCa is associated with increased milk production.

In cows with marked HYPO, treatment with intravenous solutions of Ca salts promote rapid recovery of the cow, but they become less productive and more susceptible to other metabolic and infectious diseases (GOFF et al., 1996). Through the use of acidogenic salts, control of Ca mineral and vitamin D supplementation of prepartum diets, there was an effective reduction in the incidence of CH, but the prevalence of SCH remained high (REINHARDT et al., 2011; NEVES et al., 2017).

The oral Ca (**OC**) solution was initially used in cows with CH (THILSING-HANSEN et al., 2002). The purpose of its use is to increase the concentration of Ca in the blood by

passive diffusion, but a concentration higher than 1m*M* (BRONNER, 1987). The Ca salts soluble for oral use are: Ca chloride is the most soluble of the Ca salts; Ca propionate, Ca formate, Ca acetate, Ca gluconate and Ca lactate; Ca hydroxide, calcium oxide, and calcium carbonate are relatively insoluble and unsuitable for treating hypocalcemia. Ca chloride is the most studied molecule, initially administered several days before at partum and 1 to 2 days postpartum (GOFF, 1999).

Ca formate has the high concentration required for therapy of hypocalcemia, with lower harmful effect on the mucous membranes when compared to Ca chloride; with the formula $Ca(HCOO)_2$, it is produced during the production of polyalcohols from formic acid and calcium hydroxide (SCOTT; WIJK, 2000).

The Ca formate solution is already commercialized in Brazil and in some European countries in preventive protocols for dairy cows at the beginning of lactation, however, there are few studies on their effect on animals. The objective of the study was to evaluate the effects of oral Ca formate supplementation on blood Ca concentrations, biochemical parameters, postpartum diseases occurrence, milk production, and fertility in early-lactation Holstein cows.

In the study of Goff et al. (1996) with administration at partum, 12 and 24 h postpartum, there was a reduction in the incidence of HYPO and displacement of abomasum. Oetzel and Miller (2012) OC supplementation in lame cows with a milk production greater than 105% of the herd ranking produced 2.9 kg of additional milk in the first postpartum test compared to cows with higher milk yield in the previous lactation that did not were supplemented. Cows receiving OC bolus had a very low incidence of HYPO. In Martinez et al. (2016) it was observed that OC supplementation had a greater effect on multiparous cows and with greater potential for milk production. In this category of animals, there was a reduction in the incidence and daily prevalence of SCH, in the risk of metritis, in the number of inseminations/pregnancy and reduced open days.

Calcium (**Ca**) has an intracellular function, being involved in the proliferation, differentiation and cellular motility, in the control of muscle contraction, hormonal secretion and glycogen metabolism, besides acting as second messenger and enzyme cofactor (KIMURA et al., 2006). However, its extracellular function is associated with the process of blood coagulation, cell adhesion, maintenance of bone integrity and regulation of extracellular excitability (MARTINEZ et al., 2012).

The objective of this study was to compare the effect of doses of 0, 50 and 100 g of oral Ca on calcemia in cows in the immediate postpartum period.

4.2 MATERIALS AND METHODS

4.2.1 Animals, Management of Dry Cows and Postpartum

The experiment was conducted between June of 2016 through June of 2017 at the commercial dairy farm in Castro county, Parana State, Southern Brazil (24º 47' 28" S, 50º 00' 43" W, with an altitude of 3278 feet). All animal protocols were approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Federal University of the State of Paraná, Brazil. The choice of this farm was the technological level employed, zootechnical control, production scale and the productive pattern of the animals (average of 800 dairy cows with 39 kg of milk/day). Both herds are composed of Holstein animals, and the cows are kept confined in a freestall system. The total number of females used in the experiment was 129 Holstein cows (45 primiparous and 84 multiparous) blocked by parity and by tCa status (Idexx VetTest® Chemistry Analyser, Inc., Westbrook, ME) 6 h after calving. The cows distributed among control (**C**), treatment **T1** (50 g of Ca) and **T2** (100 g of Ca) groups. The mean (±SD) lactation number 3,0 ± 1,7 for multiparous, and BW at calving of 690,5 ± 59,0 and 575,4 ± 49,0 for multiparous and primiparous, respectively. The pregnant cows were dried 57 ± 3 d before the expected calving day and separated for the batch of dry cows. All cows were moved to the prepartum group on average 17 ± 5.6 d before the day of calving. All animals calved within a 62-d window (June 2, 2016 through August 3, 2016). The diet was common to all prepartum cows was formulated to have a negative calculated DCAD, distributed twice a day as TMR and offered for ad libitum intake.

4.2.2 Diet Formulation and Composition

The diet was mixing and distribution was through equipment Faresin® leader-2200 Mixers. Pre- and postpartum cows were fed twice and three times daily consecutively. Samples of TMR after feeding and forages were collected in the first and last weeks of the experiment, frozen until they are transported to the laboratory for determination of DM (dried at 55º C for 72 in a forced-air oven) and milled in a Willey type mill with 1 mm sieves. The determination of the dry matter content (DM) will be carried out in an oven at 105ºC for 24 h and the ash content will be obtained by incineration in muffle at 600ºC for 3 hours (AOAC International, 1990). Determination of neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (FDA) will be performed according to Van Soest (1994) methodology adapted to the sequential method using ANKOM Fiber Analyzer (ANKOM® Technology Corp.) according to

the technique described by Holden (1999). The determination of Crude Protein (PB), is done using the method of Dumas Wiles, Gray and Kissling (1998), in an autoanalyzer of nitrogen (ELEMENTAR® rapid N cube). Analysis of the ether extract in the ANKOM® XT 10 extractor occurs by using petroleum ether solvent, with an extraction time of 6 hours Silva and Queiroz (2002). The levels of K and Na were determined by atomic absorption spectrophotometry at the Animal Nutrition Laboratory of the Federal University of Paraná (**UFPR**; Curitiba, Paraná, Brazil). Chlorine was determined by aqueous shaking Malavolta, Vitti e Oliveira (1989) and sulfur by turbidimetry Miyazawa, Pavan and Muraoka (1999) at the ABC Foundation Laboratory in the city of Castro, Paraná, Brazil. The mean values of the composition of TMR collected and composition estimated by the NRC (2001). With the results of the analyzes of the diets, the diet cation-anion difference (DCAD) was calculated [(K + Na) - (Cl + S)] in the first and last week of the study, using the software NRC (2001).

4.2.3 Experimental Design and Treatments

The experiment was a randomized block design. The study was conducted initially with 135 cows, however, 6 animals were eliminated from the study for presenting CH and have been treated with parenteral calcium. For the formation of the groups the 129 animals were divided according to the criteria: 1- order of calving in primiparous (n = 45) and multiparous (n = 84) and 2- tCa normocalcemic (**NC**; n = 78) and SCH (n = 51). For the determination of calcemia, the blood samples were taken 6 h postpartum, analyzed on the farm (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME) to be then categorized, and then alternated in groups C (n = 43), T1 (n=43) and T2 (n = 43). Serum samples were analyzed on farm to define the cows with SCH after calving using as the cut-point at ≤ 2.05 m*M* for tCa concentration in IDEXX VetTest® of with optimal sensitivity and specificity with the laboratory results (cut-point ≤ 2.0 m*M*), established by using the receiver operator characteristic (**ROC** curve). Within each block, fresh cows were randomly allocated in C groups (no Ca supplementation), with treated-cows being supplemented 6 h postpartum with T1 = 1 x 350 ml and T2 = 2 x 350 ml of 14.3% (w/w) calcium (50 g of Ca supplementation) as a 48.6% aqueous suspension of calcium formate (Calfon Oral®, Bayer Animal Health).

Six blood samples from each animal were collected of the coccygeal vein or artery in tube with coagulation accelerator (vacutainer), at time 6, 6.5, 7, 7.5, 8, 10, 14, 24 h (t1 to t8), day 5 and 10 after calving. The non-esterified fatty acids (**NEFA**) was determined in t1. For the minerals tCa, iCa, magnesium (**Mg**) and phosphorus (**P**), as for aspartate aminotransferase

(**AST**) enzyme, albumin protein (ALB), total cholesterol (**TC**), the at time t1 to t8; on days 5 and 10 postpartum for analysis of TC, beta-hydroxybutyrate (**BHB**), AST, ALB, P and Mg.

4.2.4 Blood and Urine Sampling

In the prepartum period, the urinary pH was measured by weekly sampling of 10 animals in each farm, using a portable pH meter with electrode (Model HI 98108 - HANNA). This evaluation aimed to evaluate the effect of anionic diet on the basic acid balance of cow. In order to have their urine samples collected, the females had to be consuming *ad libitum* the anionic diet provided in the prepartum for at least 5 days. The targeted average of urine pH in farms for cows fed whith anionic diet is was between 5.5 and 7.0.

In the postpartum period, for the evaluation of tCa on the farm, 1.5 mL of blood was collected of the coccygeal vein or artery immediately before treatment in eppendorf tube with lithium heparin. Such sample was centrifuged (Eppendorf Minispin® Plus) at 12.100 × g for 5 minutes. Before performing the tCa tests, the equipment was calibrated with standard solutions. After the calibration, the test was started with a 30µL aliquot of plasma obtained through the equipment pipettor (IDEXX VetTest® Chemistry Analyser, Inc., Westbrook, ME), which uses dry biochemistry slides with the processing and result in mg/dL available in 8 minutes. The resulting value was used for the categorization of the animals (NC or SCH) and its subsequent blocking, which undoubtedly was one of the great differentials of the present experiment.

For laboratory serum determinations, blood samples (9.0 mL) of the coccygeal vein or artery were collected in tubes with coagulation accelerator (vacutainer). The samples were centrifuged (CENTRIBIO®, model 80-2B) at 1.800 x g for 10 minutes to obtain the serum. The samples were then identified and stored in eppendorf tubes (in duplicate) at -20ºC until they were transported to the Laboratory of Veterinary Clinical Pathology of UFPR where the samples were kept frozen at -80°C and analyzed in an average period of 90 days.

4.2.5 Milk Production and Composition

The cows were milked 3 times a day, and daily dairy production records were stored through the herd management software Delpro® (DeLaval). The effects on the accumulated milk production during the first 30 and 100 days of lactation were analyzed.

The individual milk composition (fat, protein, lactose and total solids) were obtained from the first four official dairy controls performed monthly on analyzers with infrared

differential absorption technology, Bentley 2000® (Bentley Instruments) with Associação Paranaense de Criadores de Bovinos da Raça Holandesa, in Curitiba, Paraná, Brasil.

4.2.6 Fertility Rensponses and Culling

At 60 DIM, the cows were released to AI after evaluation by a veterinarian. Monitoring of estrus repetitions was performed by trained employees 3 times a day. The diagnosis of gestation by ultrasonography occurred 30 days after AI. Animals with negative pregnancy were directed to one of the farm's reproductive protocols for re-AI.

The reproductive data were obtained through the herd management software Delpro® (DeLaval), analyzing the effects of treatment on pregnancy, open days and number inseminations (AI) by pregnancy, in the period of 150 and 365 days after the calving. Discards were observed during the 365 days postpartum.

4.2.7 Concentration of minerals iCa, tCa, Mg and P

The tCa, Mg and P minerals were determined in the serum by an automated biochemical analyzer (BS-200®, MINDRAY Chemistry Analyzer). The reagents used (DIALAB®, Austria) for tCa by the ARZENAZO III colorimetric method, the Mg by the colorimetric method with Xylidyl Blue and the inorganic P by the Final Point UV Test method. The results are expressed in mg/dL. For iCa the method used was gasometry by selective ion electrode (RAPIDLab® 348 EX Blood Gas Analyzer, Siemens Healthineers). For tCa and iCa the samples were in t1 to t8, however, for Mg and P in t1 to t8 and on days 5 and 10 postpartum.

The cut point for to categorize as SCH animals were tCa ≤ 2.0 m*M*, (GOFF, 2008; REINHARDT et al., 2011; WILHELM et al., 2017). The reference values for Mg are from 1.8 to 2.3 mg/dL, and for P from 5.6 to 6.5 mg/dL (KANEKO et al., 2008).

4.2.8 Concentration of NEFA, BHBA, TC, AST and ALB

The liver function indicators analyzed were TC, AST enzyme and ALB, as well as for the energetic status of NEFA and BHBA. For the analysis of TC, DIALAB® reagents (CHOD-PAP Cholesterol) through the colorimetric enzymatic method were used for their determination after hydrolysis and enzymatic oxidation. The results are expressed in mg/dL. As normal parameters were used for TC of 80 to 120 mg/dL (Kaneko et al., 2008).

Determinations of NEFA (h = 6 postpartum) and BHB (d = 0, 5, 10 postpartum) were quantified by colorimetric enzymatic methodology using standard commercial RANDOX® reagents (Kit NEFA FA115 and Kit Ranbut D-3 Hydroxybutyrate RB 1007). The analyzes were performed in an automatic biochemical analyzer (BS-200, MINDRAY® Chemistry Analyzer). Hemolyzed samples were discarded (STOKOL; NYDAM, 2006). As normal parameters cut point postpartum were used for NEFA < 0.7 m*M,* and BHB < 1.2 m*M*, subclinical 1.2 to 2.9 m*M* and clinical > 2.9 m*M* (OETZEL, 2004; OSPINA et al., 2010; CHAPINAL et al., 2011).

For the ALB protein and the AST enzyme, aliquots of the serum were collected in times t1 to t8 and on days 5 and 10 postpartum. For the analyzes the reagents were from Dialab® reagents (Albumin-BCG), by the bromocresol green method (BCG), and the results were expressed as mg/dL and later converted to g/dL. The AST assays were performed using the UV kinetic method, optimized according to the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) using the TGO kit (ASAT), brand DIALAB® reagents, and the results expressed as U/L. As normal parameters for ALB is 3.03 a 3.55 g/dL e para o AST 78 a 132 U/L (KANEKO et al., 2008).

4.2.9 Characterization of Diseases

Standard definitions of disease were already standardized and implemented by farms in accordance with veterinarian guidelines. The standard definitions of diseases were as follows: (1) clinical hypocalcemia (**CH**) – apathy, lack of coordination, muscle tremors, cold extremities, prostration and decubitus; (2) displacement of abomasum (**DA**) – drop in food consumption, milk production, movement of the abomasum to the left side of the cow, which was detected by listening to a metallic "ping" sound on the left flank through the percussion with the finger; (3) ketosis (**KET**) – clinical ketosis (**CK**) and subclinical ketosis (**SK**) – for CK the reduction of food consumption, with decrease of milk production, without other detectable signs of disease and with positive ketosis result through test strips ≥ 2,9m*M* (FreeStyle® Optium Neo, Abbott), in days 3, 5, 7 e 11 pós-parto. For SK with positive test strips between 1.2 a 2.9 m*M*; (4) placenta retention (**PR**) - was diagnosed when fetal membranes were not completely expelled from the birth canal within 24 h of calving (KIMURA et al., 2002). (5) metrite (**ME**) - diagnosed during the daily checks of cows between days 3 and 11 of lactation, in cows that showed signs of apathy, inappetence, a temperature >39.5°C, with a foul discharge from the vulva (purulent or red to brown or both) (LeBLANC, 2010).

4.2.10 Statistical Analysis

The experiment was completely randomized, with cow being the experimental unit. Statistical models included the effects by parity (primiparous and multiparous), and tCa (NC and SHC) status 6 h after calving. For definition of NC cows the concentration of tCa in blood > 2.0 mM and SCH when tCa in blood ≤ 2.0 mM was considered. The animals were randomly assigned to the T1 (50 g of Ca), T1 (100 g of Ca) and C (did not receive oral Ca) groups. Blood samples were analyzed for group allocation using the 8.2 mg/dL as the cutpoint (IDEXX VetTest® Chemistry Analyzer, Inc., Westbrook, ME). The cutpoint was established through the optimum sensitivity (84.9) and specificity (92.7) with the tCa in the laboratory, using receiver operator characteristic (ROC), with MedCalc software (version 14.8.1). Statistical analyzes were conducted using SAS software (version 9.4; SAS/STAT®, SAS Inst. Inc., Cary, NC). All measurements repeated over time were subjected to repeated measures ANOVA using the MIXED procedure, with an autoregressive order 1 covariance structure, based on the Akaike information criterion (AIC). The data were previously tested for normality of residues by the Shapiro-Wilk Test. The model contains the effects of treatment, time, and treatment*time interaction as fixed effects and cow within treatment as a random effect. Binary traits such as the estimate of the number of AI to conceive and the risk of pregnancy at 150 days were analyzed by log-Poisson regression using the GENMOD procedure. For the association of incidence and disease prevalence, discards were analyzed by logistic regression using the GLIMMIX procedure with treatment as a binary variable (T or C) and also parity (primiparous or multiparous cows). The CORR procedure was used to determine Pearson correlation coefficients between Ca and analytes NEFA, BHBA, CHO, AST, ALB, COR after calving. Differences with $p \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered as a tendency.

4.3 RESULTS

4.3.1 Diet Composition in Pre- and Postpartum, Urinary pH in Prepartum

Analysis of diet nutrient and ingredients used in prepartum and postpartum are shown in TABLE 4.1. In the prepartum, the calculated value for the consumption of 12 kg of DM, with the use of fodder with moderate concentration of the mineral K. Thus, the average value observed for the cation K in the diet was of 0.97% in the DM, with 0.97 and 1.64% DM in corn silage and wheat straw. The DCAD of the diet was negative (-8.3 mEq/100 g DM), due to the

moderate concentration of K in forages, the supply of wet barley (mean DCAD of -15.8 mEq/100 g DM) and the use of anionic concentrate. This cation-anionic difference in the diet was able to promote a metabolic acidosis, observed through the mean pH of the urine of 6.0 ± 0.5. Another important mineral in the diet for the control of HYPO, Ca with an average content of 0.5% in DM.

TABLE 4.1 INGREDIENT COMPOSITION OF PREPARTUM AND POSTPARTUM DIET.

	Prepartum	Postpartum
Ingredient- kg/DM/d[1]		
Corn silage	5.4	6.9
Wheat straw	1.3	0.7
Brewers grain wet	0.4	1.1
Ryegrass silage	-	3.2
Corn high-moisture	-	0.53
Soybean meal	1.6	3.3
Wheat bran	3.1	-
Corn meal	-	1.6
Protected fat[2]	-	0.14
Cottonseed	-	1.35
Magnesium oxide 54%	-	0.44
Sodium bicarbonate	-	0.2
Mineral and vitamin mix[3]	0.2	-
Mineral and vitamin mix[4]	-	0.6
Chemical composition[5]		
DM, % (±SD)	34.0	43.2
NEL, Mcal/kg of DM	1.52	1.56
CP, % of DM	14.7	17.8
NDF, % of DM	44.5	25.7
ADF, % of DM	21.2	19.5
Ether extract, % of DM	4.1	4.9
Ca, % of DM	0.5	0.89
P, % of DM	0.34	0.45
Mg, % of DM	0.36	0.45
K, % of DM	0.97	1.44
Na, % of DM	0.06	0.47
S, % of DM	0.37	0.3
Cl, % of DM	0.44	0.48
DCAD[8] (mEq/100 g of DM)	-8.3	-

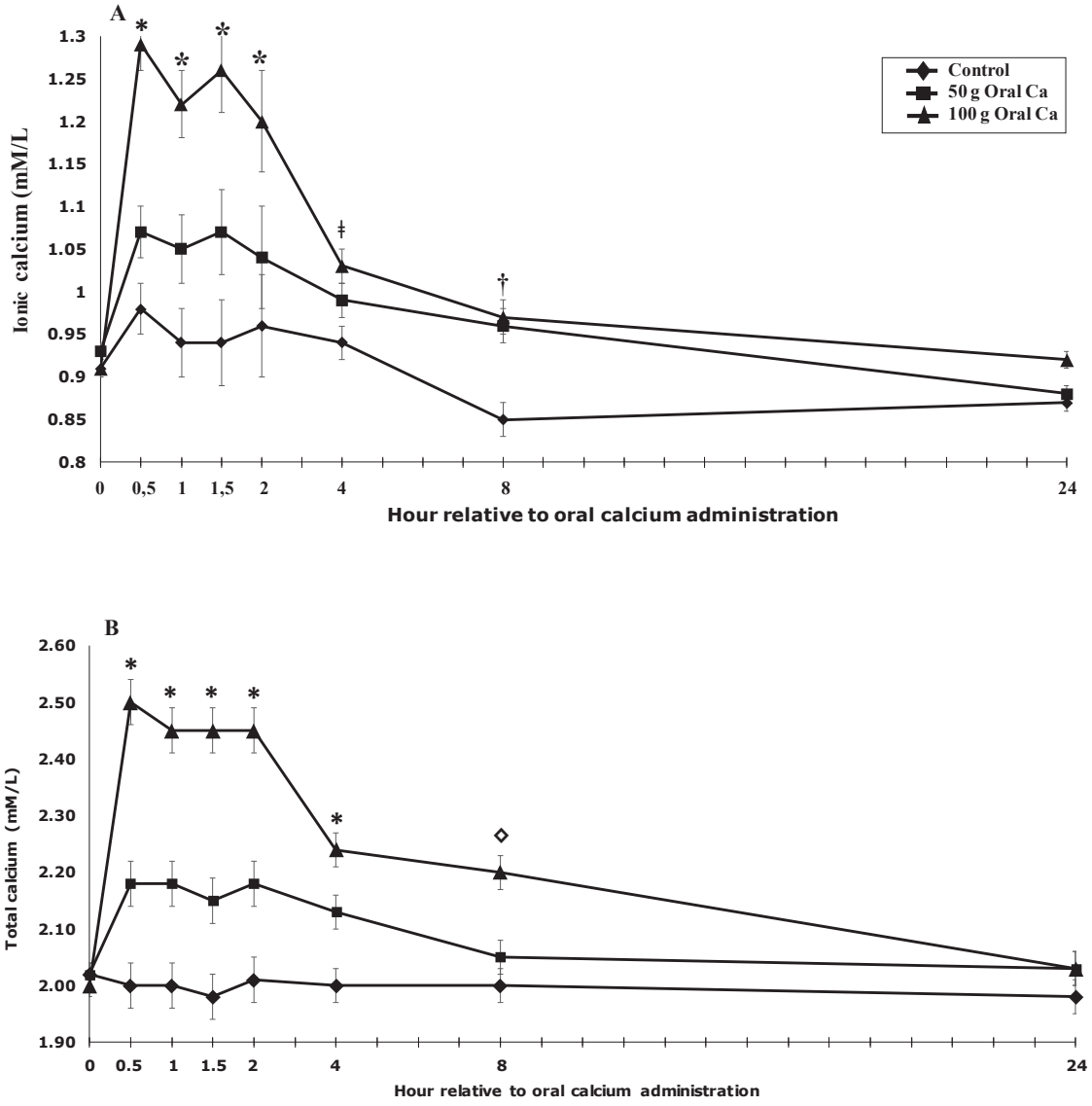
[1]Composition of diets for pre and postpartum. For pre-partum the mean time of consumption was 17 ± 5.6 d.
[2]Nutrigordura LAC Palm - calcium salts of palm oil fatty acids, composed of 82% EE, Ca 6.7%, MM 20% (Nutricorp company, county of Araçatuba, state of São Paulo, Brasil);
[3]Bovigold Pré-parto – prepartum mineral concentrate. Composed of Ca 10.6%, P 3%, S 9%, Mg 2%, Na 3.1%, Cl 13%, Co 1.2 mg/kg, Cu 600 mg/kg, Cr 30 mg/kg, Fe 600 mg/kg, I 60 mg/kg, Mn 1600 mg/kg, Se 16 mg/kg, Zn 2400 mg/kg, Biotine 80 mg/kg, Vit. A 480000 UI/kg, Vit. D3 200000 UI/kg, Vit. E 12000 UI/kg, DCAD = -794 mEq/kg (DSM Nutritional Products, São Paulo, Brasil);
[4]Núcleo Melk – mineral and vitamin premix contained 22% Ca, 4.8% P, 4.3% Mg, 2.3% S, 5.8% Na, 1.25 mg/kg Mn, 2.75 mg/kg Zn, 625 mg/kg Cu, 12.5 mg Co, 15 mg/kg Se, 50 mg/kg biotina, 200 KIU vitamina A, 60 KIU vitamina D3, 1.1 UI vitamina E, 600 mg/kg monensin (Elanco Animal Health, Greenfield, IN) (Núcleo MELK, Coop Castrolanda, Castro-Paraná/Brasil).
[5]The chemical values of the diets reflect the average of the analyzes of those collected after mixing in totalmix.
[6]DCAD was determined [$(Na^+ + K^+) - (Cl^- + S^{2-})$] by the average of the chemical analyzes of the diets with the program NRC (2001).
FONTE: o autor (2018)

4.3.2 Concentration of Minerals iCa, tCa, P and Mg in Blood

The Ca concentration in plasma did not differ between groups (FIGURE 4.1A and 4.1B) prior to administration of oral calcium. The mean value of serum tCa at t1 (6 h postpartum) was 2.0 m*M*, with the concentration rising to 2.22 m*M* in t5, returning to the initial value at t8. This effect was not observed for iCa, with the highest serum concentration 1.11 m*M* in t2, when there was a decrease until 0.89 m*M* in t8 (TABLE 4.2). The percentage of tCa found in the form of iCa the blood 6 h postpartum was 46%, from 50% at 0.5 h, decreasing to 45% at 8 h after administration of oral Ca. The percentage of tCa as iCa was 47%, with a higher proportion ($P < 0.01$) for the group with 100 g (52, 50 and 51%) of oral Ca 0.5 to 1.5 h after supplementation.

In cows with SCH, supplementation with 100 g of oral Ca increased ($P < 0.05$) iCa in the blood 0.5 to 1.5 h after calving as compared to C group. For cows classified by calcemia (for tCa in 6 h postpartum), mean tCa concentration for the NC was 2.12 ± 0.2 m*M*, whereas for HYPO of 1.85 ± 0.2 m*M*; mean iCa concentration for the NC 0.96 ± 0.1 m*M* and 0.86 ± 0.1 m*M* for HYPO.

FIGURE 4. 1 CHARACTERIZATION AT THE TOTAL Ca (A) AND IONIZED Ca (B) IN SERUM, AFTER SUPPLEMENTATION WITH ORAL Ca. *WITHIN HOUR, ADMINISTRATION OF 50 OR 100 g OF ORAL Ca DIFFERS FROM CONTROL GROUP (P<0.05). ǂ WITHIN HOUR, 100 g OF ORAL CALCIUM DIFFER FROM CONTROL GROUP (P<0.05). ◊ WITHIN HOUR, 100 g DIFFER FROM 50 g OF ORAL CALCIUM AND CONTROL GROUP (P<0.01). WITHIN HOUR, CONTROL GROUP DIFFER FROM 50 AND 100 g OF ORAL CALCIUM (P<0.01). ERROR BARS DEPICT SEM.



FONTE: o autor (2018)

In this study, cows supplemented with oral Ca formate showed a rapid serum increase for both iCa and tCa (FIGURE 4.1A and 4.1B). The highest Ca difference in blood ($P < 0.01$) was observed at 0.5 h after supplementation with oral Ca for both tCa (C vs. T1 vs. T2; 2.00 vs. 2.18 vs. 2.50 ± 0.01 m*M*, $P < 0.01$) and for iCa (0.98 vs. 1.07 vs. 1.29 ± 0.03 m*M*, $P < 0.05$). The highest increase in Ca was observed for T2, when 37% (16/43) of the cows presented a serum concentration of tCa > 2.6 m*M* and 56% (24/43) had iCa > 1.2 m*M*. For the tCa difference in serum concentration between t2 and t1 for groups C, T1 and T2 was -0.02, 0.18 and 0.47

m*M*; and iCa 0.07, 0.14 and 0.38 m*M*. This increase ($P < 0.05$) of Ca in the blood was observed up to 8 h for iCa in T1 and T2, with a tendency ($P = 0.05$) for T2 in the time 24 h after supplementation. However, for tCa the increase in Ca in the blood occurred up to 4 h for T1, with a prolonged effect for T2 up to 8 h after supplementation when compared to group C. The reduction of the effect of the treatments was observed 24 h after the supplementation, through the reduction of serum Ca.

TABLE 4.2 EFFECT OF CALCIUM FORMATE ON SERUM CALCEMIA OF POSTPARTUM DAIRY COWS

Post-oral Ca	Treatment				*P-Value*		
time (h)	Control	T1	T2	SEM	C x T1	C x T2	T1 x T2
				Ionic Calcium (m*M*)			
0	0.90	0.93	0.91	0.01	0.93	0.79	0.44
0.5	0.98	1.07	1.29	0.03	<0.05	<0.01	<0.01
1	0.94	1.05	1.22	0.04	<0.01	<0.01	<0.01
1.5	0.94	1.07	1.26	0.05	<0.01	<0.01	<0.01
2	0.96	1.04	1.20	0.06	<0.05	<0.01	<0.01
4	0.94	0.99	1.03	0.02	<0.05	<0.05	<0.05
8	0.85	0.96	0.97	0.02	<0.01	<0.01	0.71
24	0.87	0.88	0.92	0.01	0.73	0.05	0.12
				Total Calcium (m*M*)			
0	2.02	2.02	2.00	0.06	0.92	0.33	0.38
0.5	2.00	2.18	2.48	0.01	<0.01	<0.01	<0.01
1	2.00	2.18	2.45	0.14	<0.01	<0.01	<0.01
1.5	1.98	2.15	2.45	0.14	<0.01	<0.01	<0.01
2	2.00	2.18	2.45	0.14	<0.01	<0.01	<0.01
4	2.00	2.13	2.25	0.12	<0.01	<0.01	<0.01
8	2.00	2.05	2.20	0.10	0.11	<0.01	<0.01
24	1.98	2.03	2.03	0.10	0.31	0.14	0.64

Supplementation with oral Ca formate was in the mean time of 6 h postpartum, shortly after collection of blood for calcemia determination.
FONTE: o autor (2018)

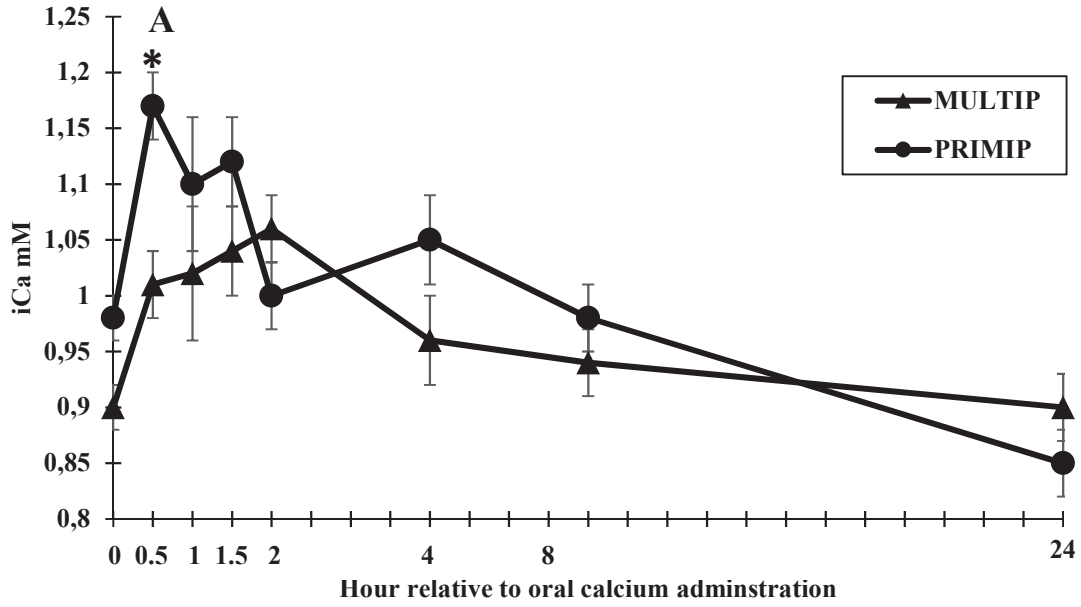
The highest value was observed in the seric concentration of primiparous when compared to multiparous cows (FIGURE 4.2). This effect of T2 vs. C group was observed ($P < 0.05$) also in the serum concentration of iCa in t2 (Mult vs. Primip; 1.23 vs. 1.40 ± 0.05 mM) and ratio of iCa to tCa (50.1 vs. 54.8 ± 1.05 % of ionic calcium; $P < 0.01$). This effect of T1 vs. C group was observed ($P < 0.01$) also in the serum concentration of iCa in t2 (Mult vs. Primip;

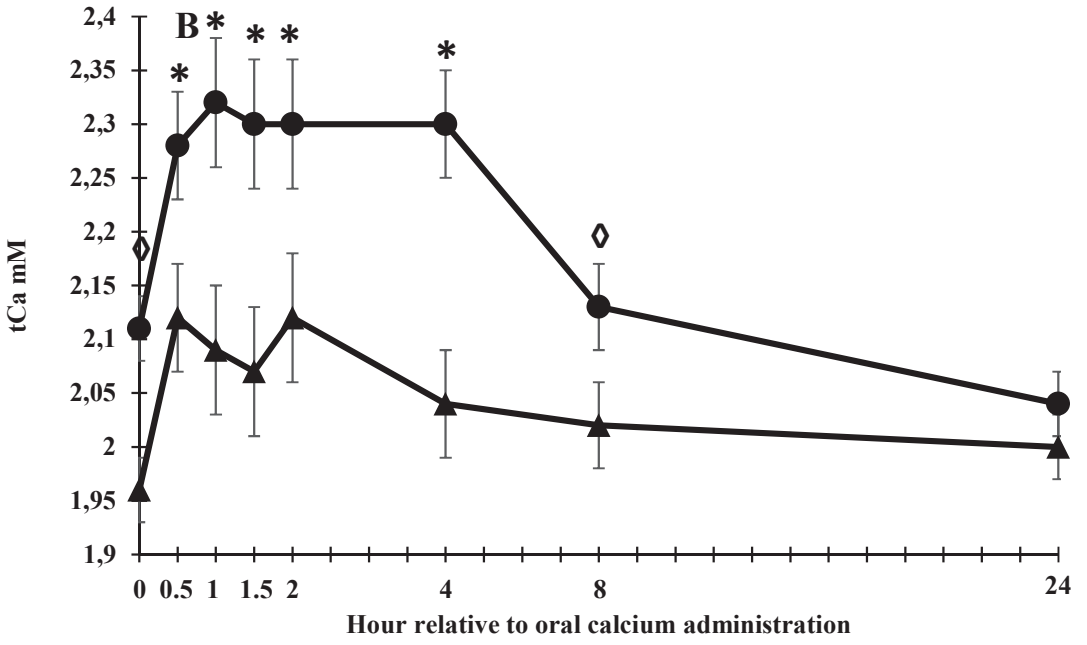
1.01 vs. 1.17 ± 0.03 mM) and ratio of iCa to tCa (48.2 vs. 51.4 ± 0.8 % of ionic calcium; P < 0.01).

For mineral P the mean value was 5.5 ± 1.5 mg/dL, with the highest concentration observed in the t1(5.8 mg/dL) and the lowest on t2, t3 and t5 (5.3mg/dL). By the effect of parity, the multiparous had higher serum values when compared to primiparous in t1 (6.0 vs. 5.3 mg/dL; $P < 0.01$). This difference was also observed in t6 and t7 ($P < 0.05$). For the treatment effect, a reduction of serum P was observed when compared to T1 vs. C in t6 (5.4 vs. 5.9 mg/dL; $P < 0.05$) and t7 (5.5 vs. 6.4 mg/dL; $P<0.01$). A similar effect was observed for T2 vs. C in the t6 (5.6 vs. 5.9 mg/dL; $P=0.10$), but also for the t7 (5.5 vs. 6.4 mg/dL; $P < 0.01$). In cows with SCH, there was an effect (P <0.05) of Ca formate between T2 vs. C in t2 (5.8 vs. 4.5 mg/dL), t3 (5.8 vs. 4.8 mg/dL), t4 (5.8 vs. 4.7 mg/dL) and t5 (5.6 vs. 4.7 mg/dL).

Mg with mean serum concentration of 2.2 ± 0.3 mg/dL in t1, showed a decrease in mean values over time, with the lowest value observed on day 5 postpartum (1.94 mg/dL). For the parity effect, the primiparous cow presented higher concentration of Mg in the t1 (2.3 vs. 2.1 ± 0,03 mg/dL; $P < 0.01$), however, the value was higher in multiparous patients in t6 (2.1 vs. 1.93 ± 0,03 mg/dL; $P < 0.01$). For the treatment effect, the animals that received the Ca formate had a more progressive reduction in the serum concentration of Mg at the times sampled. This fact was observed for T1($P < 0.01$) and T2 ($P < 0.05$) for the times from t4 to t8. However, the largest falls in values occurred for the T2 (TABLE 4.3). The correlation was low but significant between Mg and tCa (r = -0.24, P < 0.01), but also for iCa (r = -0.20; $P < 0.05$).

FIGURE 4. 2 CHARACTERIZATION OF BLOOD SERUM iCa (A) AND tCa (B) IN PRIMIPAROUS AND MULTIPAROUS COWS. WHEN ANALYZED FOR EFFECT OF ORAL Ca FORMATE (CALFON ORAL®, BAYER ANIMAL HEALTH) ON PARITY WHEN COMPARING THE TREATMENT WITH 100 g OF Ca AND THE CONTROL GROUP. * EFFECT OF PARITY IN CONCENTRATION OF Ca ($P < 0.01$). ◊ EFFECT OF PARITY IN CONCENTRATION OF Ca ($P < 0.05$).





FONTE: o autor (2018)

4.3.3 Concentrations of NEFA, BHBA, TC, ALB and AST in Blood

The mean concentration of the metabolite NEFA in t1 was 0.68 ± 0.3 m*M*. It was observed that 39% (50/129) of the cows had an average 1,01 m*M*, being in 34.5% (29/84) of multiparous cows, however, the highest frequency was in the primiparous 46% (21/45). For the BHBA metabolite the mean values were 0.40 ± 0.2, 0.66 ± 0.40 and 0.67 ± 0.80 m*M* for days 0, 5 and 10, consecutively.

TABLE 4.3 EFFECT OF CALCIUM FORMATE ON SERUM MINERALS P AND Mg IN POSTPARTUM DAIRY COWS

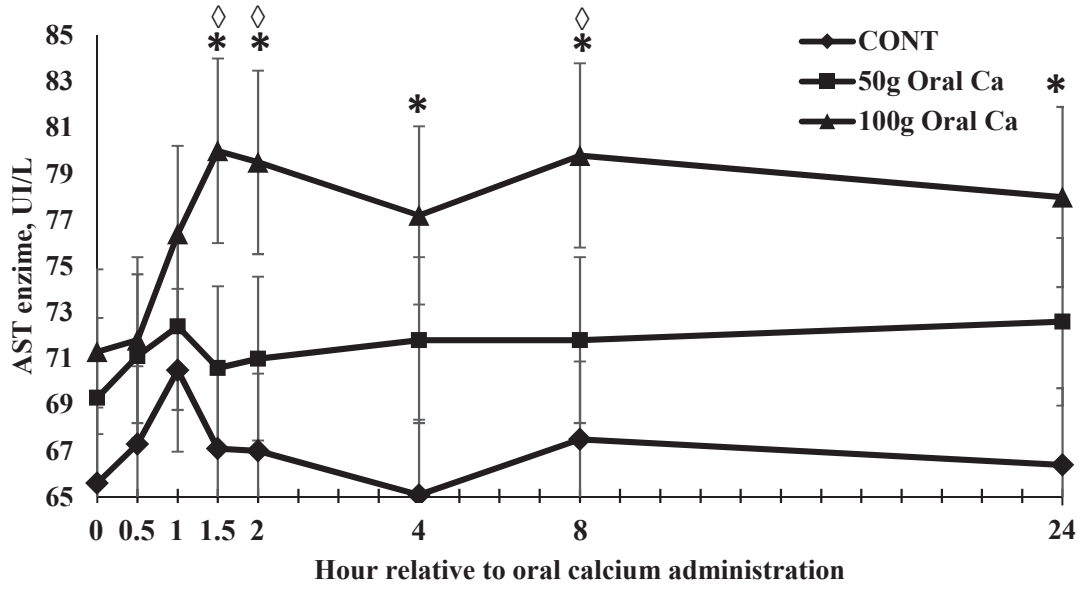
Post-oral Ca time (h)	Treatment				*P-Value*		
	Control	T1	T2	SEM	C x T1	C x T2	T1 x T2
			Phosphor		**(mg/dL)**		
0	5.6	5.8	5.9	0.2	0.54	0.31	0.70
0.5	5.0	5.3	5.5	0.2	0.48	0.61	0.22
1	5.3	5.3	5.5	0.2	0.96	0.35	0.32
1.5	5.3	5.3	5.0	0.2	0.83	0.38	0.50
2	5.3	5.3	5.4	0.2	0.89	0.70	0.59
4	5.9	5.4	5.6	0.2	<0.05	0.10	0.45
8	6.4	5.5	5.5	0.2	<0.01	<0.01	0.98
24	5.8	5.8	5.9	0.2	0.94	0.48	0.43
			Magnesium		**(mg/dL)**		
0	2.30	2.20	2.20	0.04	0.74	0.09	0.17
0.5	2.20	2.15	2.13	0.04	0.09	0.05	0.80
1	2.20	2.12	2.10	0.05	0.82	0.30	0.41
1.5	2.20	2.12	2.10	0.04	0.10	<0.05	0.66
2	2.20	2.10	2.00	0.03	<0.01	<0.05	0.11
4	2.20	2.04	1.90	0.04	<0.01	<0.01	<0.01
8	2.20	2.00	1.90	0.04	<0.01	<0.01	<0.05
24	2.10	1.90	1.80	0.04	<0.01	<0.01	<0.05

FONTE: o autor (2018)

The value for the AST enzyme was 71.9 ± 16.1UI/L, with the lowest value in the t1 (68.9 UI/L) and average highest value on day 5 (81.6 UI/L), but these results were within the reference values. On day 5 the highest concentration in individual value was observed (mean 143.4; range of 101 to 316 U/L), in16% of cows (20/129), and most (85%) in multiparous. This fact is related to the cows that presented the incidence of 1 or more diseases at the beginning of lactation. In primiparous cows, AST was higher in t6 and t8 ($P < 0.05$) with a tendency in t7 (*P*

= 0.08). Cows supplemented with calcium formate had higher serum AST levels. This effect was observed between T2 vs. C, and also for T2 vs. T1 ($P < 0.01$), from t4 to t8 (FIGURE 4.3).

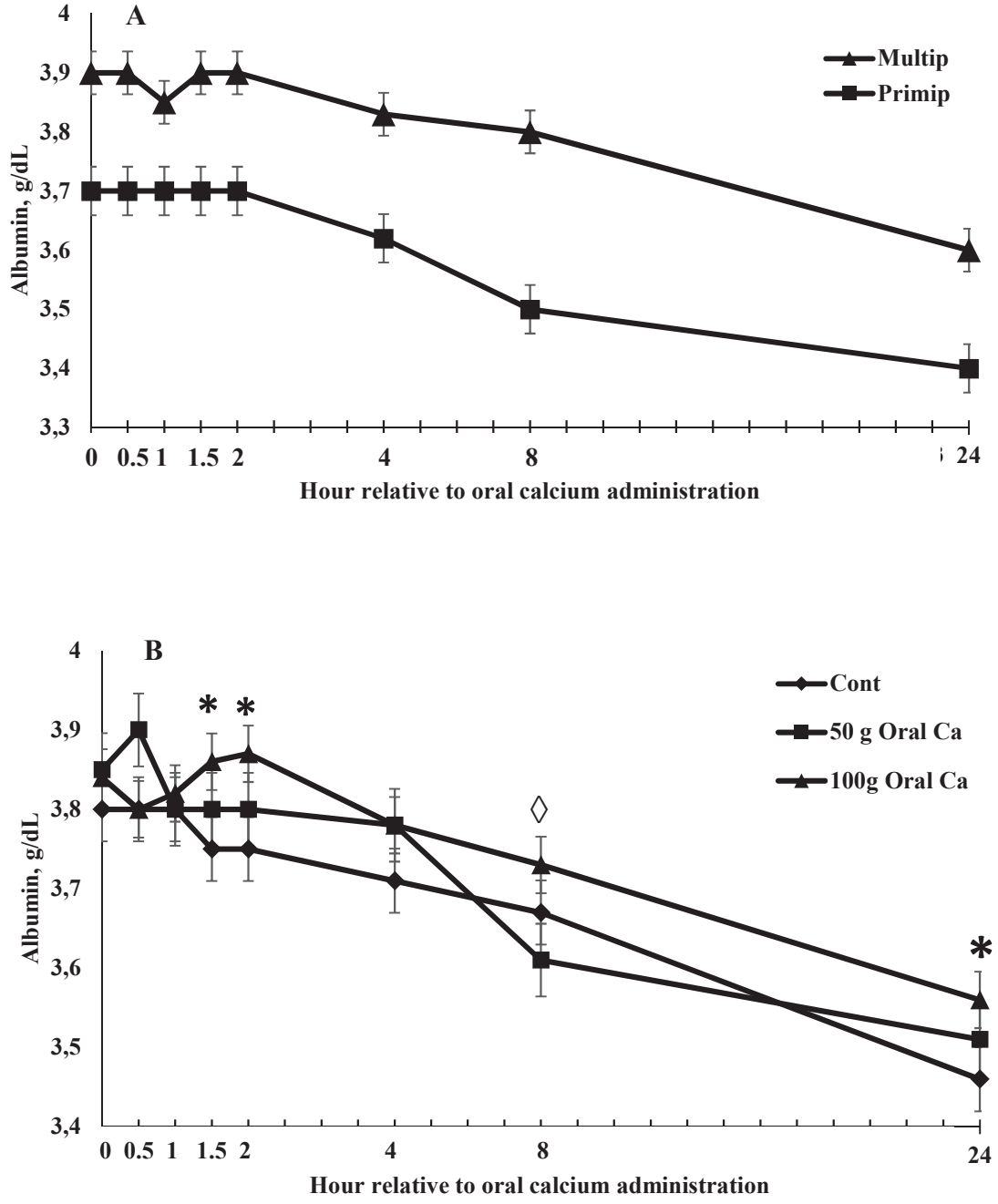
FIGURE 4. 3 - EFFECT OF Ca FORMATE ON AST ENZYME. *WITHIN HOUR, 100 g ORAL Ca DIFFER FROM CONTROL GROUP (P<0.01). ◊ WITHIN HOUR, 50 g DIFFER FROM 100 g GROUP (P<0.01). ERROR BARS DEPICT SEM.



FONTE: o autor (2018)

ALB had an average concentration of 3.76 ± 0.25 g/dL, with the highest value in t1 of 3.82 ± 0.26 g/dL and the lowest value in t8 with 3.5 ± 0.25 g/dL. In the FIGURE 4.4 A, the multiparous cows presented higher average values for the ALB when compared to the primiparous ones in all samples analyzed (t1 a t8; $P < 0.01$). After the use of oral Ca formate, concentrations of ALB increased and were greater ($P < 0.05$) in cows receiving 100 g of oral Ca compared with cows receiving 0 g of Ca, at t4, t5 and t8 (FIGURE 4.4 B).

FIGURE 4. 4 CHARACTERIZATION OF BLOOD SERUM ALBUMIN. (A) EFFECT PARITY, THE MULTIPAROUS COWS HAD A HIGHER MEAN CONCENTRATION WHEN COMPARED TO THE PRIMIPAROUS (P<0.01). (B) SERUM ALB CONCENTRATIONS AFTER ORAL Ca SUPPLEMENTATION WITH 0, 50 AND 100 g (CALFON ORAL, BAYER ANIMAL HEALTH). EFFECT OF Ca FORMATE ON ALBUMIN PROTEIN AND INTERACTION BETWEEN TREATMENT AND HOUR (P<0.05). *WITHIN HOUR, 100 g ORAL Ca DIFFER FROM CONTROL GROUP. ◊ WITHIN HOUR, 50 g ORAL DIFFER FROM 100 g ORAL GROUP. ERROR BARS DEPICT SEM.



FONTE: o autor (2018)

For the TC metabolite an average value of 68 ± 16.1 mg/dL, with the highest concentration of TC at t4 (73 ± 17 mg/dL) and the lowest at t8 (60 ± 14.7 mg/dL). When comparing the results with the reference value (above 80 mg/dL), 76% (mean 62.5 mg/dL) of

cows had values lower in t1, 97% (mean of 57.5 mg/dL) in t2. No differences were detected ($P > 0.05$) for TC concentrations in parity order and between cows receiving oral Ca compared with cows receiving 0 g of Ca.

4.3.4 Fertility Responses, Milk Yield and Culling

The mean time between calving and the first AI was 84 DMI. Forty-one percent (53/129) of the cows were pregnant at the first insemination, 53.4% (69/129) at 150 DIM. The mean time to pregnancy was 76 DIM, with 1.54 AI per pregnancy. There was no effect from the use of Ca formate on pregnancy in the first AI and at 150 DIM ($P = 0,82$).

The average milk production was 36 ± 8.8 and 43 ± 8.4 kg/milk/day in 30 and 100 DIM, consecutively. When analyzing the effect of treatments in the first 30 DIM, there was a tendency ($P = 0.05$) of lower average milk yield for T2 (35.6 ± 0.92 kg of milk) when compared to group C (38.6 ± 0.92 kg of milk/milk/day). However, Ca formate had no effect on primiparous, but in multiparous cows there was a trend ($P = 0.09$) of lower milk yield when compared to T2 vs. C (39.9 vs. 43.8 ± 1.2 kg/milk/day) for the first 30 DIM.

Multiparous and primiparous cows on average produced 17.4 ± 9.8 and 14.7 ± 6.7 kg of at the first milking, consecutively. Multiparous cows with SCH showed a higher average milk in 100 DIM when compared to NC cows (45.7 vs. 43.3 kg/milk/day, $P < 0.01$), a fact not observed in primiparous animals (34.5 vs. 33.7; $P = 0.26$).

The main causes of cow discards were due to reproductive and locomotor system problems. There was no effect of Ca formate on the cow discard rate ($P > 0.05$).

4.3.5 Prevalence and Health Disorders

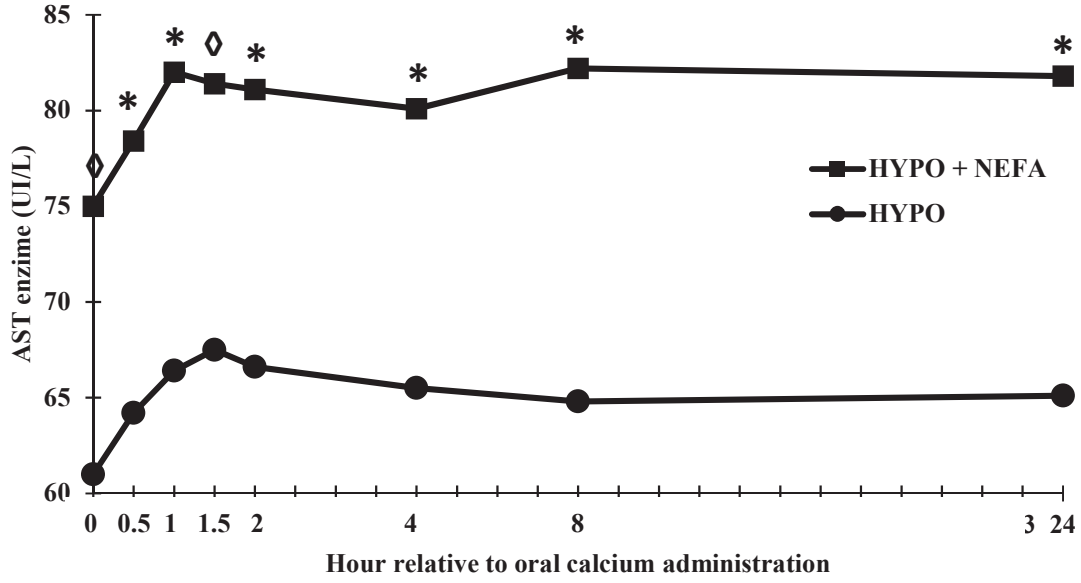
For the present study SCH was determined in t1, before the application of Ca formate. The incidence rates were 38 % using on-farm tCa the VetTest (≤ 2,175 m*M*), and analized in laboratory, 40 % using tCa (≤ 2.0 m*M*) and 71 % for iCa (concentration ≤ 1.0 m*M*). Through the tCa in time t1 the primiparous presented an average incidence rate of SCH of 11 % and multiparous 54% (33% for second-lactation cows, 67% for third or more lactation). When analyzed at 24 h after calving, the prevalence was 53% (23/43), 51% (22/43) and 37% (16/43) SCH for groups C, T1 and T2, consecutively. The lowest Ca values were observed at 6 and 24 h postpartum for multiparous and primiparous.

Of the 51 cows categorized with SCH, 29 cows had NEFA < 0.7 m*M* (mean value of 0.49 mM; SCH) whereas 22 presented NEFA > 0.7 m*M* (mean of 0.96 m*M*, SCH + NEFA). In the SCH + NEFA cows, a higher concentration of AST (P <0.05) was observed in t2, t3, t5 to t8, and in day 10 postpartum (FIGURE 4.5) when compared to SCH. For BHBA, a higher concentration was observed in t1 (SCH vs. SCH + NEFA, 0.33 vs. 0.46 ± 0.04 m*M*, $P < 0.05$) and day 5 (0.47 vs. 0.94 ± 0.15 m*M*, $P < 0.05$) for animals with SCH + NEFA, and a tendency on day 10 (0.35 vs 1.05 ± 0.27 m*M*, $P = 0.09$).

The occurrence of diseases was higher for cows in group C, SK = 68% (11/16), PR = 71% (5/7), MET = 60% (6/10) when compared to groups that received Ca. For the order of parturition, multiparous cows presented a higher number of diseases (SK = 87%, PR = 85%, MET = 80%, CULLING = 89%) in relation to primiparous cows.

The incidence of other diseases such as of SK, MET, RP, and DA in the current study was 12, 7.8, 5.4, and 0.8 %, respectively. There was no effect of Ca formate on the cow diseases prevalence ($P > 0.05$).

FIGURE 4. 5 CHARACTERIZATION OF BLOOD SERUM AST. EFFECT OF HYPO + NEFA VS. HYPO IN COWS. *WITHIN HOUR, DIFFER (P < 0.01). ◊ WITHIN HOUR (P < 0.05)



FONTE: o autor (2018)

4.4 DISCUSSION

In both farm adopts as a preventive strategy for HYPO, a prepartum diet with control of the concentration of minerals K and Ca, as well as through the inclusion of anionic salts. In this way, in the present study, the calculated mean DCAD of the prepartum diet cows was -8.3 mEq/100 g DM. These values are in agreement with recommendations for anionic diets of -5 to -15mEq/100g DM to promote metabolic acidosis and contribute to the reduction of the incidence of hypocalcemia (MOORE et al. 2000; LENO et al., 2017). The use of the anionic diet in the prepartum was effective to control of CH in the experimental period, but was moderate for SCH with 40% (51/129). However, feeding anionic salts has decreased the incidence of SCH in some studies (MOORE et al. 2000), but failed to do so in others (RAMOS-NIEVES et al., 2009). In Leno et al. (2017) the plasma Ca concentrations in postpartum increased linearly with decreasing prepartum DCAD and had decreased prevalence of SCH in the days immediately after parturition.

Mean of Ca mineral concentration in the prepartum diet was 0.5% in DM. In practice, prepartum diet with high dietary calcium content (1.1 to 1.5% of Ca) leads the cow to maximum risk, while low levels of Ca in the diet (< 0.5%) predict a low incidence of HYPO (LEAN et al., 2006).

The effect of the use of negative DCAD can be observed through the pH of the average urine of 6.0 (pH 5.7 - 6.2) of the cows in the 4 experimental weeks. The benefits in the use of anionic diet in the prepartum in Holsteins cows are observed with the urine pH between 5,5 and 6,8 (GOFF, 2008; MOORE et al. 2000; BLANC et al. 2014; LENO et al. 2017). However, the restoration of target tissue PTH sensitivity has been demonstrated at a relatively low urine pH (< 6.0) (GOFF; LIESEGANG; HORST, 2014).

Another strategy for the prevention of HYPO is the use of oral Ca in dairy cows. It is common to recommend four doses of approximately 50 g of Ca in the form bolus or gel 12 h before expected calving, immediately after calving, 12 and 24 h after calving (OETZEL, 1996; GOFF et al., 1996). This study analyzed the effect of Ca formate (Calfon oral®) with concentrations of 50 and 100 g of oral Ca in a single dose, in time of 6 h postpartum. However, the effects the Ca in blood are more likely to be obtained if oral Ca is administered before parturition or within 24 h of calving (THILSING-HANSEN et al., 2002 ; MARTINEZ et al., 2016a; MEGAHED et al., 2018).

Cutoff used to identify SCH has been 8.0 mg/dL (2.0 m*M*) tCa, and 4.0 mg/dL (1.0 m*M*) iCa, in time 6 h postpartum, due to an assumption that total blood calcium is composed of 50% ionized calcium (OETZEL, 2004; REINHARDT et al., 2011; WILHELM et al., 2017). The criterion for the definition of the reference value for the definition of HYPO, especially from a borderline point of view, is not clear for determining the risk of diseases for cows. The studies on Ca up to then present different methodologies both for the time of blood collection and also for the cutoff value in the classification of calcemia in postpartum.

The nadir for iCa (0.89 ± 0,13 m*M*) and tCa (2.00 ± 0,15 m*M*) was observed in the mean time of 24 h postpartum, which is in agreement with the described in literature (OETZEL, 2004; GOFF, 2008; BLANC et al., 2014). Megahed et al. (2018) found in their study evaluating the tCa in multiparous cows the values of 2.32 and 2.13 m*M* at 72 and 9 h before calving, with nadir occurring at 28 h postpartum with a mean value of 1.87 m*M*. The conclusions this finding was that plasma tCa in 12-h period before parturition was negatively associated with age and colostrum volume, and 24-h after parturition with age. In Goff (2008) the blood Ca concentrations reach a nadir at approximately 12 to 24 h after calving, with high risk of metritis of animals having the lowest concentrations between 24 and 48 h after parturition (MARTINEZ et al., 2012).

In the results of studies on the effect of postpartum oral Ca use have been promising. In the present experiment there was an effect of Ca formate supplementation with a rapid increase in serum Ca in the first hours after supplementation. Martinez et al. (2012) found that serum tCa concentration was higher for cows supplemented with oral Ca ($CaCl_2$ and $CaSO_4$) compared to control cows as well as primiparous cows over multiparous cows. Afshar Farnia et al. (2018) compared the effect of dietary acidogenic at the end of gestation and oral Ca ($CaCl_2$) postpartum, concluding that the combination of both provided a higher concentration of blood Ca at 6 and 12 h postpartum when compared after calving alone.

A rapid serum increase was observed for both iCa and tCa after supplementation with oral Ca formate. When compared to group C, the highest Ca difference in blood ($P < 0.01$) was observed at 0.5 h after supplementation, persisting ($P < 0.05$) up to 8 h for iCa at T1 and T2, with a tendency ($P = 0.05$) for T2 at 24 h after supplementation. However, for tCa this increase was observed up to 4 h for T1 but extending to T2 up to 8 h. Our results are in accordance with previous studies where supplementation was with Ca chloride or propionate with an increase in blood Ca concentrations reaching peak at 30 minutes post administration and lasting approximately 4 to 6 h (GOFF; HORST, 1993; OETZEL, 2013; MARTINEZ et al., 2016a)

The reduction of the effect of the treatments was observed 24 h after the supplementation, through the reduction of serum Ca. Drainage via the mammary gland and the low systemic supply may explain the low persistence of serum Ca concentrations following oral administration. According to Oetzel (2013) the cows absorb an effective amount of calcium into the bloodstream within approximately 0.5 h of supplementation keeping the concentration in the blood for 4 to 6 hours. In Oetzel e Miller (2012) if samples are taken after 8 h of treatment, the differences are minimized or even cease to exist, which may probably raise the values for the prevalence and incidence of SCH in cows receiving oral Ca.

With supplementation of 100 g of oral Ca had a higher serum Ca response when compared to the use of 50 g Ca and the control group. This effect occurs due to passive transport by increasing the luminal concentration of Ca above 1 m*M*, and for ruminal absorption >1.5 m*M* (GOFF; HORST, 1993). The iCa absorption is unlimited and independent of stimulation by 1.25-dihydroxyvitamin D, the net absorption of free Ca increases linearly with increasing luminal Ca concentrations (THILSING-HANSEN et al., 2002). The rapid increase observed in blood Ca has a large participation of passive diffusion of iCa across the reticulum and the rumen Schröder et al. (2015). In the first days postpartum a cow has about 60 L of liquid in the rumen, and the supply of a dose close to 100 g of oral Ca, if all solubilized, can promote a concentration of Ca greater than 10 m*M* (MARTINEZ et al., 2016a). This concentration is greater than that required to cross the epithelium, which facilitates tissue diffusion and provides a rapid increase of Ca in the blood (SCHRÖDER et al., 2015).

The primiparous cows presented the largest increase in serum Ca with the use of Ca formate when compared to the multiparous, with the greatest difference observed for tCa. The different responses in blood tCa between primiparous and multiparous receiving the same dose of oral calcium are explained for physiological differences, and the way they cope with the increased energy and calcium demands in the transition period (CHAPINAL et al., 2012). The differences that multiparous cows present with higher volume of rumen (higher absorption rate) produce a higher volume of colostrum compared to primiparous cows, higher DMI (which causes a faster passage rate of the digestive contents) and which dilutes the volume of absorbed Ca (MARTINEZ et al., 2016b). The responses for supplementation with oral Ca are likely related to susceptibility to HYPO, apart from that the oral Ca administration affected acid-base status with a compensated metabolic acidosis; perhaps primiparous cows are more sensitive to the potential negative effects of metabolic acidosis during early lactation (MARTINEZ et al., 2016a). However, the benefits of the additional acidification from oral Ca administration cause

more benefits via improved Ca metabolism than potentially detrimental effects from systemic acidification (OETZEL; MILLER, 2012).

The concentration of the P mineral presented a slight decrease between the times t2 to t5, which for Goff (2006) occurs secondary to hypocalcemia, since parathyroid hormone secreted in response to hypocalcemia affects phosphorus metabolism. After oral administration of Ca, blood P concentrations increased and were greater in cows with SCH. The administration of Ca promotes recovery of P levels in the blood due to the reduction of PTH secretion, which reduces the urinary and salivary loss of P (GOFF, 2006). According to this author generally causes this Ca apport the resumption of gastrointestinal motility, which allows absorption of dietary P and reabsorption of salivary P secretions.

Serum values for Mg had a progressive but small reduction in all collections when compared to t1. This shows the enormous drain of Mg from and failure to mobilize Mg from the bones to maintain Mg homeostasis during onset of lactation. A similar condition was observed by Dhiman and Sasidharan (1999) when using supplementation with oral Ca sources. Blood concentrations of Mg generally decrease after calving (MELENDEZ et al., 2003; RAMOS-NIEVES et al., 2009), and Ca supplementation in large quantities (activation of receptors by the iCa may) decrease the PTH level and increase the urinary excretion of Mg (ROSOL;CAPEN, 1996; REINHARDT et al., 1988; MARTINEZ et al., 2016a). The fecal and urinary excretions of Mg were not measured in this study. In fact, in our study cows that were supplemented with Ca formate had lower serum Mg values. Thus, the hypercalcemia induced by use of oral Ca probably promoted an increase in the urinary excretion of Mg, with reduction of its concentration in the blood during onset of lactation.

Energetic metabolism through the concentration of NEFA (day of calving) and BHBA (calving, day 5 and 10 postpartum) did not present challenging values for cows. Adequate nutritional management and the division of primiparous and multiparous cows in both pre- and postpartum were important points for nutritional balance and reduction of cow's energy challenge. Thus, no effect of the use of Ca formate on these TC metabolites was observed, a metabolite that represents the energetic metabolism pathway.

Liver function indicators analyzed were TC, the enzyme AST and the ALB. In our study it was observed a large percentage of cows with values lower than those recommended for TC, below borderline for this metabolite, with a higher occurrence on day of calving and 24 h postpartum. The concentration of TC is related to food intake, so the decrease in TC synthesis occurs when there is food restriction, leading to a lower insulin concentration and an increase in glucagon (GRUMMER, 1995). According to Guretzky et al., 2006 the TC concentration

below 77.34 mg/dL is associated with low voluntary consumption or metabolic problems, and the limit of 80mg/dL is recommended by Kaneko et al. (2008). Stengärde et al. (2008) observed that in cows that presented increased DIM after calving, there was an increase in TC concentrations, with elevated lipid uptake by the liver and an improvement in the negative energy balance.

Plasma values of the AST enzyme were higher in primiparous cows, however, still within physiological values, fact that according to Reynolds et al. (2003) reflects the change in hepatic metabolism, mainly due to milk production. However, the highest values (values higher than 100 U/L) for AST were found on day 5 and most (85%) in multiparous cows. This fact is related to the cows that presented the incidence of 1 or more diseases at the beginning of lactation. In this case, proinflammatory cytokines reduce the synthesis of some proteins, such as albumin, in addition to altering the normal metabolic pathways to lactation, with increased fat mobilization, fat deposition and induction of acute phase response in the liver (DRACKLEY et al., 2005).

The AST enzyme is found primarily in the liver, erythrocytes and in the skeletal and cardiac muscles. It is used as an indicator of necrosis in these tissues, especially in hepatic lesions (HOFFMANN; SOLTER, 2008). Values above 100 U/L are indicative of hepatic lesions (OETZEL, 2004; XU; WANG, 2008). It is considered the most sensitive indicator in the diagnosis of fatty liver in cows. In cattle, sensitivity is reported in 94% for hepatic lipidosis (KANEKO et al., 2008).

In this study, the ALB the multiparous cows presented higher average values when compared to the primiparous ones in all samples analyzed. This may be related with elevated inflammatory response during the postpartum period had decreased serum albumin concentrations and changes in other blood component (BERTONI; TREVISI, 2013). Cows diagnosed with endometritis and intramammary infections at the onset of lactation had lower concentrations of plasma albumin (BOSSAERT et al., 2012; REZAMAND et al., 2007). In addition others significant components can explain the variation of albumin in the herd, such as parity and milk production season on total protein and globulin (COZZI et al., 2011). No significant effect of dietary protein concentration (high or low protein) of cows in the dry period on serum concentrations of cows was found. (BURHANS, 2006).

Cows with NEFA (> 0.7 mM) + SCH resulted in a higher concentration of BHBA and AST. In this condition, according to Goff et al. (1989) hypocalcemia leads to an increased risk of ketosis and AD, by decreasing DMI fill-reduction and rumen mat thickness, allowing more volatile fatty acids (**VFA**) to reach the abomasum and reduce contractility. According LeBlanc

et al. (2005) other diseases are also related to PR, MET, elevation of NEFA in prepartum and postpartum BHBA but not hypocalcemia as factors that increase the risk for AD. However, in this study Ca formate had an important role in the control of AD, through the greater contribution of Ca, which contributed to the maintenance of muscular functions and with that, the improvement of the energetic metabolism of the cows of the T group.

We observed that supplementation with 100 g of oral Ca reduced the prevalence of SCH when compared to group C at t8 (24 h after supplementation). In other studies, supplementation with 43 and 86 g of oral Ca reduced the incidence and daily prevalence of SCH (GOFF et al., 1989; MARTINEZ et al., 2016b), but with greater effect in multiparous (MARTINEZ et al., 2016). It may also be economically beneficial for herds with a high incidence of hypocalcemia, cows with high milk production at the previous lactation and lame cows have the best response to oral calcium supplementation (OETZEL, 2013).

The observations on general health did not suggest any significant effect of oral Ca supplementation on the diseases, which may be related to the number of animals studied and their low incidence during the experimental period. The observations on general health were not suggestive of any major effects of oral Ca supplementation. However, the number of animals used per treatment was not adequate to accurately measure the treatment effects on health performance. This effect also did not observe effect of the use of oral Ca (salts of sulfates and chlorides) on the incidence of diseases between groups C vs. T in primiparous, a fact described by Martinez et al., (2016a), where it even doubled the incidence of metritis in the primiparous of the T group.

In the present study, in multiparous cows with SCH the mean adjusted for milk production was higher ($P < 0.01$) in the 100 DIM than the cows in group C, a fact not observed in primiparous animals (P = 0.26). This condition was reported by Rajala-Schultz et al. (1999), where multiparous cows (2 or more deliveries) with CH produced 1.6 kg / d more milk in lactation compared to healthy ones. A similar situation was found by Jawor et al. (2012) according to whom cows with SCH produced, on average, 5.7 kg/d more milk during the first week 4 compared to control cows multiparous. They describe that the mechanism between hypocalcemia and milk production has not been fully clarified, but cows with higher milk production capacity, although transient, are more prone to greater net loss of calcium soon after calving.

Regarding the effect of oral Ca, cows receiving supplementation with 100 g oral Ca (T2) presented a tendency ($P = 0.05$) for lower average daily production than those of group C in the first 30 DIM. However, this effect was not observed on primiparous, but in multiparous

cows (P = 0.09). In a study with oral Ca supplementation Martinez et al. (2016b) concluded that when analyzing the results with only multiparous cows, an interaction between oral Ca supplementation and milk production was found in the first 30 DIM. In Oetzel and Miller (2012) the response to in cows with a high previous lactation milk yield (previous lactation mature-equivalent milk yield > 105% or ≤105% of herd rank) produced +2.9 kg of milk in the first control (DHI). Other studies did not show effect of oral Ca supplementation on milk production (GOFF et al., 1996; MELENDEZ et al., 2002; MARTINEZ et al., 2016b). With the number of animals involved in the experiment and the variables analyzed, it was not possible to identify the factors involved in the reduction of average daily milk production to T2 in the first 30 days. Further studies are needed for such clarifications.

4.5 CONCLUSIONS

The supplementation with oral Ca formate in concentrations of 50 and 100 g of Ca was effective in the increase of serum Ca, with the highest effect occurring 0.5 h after its administration. The duration of this effect was 8 h for iCa in the 2 treatments, however, for tCa this increase was up to 4 h for the treatment with 50 g and 8 h for 100 g of Ca. This increase raises the ratio of iCa to to tCa, a fraction readily available to the cow. With the parity order, the primiparous cows presented the greatest increase when compared to the multiparous cows. Supplementation with 100 g of oral Ca reduced the incidence of SCH, 24 h after administration when compared to group C. With the low incidence of the diseases analyzed and only one dose of oral Ca, it was not possible to evaluate its effect, on reproductive indices. The use of Ca formate is effective in the supply of readily absorbable Ca with a rapid increase in serum calcium at a critical moment for the dairy cow.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Bayer Animal Health for the financial support of this project and the Brazilian government institution CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for the Ph.D. scholarship. We also thank the staff from the Fini and Tina Farms, herds where this study was conducted.

REFERENCES

AFSHAR FARNIA, S.; RASOOLI, A.; NOURI, M.; et al. Effect of postparturient oral calcium administration on serum total calcium concentration in Holstein cows fed diets of different dietary cation-anion difference in late gestation. **Research in Veterinary Science**, v. 117, n. November 2017, p. 118–124, 2018. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0034528817307324>. .

AOAC INTERNATIONAL. Official methods of analysis. **Journal of Pharmaceutical Sciences**, v. 1, n. Volume 1, p. 1230, 1990. Disponível em: <http://sutlib2.sut.ac.th/sut_contents/H125800.pdf%5Cnhttp://doi.wiley.com/10.1002/jps.2600650148>. .

BAUMAN, D. E. AND CURRIE, W. B. Partitioning of Nutrients During Pregnancy and Lactation : A Review of Mechanisms Involving Homeostasis and Homeorhesis. **Journal of Dairy Science**, v. 63, n. 9, p. 1514–1529, 1980.

BERTONI, G.; TREVISI, E. 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, 2013.

BLANC, C. D.; VAN DER LIST, M.; ALY, S. S.; ROSSOW, H. A.; SILVA-DEL-RÍO, N. Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium. **Journal of Dairy Science**, v. 97, n. 11, p. 6901–6906, 2014. Elsevier.

BLOCK, E. Manipulating Dietary Anions and Cations for Prepartum Dairy Cows to Reduce Incidence of Milk Fever. **Journal of Dairy Science**, v. 67, n. 12, p. 2939–2948, 1984. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030284816574>. .

BOSSAERT, P.; TREVISI, E.; OPSOMER, G.; et al. The association between indicators of inflammation and liver variables during the transition period in high-yielding dairy cows: An observational study. **Veterinary Journal**, v. 192, n. 2, p. 222–225, 2012. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2011.06.004>. .

BRONNER, F. Intestinal calcium absorption: mechanisms and applications. **The Journal of nutrition**, v. 117, n. 8, p. 1347–1352, 1987.

BURHANS, W. S. **No TitleEffects of Nutritional Management on Peripartum Glucose and Energy Metabolism and Postpartum Health in Transition Dairy Cows**, 2006. Cornell University, Ithaca, NY.

CHAPINAL, N.; CARSON, M.; DUFFIELD, T. F.; et al. The association of serum metabolites with clinical disease during the transition period. **Journal of Dairy Science**, v. 94, n. 10, p. 4897–4903, 2011. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021100511X>. .

CHAPINAL, N.; CARSON, M. E.; LEBLANC, S. J.; et al. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. **Journal of Dairy Science**, v. 95, n. 3, p. 1301–1309, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212000872>. .

COZZI, G.; RAVAROTTO, L.; GOTTARDO, F.; et al. Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. **Journal of Dairy Science**, v. 94, n. 8, p. 3895–3901, 2011. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030211003948>. .

DEGARIS, P. J.; LEAN, I. J. Milk fever in dairy cows: A review of pathophysiology and control principles. **Veterinary Journal**, v. 176, n. 1, p. 58–69, 2008. Elsevier Ltd.

DHIMAN, T. R.; SASIDHARAN, V. Effectiveness of calcium chloride in increasing blood calcium concentrations of periparturient dairy cows. **Journal of Animal Science**, v. 77, n. 6, p. 1597–1605, 1999.

DRACKLEY, J. K.; DANN, H. M.; DOUGLAS, G. N.; et al. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. **Italian Journal of Animal Science**, v. 4, n. 4, p. 323–344, 2005.

GOFF, J.; HORST, R. L. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. , v. 302, n. May 2016, 1993.

GOFF, J. P. Treatment of Calcium, Phosphorus, and Magnesium Balance Disorders. **Veterinary Clinics of North America: Food Animal Practice**, v. 15, n. 3, p. 619–639, 1999. Elsevier Masson SAS. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0749072015301675>. .

GOFF, J. P. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. **Animal Feed Science and Technology**, v. 126, n. 3–4, p. 237–257, 2006.

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2007.12.020>. .

GOFF, J. P.; HORST, R. L.; JARDON, P. W.; BORELLI, C.; WEDAM, J. Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows. **Journal of Dairy Science**, v. 79, n. 10, p. 378–383, 1996. Disponível em: <http://jds.fass.org/cgi/content/abstract/79/3/378%5Cnhttp://www.journalofdairyscience.org/article/S0022-0302(96)76375-0/abstract%5Cnhttp://www.sciencedirect.com/science/article/pii/S0022030296763750>. .

GOFF, J. P.; KEHRLI, M. E.; HORST, R. L. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. **Journal of dairy science**, v. 72, n. 5, p. 1182–7, 1989. Elsevier. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/2745825%5Cnhttp://www.journalofdairyscience.org/article/S0022-0302(89)79222-5/pdf>. .

GOFF, J. P.; LIESEGANG, A.; HORST, R. L. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. **Journal of Dairy Science**, v. 97, n. 3, p. 1520–1528, 2014. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030214000228>. .

GOFF J, HORST R, JARDON P, BORELLI C, W. J. Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows 1. **Journal of Dairy Science**, v. 79, n. 10, p. 378–383, 1996.

GRUMMER, R. R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow Impact of Changes in Organic Nutrient Metabolism Feeding the Transition Dairy Cowl. **Journal of Animal Science**, v. 73, p. 2820–2833, 1995.

GURETZKY, N. A. J.; CARLSON, D. B.; GARRETT, J. E.; DRACKLEY, J. K. Lipid Metabolite Profiles and Milk Production for Holstein and Jersey Cows Fed Rumen-Protected Choline During the Periparturient Period. **Journal of Dairy Science**, v. 89, n. 1, p. 188–200,

2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206720835>. .

HOFFMANN, W. E.; SOLTER, P. F. Diagnostic Enzymology of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 351–378, 2008.

HOLDEN, L. A. Comparison of Methods of In Vitro Dry Matter Digestibility for Ten Feeds. **Journal of Dairy Science**, v. 82, n. 8, p. 1791–1794, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299754093>. .

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A. Adapting to the transition between gestation and lactation: Differences between rat, human and dairy cow. **Journal of Mammary Gland Biology and Neoplasia**, v. 10, n. 2, p. 141–156, 2005.

JAWOR, P. E.; HUZZEY, J. M.; LEBLANC, S. J.; VON KEYSERLINGK, M. A. G. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. **Journal of Dairy Science**, v. 95, n. 3, p. 1240–1248, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212000823>. .

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. Clinical Biochemistry of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 661, 890–894, 2008. Disponível em: <http://medcontent.metapress.com/index/A65RM03P4874243N.pdf%5Cnhttp://books.google.com/books?hl=en&lr=&id=spsD4WQbL0QC&oi=fnd&pg=PP1&dq=Clinical+Biochemistry+of+Domestic+Animals&ots=T_f6aJml8f&sig=sKSGfll8B1Rjre6_UTJHLMZSxbI%5Cnhttp://books.google.at/books>. .

KIMURA, K.; GOFF, J. P.; KEHRLI, M. E.; REINHARDT, T. A. Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. **Journal of Dairy Science**, v. 85, n. 3, p. 544–550, 2002. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030202741076>. .

KIMURA, K.; REINHARDT, T. A.; GOFF, J. P. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. **Journal of Dairy Science**, v. 89, p. 2588–2595, 2006. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(06)72335-9%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/16772578%5Cnhttp://pubag.nal.usda.gov/pubag

/downloadPDF.xhtml?id=2094&content=PDF>. .

LEAN, I. J.; DEGARIS, P. J.; MCNEIL, D. M.; BLOCK, E. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. **Journal of dairy science**, v. 89, n. 2, p. 669–684, 2006.

LEBLANC, S. Monitoring metabolic health of dairy cattle in the transition period. **The Journal of reproduction and development**, v. 56 Suppl, p. S29-35, 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20629214>. .

LEBLANC, S. J.; LESLIE, K. E.; DUFFIELD, T. F. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. **Journal of Dairy Science**, v. 88, n. 1, p. 159–170, 2005. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030205726746>. .

LENO, B. M.; NEVES, R. C.; CURLER, M. D.; et al. Association of immediate postpartum plasma Ca concentration with early- lactation clinical diseases , culling , reproduction , and milk production. **Journal of Dairy Science Vol. 100, Suppl. 2**, v. 100, p. 39, 2017. Disponível em: <https://blogs.cornell.edu/jessmcartlab/files/2017/06/ADSA-Poster_Neves-2017_Final_06.23.2017-129b0t3.pdf>. .

LENO, B. M.; RYAN, C. M.; STOKOL, T.; et al. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217303259>. .

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações. **Associação Brasileira para Pesquisa da Potassa e do Fosfato,**. p.139–169, 1989. Piracicaba, São Paulo, Brasil.

MARTINEZ, N.; RISCO, C. A.; LIMA, F. S.; et al. Evaluation of peripartal calcium status , energetic profile , and neutrophil function in dairy cows at low or high risk of developing uterine disease. , p. 7158–7172, 2012.

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; LOPERA, C.; et al. Effects of oral calcium supplementation on mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8397–8416,

2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304520>. .

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; RISCO, C. A.; et al. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8417–8430, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304519>. .

MEGAHED, A. A.; HIEW, M. W. H.; EL BADAWY, S. A.; CONSTABLE, P. D. Plasma calcium concentrations are decreased at least 9 hours before parturition in multiparous Holstein-Friesian cattle in a herd fed an acidogenic diet during late gestation. **Journal of Dairy Science**, p. 1–14, 2018. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2017-13376>. .

MELENDEZ, P.; DONOVAN, A.; RISCO, C. A.; et al. Metabolic Responses of Transition Holstein Cows Fed Anionic Salts and Supplemented at Calving with Calcium and Energy 1. **Journal of Dairy Science**, v. 85, n. 5, p. 1085–1092, 2002. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(02)74169-6>. .

MELENDEZ, P.; DONOVAN, G. A.; RISCO, C. A.; LITTELL, R.; GOFF, J. P. Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets. **Theriogenology**, v. 60, n. 5, p. 843–854, 2003.

MIYAZAWA, M.; PAVAN, M. A.; MURAOKA, T. ET AL. **Manual de Análises Químicas de Solos, Plantas e Fertilizantes.** Brasília, DF, 1999.

MOORE, S. J.; VANDEHAAR, M. J.; SHARMA, B. K.; et al. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. **Journal of Dairy Science**, v. 83, n. 9, p. 2095–2104, 2000. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(00)75091-0>. .

NEVES, R. C.; LENO, B. M.; STOKOL, T.; OVERTON, T. R.; MCART, J. A. A. Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. **Journal of Dairy Science**, v. 100, n. 5, p. 3796–3804, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021730231X>. .

NRC. NRC. 2001. **Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press , Washington, DC**, p. 1–333, 2001.

OETZEL, G. R. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. **J. Am. Vet. Med. Assoc.**, v. 209, p. 958–961, 1996.

OETZEL, G. R. Monitoring and testing dairy herds for metabolic disease. **Veterinary Clinics of North America - Food Animal Practice**, v. 20, n. 3 SPEC. ISS., p. 651–674, 2004.

OETZEL, G. R. Oral calcium supplementation in peripartum dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 447–455, 2013. Elsevier Inc.

OETZEL, G. R.; MILLER, B. E. Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. **Journal of Dairy Science**, v. 95, n. 12, p. 7051–7065, 2012. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2012-5510>.

OSPINA, P. A; NYDAM, D. V; STOKOL, T.; OVERTON, T. R. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. **Journal of dairy science**, v. 93, n. 8, p. 3595–3601, 2010. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2010-3074>. .

RAJALA-SCHULTZ, P. J.; GRÖHN, Y. T.; MCCULLOCH, C. E.; GUARD, C. L. Effects of Clinical Mastitis on Milk Yield in Dairy Cows. **Journal of Dairy Science**, v. 82, n. 6, p. 1213–1220, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299753440>. .

RAMOS-NIEVES, J. M.; THERING, B. J.; WALDRON, M. R.; JARDON, P. W.; OVERTON, T. R. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. **Journal of dairy science**, v. 92, n. 11, p. 5677–5691, 2009.

REINHARDT, T. A.; HORST, R. L.; GOFF, J. P. Calcium, Phosphorus, and Magnesium Homeostasis in Ruminants. **Veterinary Clinics of North America: Food Animal Practice**, v. 4, n. 2, p. 331–350, 1988. Elsevier Masson SAS. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0749072015310525>. .

REINHARDT, T. A.; LIPPOLIS, J. D.; MCCLUSKEY, B. J.; GOFF, J. P.; HORST, R. L. Prevalence of subclinical hypocalcemia in dairy herds. **Veterinary Journal**, v. 188, n. 1, p. 122–124, 2011. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2010.03.025>. .

REYNOLDS, C. K.; AIKMAN, P. C.; LUPOLI, B.; HUMPHRIES, D. J.; BEEVER, D. E. Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. **Journal of Dairy Science**, v. 86, n. 4, p. 1201–1217, 2003. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030203737047>. .

REZAMAND, P.; HOAGLAND, T. A.; MOYES, K. M.; SILBART, L. K.; ANDREW, S. M. Energy Status, Lipid-Soluble Vitamins, and Acute Phase Proteins in Periparturient Holstein and Jersey Dairy Cows With or Without Subclinical Mastitis. **Journal of Dairy Science**, v. 90, n. 11, p. 5097–5107, 2007. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030207719793>. .

ROSOL, T. J.; CAPEN, C. C. Pathophysiology of calcium, phosphorus, and magnesium metabolism in animals. **Veterinary Clinics of North America - Small Animal Practice**, v. 26, n. 5, p. 1155–1184, 1996. Elsevier. Disponível em: <http://dx.doi.org/10.1016/S0195-5616(96)50060-4>. .

SCHRÖDER, B.; WILKENS, M. R.; RICKEN, G. E.; et al. Calcium transport in bovine rumen epithelium as affected by luminal Ca concentrations and Ca sources. **Physiological Reports**, v. 3, n. 11, 2015. Disponível em: <http://physreports.physiology.org/content/3/11/e12615>. .

SCOTT, D.; WIJK, N. VAN. Comparison in dairy cattle of mucosal toxicity of calcium formate and calcium chloride in oil. **New Zealand Veterinary Journal**, v. 48, n. 1, p. 24–26, 2000. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/00480169.2000.36153>.

SILVA, D.J.; QUEIROZ, A. . **Análises de alimentos (métodos químicos e biológicos)**. 3º ed. Viçosa, MG, Brasil, 2002.

SOEST, P. J. V. S. Sumário. **Nutrition ecology of ruminants. Ithaca. Cornell University Press,** v. 2, p. 1–117, 1994.

STENGÄRDE, L.; TRÅVÉN, M.; EMANUELSON, U.; et al. Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. **Acta Veterinaria Scandinavica**, v. 50, n. 1, p. 1–11, 2008.

STOKOL, T.; NYDAM, D. V. Effect of Hemolysis on Nonesterified Fatty Acid and -Hydroxybutyrate Concentrations in Bovine Blood. **Journal of Veterinary Diagnostic Investigation**, v. 18, n. 5, p. 466–469, 2006. Disponível em: <http://vdi.sagepub.com/lookup/doi/10.1177/104063870601800507>. .

THILSING-HANSEN, T.; JØRGENSEN, R. J.; ØSTERGAARD, S. Milk fever control principles: a review. **Acta veterinaria Scandinavica**, v. 43, n. 1, p. 1–19, 2002. Disponível em: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1764183/>. .

VIEIRA-NETO, A.; LIMA, I. R. P.; JR, F. L.; et al. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2016-12506>. .

WILES, P. G.; GRAY, I. K.; KISSLING, R. C. Routine analysis of protein by Kjeldahl and Dumas methods: review and interlaboratory study dairy products. **. J. AOAC Int.**, v. 81, p. 620–632, 1998.

WILHELM, A. L.; MAQUIVAR, M. G.; BAS, S.; et al. Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. **Journal of Dairy Science**, p. 1–9, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217301224>. .

XU, C.; WANG, Z. Comparative proteomic analysis of livers from ketotic cows. **Veterinary Research Communications**, v. 32, n. 3, p. 263–273, 2008.

5. CONSIDERAÇÕES FINAIS

Ao realizar esse trabalho em um sistema com alta produtividade animal, foi perceptível o quão importante dentro do manejo desses rebanhos é o planejamento e a implementação de medidas preventivas.

São muitos os fatores envolvidos na saúde dos animais principalmente no período próximo ao parto e início da lactação. É um momento crítico na vida da vaca, onde já foi descrito como como a "fronteira final" no estudo de vacas leiteiras. Nesse período, a hipocalcemia tem um papel importante, com uma baixa incidência na forma clínica, porém uma alta prevalência na forma subclínica.

A hipocalcemia subclínica direta e indiretamente promove perdas incalculáveis aos rebanhos leiteiros. É responsável, entre outros fatores, pela queda na ingestão de alimentos e na resposta imune das vacas próximo ao parto, o que predispõe a enfermidades metabólicas e infecciosas.

Há pouca informação sobre a prevalência da hipocalcemia no período de transição em rebanhos brasileiros. Esse fato está associado a dificuldade de realizar os testes de forma rápida ao pé da vaca (falta de equipamentos ou custos elevados por análise), como também pela pouca relevância de seus efeitos sobre a saúde da vaca em boa parte das fazendas. Dessa forma, a hipocalcemia, principalmente na forma subclínica passa desapercebida para a maioria dos técnicos e proprietários. No entanto, foi demonstrado através desse trabalho, a alta prevalência da hipocalcemia subclínica nos rebanhos estudados, principalmente por se tratar de animais com alta produtividade de leite.

Para a determinação da calcemia sérica, a amostra de cálcio total apresentou maior estabilidade no decorrer do tempo de estocagem, proporcionando menor variação nos resultados quando comparado ao cálcio iônico após o tempo de estocagem das amostras. A elevação do pH da amostra promove a fixação do cálcio iônico, alterando os resultados da sua concentração.

Quando a intensão for aferir o cálcio iônico, há necessidade de realização imediata da análise após a obtenção da amostra. Isso pode ser realizado com equipamento portátil, porém o custo da análise torna o processo oneroso, sendo utilizado basicamente em estudos científicos.

Dessa forma, mesmo sem ter levantamentos do grau de desafio de hipocalcemia em seus rebanhos, medidas preventivas já estão sendo implementadas nas fazendas leiteiras, sendo as principais a inclusão de sais acidogênicos e controle da qualidade das forragens (concentração de K) nas dietas no pré-parto. No entanto, os resultados de pesquisa, inclusive

em nossos estudos, demonstraram que com essas medidas há uma redução na incidência de hipocalcemia clínica, porém se mantém uma alta prevalência de hipocalcemia subclínica.

Neste contexto, observamos que as vacas hipocalcêmicas produziram mais leite nos 100 primeiros dias de lactação, no entanto, apresentaram maior NEFA no parto, BHBA e cortisol no dia 5 pós-parto. Esta condição de desafio metabólico levou este grupo de animais a apresentar a maior incidência de doenças no início da lactação, bem como de descartes.

Como medida preventiva no pós-parto, a suplementação de formiato de Ca em duas concentrações, 50 e 100 g de Ca oral foi efetiva no aumento de Ca sérico, ocorrendo o maior efeito 0,5 h após sua administração e com uma duração entre 4 e 8 h. Esses resultados demonstram que o uso de formiato de Ca foi eficaz no fornecimento de Ca prontamente absorvível com um rápido aumento do cálcio sérico em um momento crítico para a vaca leiteira.

No outro estudo, com o uso de duas doses de formiato de Ca oral (50 g de Ca), sendo uma dose no parto e uma segunda dose no dia 1 após o parto, as vacas que receberam Ca oral apresentaram uma menor concentração de BHBA no 5º dia pós-parto, condição que demonstra um efeito positivo sobre o balanço energético no início da lactação.

Outro ponto importante no uso de suplementação de Ca oral está na observação da redução na incidência de deslocamento de abomaso, com 80% dos casos ocorrendo no grupo controle. Esse fato se deve a administração de cálcio oral, com a elevação da concentração do cálcio iônico sérico, o que demonstra que a hipocalcemia é um potencial fator desencadeante dessa patologia.

Todos os resultados desse trabalho com a suplementação de Ca oral foram obtidos no pós-parto, no entanto, há um potencial efeito no seu uso entre 24 e 12 h antes do parto. A dificuldade da administração nesse momento está na acurácia da previsão de parto e, portanto, a sua inclusão nos protocolos experimentais e na rotina das fazendas leiteiras.

Dessa forma, o uso da suplementação oral com o formiato de cálcio é uma forma de fornecer à vaca em um momento muito crítico para o seu metabolismo, uma fonte de cálcio altamente solúvel e de rápida absorção pelo sistema digestivo da vaca. Trata-se de uma indicação estratégica, que pode complementar as demais formas preventivas já em uso, possibilitando um efeito positivo no controle da hipocalcemia.

Pelo maior desafio metabólico, os efeitos positivos e dessa forma com indicação promissora, foram observados para as vacas pluríparas. No entanto, entendemos que há necessidade de maiores estudos para determinação do melhor momento para a administração, o ajuste da concentração de cálcio e frequência do uso do cálcio na forma oral.

REFERÊNCIAS GERAIS

AFSHAR FARNIA, S.; RASOOLI, A.; NOURI, M.; et al. Effect of postparturient oral calcium administration on serum total calcium concentration in Holstein cows fed diets of different dietary cation-anion difference in late gestation. **Research in Veterinary Science**, v. 117, n. November 2017, p. 118–124, 2018. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0034528817307324>. .

AOAC INTERNATIONAL. Official methods of analysis. **Journal of Pharmaceutical Sciences**, v. 1, n. Volume 1, p. 1230, 1990. Disponível em: <http://sutlib2.sut.ac.th/sut_contents/H125800.pdf%5Cnhttp://doi.wiley.com/10.1002/jps.2600650148>. .

BAUMAN, D. E. AND CURRIE, W. B. Partitioning of Nutrients During Pregnancy and Lactation : A Review of Mechanisms Involving Homeostasis and Homeorhesis. **Journal of Dairy Science**, v. 63, n. 9, p. 1514–1529, 1980.

BEEDE, D.K.; SHIRE, J. . DCAD revisited: Prepartum use to optimize health and lactational performance. In: Proc. Southwest Dairy Nutrition and Management Conf. **Anais...** . p.1–11, 2013. AZ, EUA. Disponível em: <https://www.researchgate.net/publication/237144494_DCAD_revisited_Prepartum_use_to_optimize_health_and_lactational_performance>. .

BERTONI, G.; TREVISI, E. 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, 2013.

BLANC, C. D.; VAN DER LIST, M.; ALY, S. S.; ROSSOW, H. A.; SILVA-DEL-RÍO, N. Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium. **Journal of Dairy Science**, v. 97, n. 11, p. 6901–6906, 2014. Elsevier.

BLOCK, E. Manipulating Dietary Anions and Cations for Prepartum Dairy Cows to Reduce Incidence of Milk Fever. **Journal of Dairy Science**, v. 67, n. 12, p. 2939–2948, 1984. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030284816574>. .

BLUNT, J. W.; DELUCA, H. F.; SCHNOES, H. K. 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D3. **Biochemistry**, v. 7, n. 10, p. 3317–3322, 1968.

BOSSAERT, P.; TREVISI, E.; OPSOMER, G.; et al. The association between indicators of inflammation and liver variables during the transition period in high-yielding dairy cows: An observational study. **Veterinary Journal**, v. 192, n. 2, p. 222–225, 2012. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2011.06.004>.

BREVES, G.; SCHRÖDER, B. Comparative aspects of gastrointestinal phosphorus metabolism. **Nutrition research reviews**, v. 4, n. 1991, p. 125–140, 1991.

BRONNER, F. Intestinal calcium absorption: mechanisms and applications. **The Journal of nutrition**, v. 117, n. 8, p. 1347–1352, 1987.

BROWN, J. L.; PH, D.; WALKER, S.; STEINMAN, K.; ROYAL, F. Endocrine Manual for Reproductive Non-Domestic Species. **Smithsonian's National Zoological Park Conservation & Research Center Endocrine Workbook**, p. 69, 2004.

BURHANS, W. S. **No TitleEffects of Nutritional Management on Peripartum Glucose and Energy Metabolism and Postpartum Health in Transition Dairy Cows**, 2006. Cornell University, Ithaca, NY.

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. **Journal of dairy science**, v. 94, n. suppl_5, p. 589–590, 2016. Disponível em: <https://academic.oup.com/jas/article/94/suppl_5/589-590/4767055>. .

CARNEIRO, E. W; HONORATO, S. H.; ICHIKAWA E. E.; ALMEIDA, R. Increased serum calcium in dairy cows with oral calcium formate supplementation in the postpartum period. **Journal of dairy science**, v. 100, n. American Dairy Science Association – ADSA Annual Meeting, June 25-28, 2017 ., p. p.87-88, 2017. Pittsburgh, Pennsylvania, EUA. Disponível em: <http://m.adsa.org/2017/abs/t/70424>. .

CAVALIERI, F.L.B.; SANTOS, G. . Balanço catiônico-aniônico em vacas leiteiras no pré-parto. Disponível em: <http://www.nupel.uem.br/balanco.pdf.>. .

CHAMBERLIN, W. G.; MIDDLETON, J. R.; SPAIN, J. N.; et al. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. **Journal of Dairy Science**, v. 96, n. 11, p. 7001–7013, 2013. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030213006474>. .

CHAPINAL, N.; CARSON, M.; DUFFIELD, T. F.; et al. The association of serum metabolites with clinical disease during the transition period. **Journal of Dairy Science**, v. 94, n. 10, p. 4897–4903, 2011. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021100511X>. .

CHAPINAL, N.; CARSON, M. E.; LEBLANC, S. J.; et al. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. **Journal of Dairy Science**, v. 95, n. 3, p. 1301–1309, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212000872>. .

CHAPINAL, N.; CARSON, M. E.; LEBLANC, S. J.; et al. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. **Journal of Dairy Science**, v. 95, n. 3, p. 1301–1309, 2012. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2011-4724>. .

CHARBONNEAU, E.; PELLERIN, D.; OETZEL, G. R. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. **Journal of Dairy Science**, v. 89, n. 2, p. 537–548, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206721166>. .

COZZI, G.; RAVAROTTO, L.; GOTTARDO, F.; et al. Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. **Journal of Dairy Science**, v. 94, n. 8, p. 3895–3901, 2011. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030211003948>. .

CURTIS, C. R.; ERB, H. N.; SNIFFEN, C. J.; SMITH, R. D.; KRONFELD, D. S. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. **Journal of Dairy Science**, v. 68, n. 9, p. 2347–2360, 1985. Disponível em: <http://jds.fass.org/cgi/content/abstract/68/9/2347>. .

DEGARIS, P. J.; LEAN, I. J. Milk fever in dairy cows: A review of pathophysiology and control principles. **Veterinary Journal**, v. 176, n. 1, p. 58–69, 2008. Elsevier Ltd.

DEGROOT, M. A.; BLOCK, E.; FRENCH, P. D. Effect of prepartum anionic supplementation on periparturient feed intake, health, and milk production. **Journal of Dairy Science**, v. 93, n. 11, p. 5268–5279, 2010. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030210005692>. .

DHIMAN, T. R.; SASIDHARAN, V. Effectiveness of calcium chloride in increasing blood calcium concentrations of periparturient dairy cows. **Journal of Animal Science**, v. 77, n. 6, p. 1597–1605, 1999.

DRACKLEY, J. K. Biology of dairy cows during the transition period: The final frontier? **Journal of Dairy Science**, v. 82, n. 11, p. 2259–2273, 1999. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(99)75474-3>. .

DRACKLEY, J. K.; DANN, H. M.; DOUGLAS, G. N.; et al. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. **Italian Journal of Animal Science**, v. 4, n. 4, p. 323–344, 2005.

DUKES, H. H. **Fisiologia dos Animais Domésticos**. 11 Ed ed. Rio de Janeiro, 1996.

ENDER, F.; DISHINGTON, I.W.; HELGEBOSTAD, A. Parturient paresis and related forms of hypocalcaemic disorders induced experimentally in dairy cows. **Acta Vet. Scand**, v. 3, n. Suppll.1, p. 5–52, 1962.

GERARDO, F. Q. R.; LEBLANC, S. J.; DUFFIELD, T. F.; et al. Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. **Canadian Veterinary Journal**, v. 50, n. 4, p. 383–388, 2009.

GOFF, J. P.; KEHRLI, M. E.; HORST, R. L. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. **Journal of dairy science**, v. 72, n. 5, p. 1182–7, 1989. Elsevier. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/2745825%5Cnhttp://www.journalofdairyscience.org/article/S0022-0302(89)79222-5/pdf>. .

GOFF, J. P.; HORST, R. L. Effect of Subcutaneously Released 24F-1,25-Dihydroxyvitamin D3 on Incidence of Parturient Paresis in Dairy Cows. **Journal of Dairy Science**, v. 73, n. 2, p. 406–412, 1990. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030290786870>. .

GOFF, J.; HORST, R. L. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. , v. 302, n. May 2016, 1993.

GOFF, J. P.; HORST, R. L.; JARDON, P. W.; BORELLI, C.; WEDAM, J. Field Trials of an Oral Calcium Propionate Paste as an Aid to Prevent Milk Fever in Periparturient Dairy Cows. **Journal of Dairy Science**, v. 79, n. 10, p. 378–383, 1996. Disponível em: <http://jds.fass.org/cgi/content/abstract/79/3/378%5Cnhttp://www.journalofdairyscience.org/article/S0022-0302(96)76375-0/abstract%5Cnhttp://www.sciencedirect.com/science/article/pii/S0022030296763750>.

GOFF, J. P.; HORST, R. L. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders 1 , 2. **Journal of Dairy Science**, v. 80, n. 7, p. 1260–1268, 1997. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(97)76055-7>. .

GOFF, J. P. Treatment of Calcium, Phosphorus, and Magnesium Balance Disorders. **Veterinary Clinics of North America: Food Animal Practice**, v. 15, n. 3, p. 619–639, 1999. Elsevier Masson SAS. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0749072015301675>. .

GOFF, JESSE P., JORGE ML SÁNCHEZ, AND R. L. H. Mineral Nutrition Conference. University of Tennessee. Hypocalcemia biological effects and strategies for prevention. **Anais...** . p.5–10, 2005. Disponível em: <ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/TNC2005/Goff.pdf>. .

GOFF, P. J.; SÁNCHEZ, J. M.; HORST, R. L. Hypocalcemia: Biological Effects and Strategies for Prevention. **Nutrition Conference, Departament of Animal Science, University of Tennessee**, p. 5–10, 2005. Disponível em: <ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/TNC2005/Goff.pdf>. .

GOFF, J. P. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. **Animal Feed Science and Technology**, v. 126, n. 3–4, p. 237–257, 2006.

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2007.12.020>. .

GOFF, J. P. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. **Veterinary Journal**, v. 176, n. 1, p. 50–57, 2008b.

GOFF, J. P. Transition period management and nutrition problems - A few solutions. **High Plains Dairy Confernce**, p. 123–138, 2008. Disponível em: <http://www.highplainsdairy.org/2008/21 Goff.pdf>. .

GOFF, J. P.; HORST, R. L. Oral Administration of Calcium Salts for Treatment of Hypocalcemia in Cattle. **Journal of Dairy Science**, v. 76, n. 1, p. 101–108, 1993. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030293773282>. .

GOFF, J. P.; HORST, R. L.; JARDON, P. W.; BORELLI, C.; WEDAM, J. Field trials of an oral calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. **Journal of dairy science**, v. 79, n. 3, p. 378–383, 1996.

GOFF, J. P.; HORST, R. L.; MUELLER, F. J.; et al. Addition of Chloride to a Prepartal Diet High in Cations Increases 1,25-Dihydroxyvitamin D Response to Hypocalcemia Preventing Milk Fever. **Journal of Dairy Science**, v. 74, n. 11, p. 3863–3871, 1991. Elsevier. Disponível

em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030291785792>. .

GOFF, J. P.; LIESEGANG, A.; HORST, R. L. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. **Journal of Dairy Science**, v. 97, n. 3, p. 1520–1528, 2014. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030214000228>. .

GONZÁLEZ, F. H. D.; SILVA, S. C. **Introdução a bioquímica clínica veterinária.** Editora da UFRGS. 2 ed. 355 p. 2006.

GRUMMER, R. R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow Impact of Changes in Organic Nutrient Metabolism Feeding the Transition Dairy Cowl. **Journal of Animal Science**, v. 73, p. 2820–2833, 1995.

GURETZKY, N. A. J.; CARLSON, D. B.; GARRETT, J. E.; DRACKLEY, J. K. Lipid Metabolite Profiles and Milk Production for Holstein and Jersey Cows Fed Rumen-Protected Choline During the Periparturient Period. **Journal of Dairy Science**, v. 89, n. 1, p. 188–200, 2006. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030206720835>. .

HENRY, J. B. **Diagnósticos clínicos e tratamento por métodos laboratoriais**. 1995.

HOFFMANN, W. E.; SOLTER, P. F. Diagnostic Enzymology of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 351–378, 2008.

HOLDEN, L. A. Comparison of Methods of In Vitro Dry Matter Digestibility for Ten Feeds. **Journal of Dairy Science**, v. 82, n. 8, p. 1791–1794, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299754093>. .

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A. Calcium and Vitamin D Metabolism in the Dairy Cow. **Journal of Dairy Science**, v. 77, n. 7, p. 1936–1951, 1994. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203029477140X>. .

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A; BUXTON, D. R. Strategies for preventing milk fever in dairy cattle. **Journal of dairy science**, v. 80, n. 7, p. 1269–1280, 1997. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(97)76056-9>. .

HORST, R.L.; GOFF, J.P.; MCCLUSKEY, B. J. Prevalence of subclinical hypocalcemia in US dairy operations. **Journal of Dairy Science**, v. 86, n. Suppl. 1, p. 247, 2003.

HORST, R. L.; GOFF, J. P.; REINHARDT, T. A. Adapting to the transition between gestation and lactation: Differences between rat, human and dairy cow. **Journal of Mammary Gland Biology and Neoplasia**, v. 10, n. 2, p. 141–156, 2005.

JAWOR, P. E.; HUZZEY, J. M.; LEBLANC, S. J.; VON KEYSERLINGK, M. A G. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. **Journal of dairy science**, v. 95, n. 3, p. 1240–8, 2012. Elsevier. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/22365207>. .

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. Clinical Biochemistry of Domestic Animals. **Clinical Biochemistry of Domestic Animals**, p. 661, 890–894, 2008. Disponível

em:
<http://medcontent.metapress.com/index/A65RM03P4874243N.pdf%5Cnhttp://books.google.com/books?hl=en&lr=&id=spsD4WQbL0QC&oi=fnd&pg=PP1&dq=Clinical+Biochemistry+of+Domestic+Animals&ots=T_f6aJml8f&sig=sKSGfll8B1Rjre6_UTJHLMZSxbI%5Cnhttp://books.google.at/books>. .

KICHURA, T. S.; HORST, R. L.; BEITZ, D. C.; LITTLEDIKE, E. T. Relationships between prepartal dietary calcium and phosphorus, vitamin D metabolism, and parturient paresis in dairy cows. **The Journal of nutrition**, v. 112, n. 3, p. 480–7, 1982.

KIMURA, K.; GOFF, J. P.; KEHRLI, M. E.; REINHARDT, T. A. Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. **Journal of Dairy Science**, v. 85, n. 3, p. 544–550, 2002. Disponível em:
<http://linkinghub.elsevier.com/retrieve/pii/S0022030202741076>. .

KIMURA, K.; REINHARDT, T. A.; GOFF, J. P. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. **Journal of dairy science**, v. 89, n. 7, p. 2588–2595, 2006. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(06)72335-9>. .

LEAN, I. J.; DEGARIS, P. J.; MCNEIL, D. M.; BLOCK, E. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. **Journal of dairy science**, v. 89, n. 2, p. 669–684, 2006.

LEBLANC, S. J.; LESLIE, K. E.; DUFFIELD, T. F. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. **Journal of Dairy Science**, v. 88, n. 1, p. 159–170, 2005. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030205726746>. .

LEBLANC, S. J.; LISSEMORE, K. D.; KELTON, D. F.; DUFFIELD, T. F.; LESLIE, K. E. Major Advances in Disease Prevention in Dairy Cattle. **Journal of Dairy Science**, v. 89, n. 4, p. 1267–1279, 2006. Elsevier. Disponível em:
<http://linkinghub.elsevier.com/retrieve/pii/S0022030206721956>. .

LEBLANC, S. Monitoring metabolic health of dairy cattle in the transition period. **The Journal of reproduction and development**, v. 56 Suppl, p. S29-35, 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20629214>. .

LENO, B. M.; NEVES, R. C.; CURLER, M. D.; et al. Association of immediate postpartum plasma Ca concentration with early- lactation clinical diseases , culling , reproduction , and milk production. **Journal of Dairy Science Vol. 100, Suppl. 2**, v. 100, p. 39, 2017. Disponível em: <https://blogs.cornell.edu/jessmcartlab/files/2017/06/ADSA-Poster_Neves-2017_Final_06.23.2017-129b0t3.pdf>. .

LENO, B. M.; RYAN, C. M.; STOKOL, T.; et al. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em:
<http://linkinghub.elsevier.com/retrieve/pii/S0022030217303259>. .

LITTLEDIKE, E. T.; HORST, R. L. Vitamin D3Toxicity in Dairy Cows,. **Journal of Dairy Science**, v. 65, n. 5, p. 749–759, 1982. Disponível em:
<http://linkinghub.elsevier.com/retrieve/pii/S0022030282822637>. .

LOMBA, F.; CHAUVAUX, G.; TELLER, E.; LENGELE, L.; BIENFET, V. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. **The British journal of nutrition**, v. 39, n. 3, p. 425–9, 1978.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações. **Associação Brasileira para Pesquisa da Potassa e do Fosfato,**. p.139–169, 1989. Piracicaba, São Paulo, Brasil.

MARTINEZ, N.; RISCO, C. A.; LIMA, F. S.; et al. Evaluation of peripartal calcium status , energetic profile , and neutrophil function in dairy cows at low or high risk of developing uterine disease. , p. 7158–7172, 2012.

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; RISCO, C. A.; et al. Effects of oral calcium supplementation on productive and reproductive performance in Holstein cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8417–8430, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304519>. .

MARTINEZ, N.; SINEDINO, L. D. P.; BISINOTTO, R. S.; DAETZ, R.; LOPERA, C.; et al. Effects of oral calcium supplementation on mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. **Journal of Dairy Science**, v. 99, n. 10, p. 8397–8416, 2016. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030216304520>. .

MCART, J. A. A.; OETZEL, G. R. A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. **Journal of Dairy Science**, v. 98, n. 10, p. 7408–7418, 2015. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030215005354>. .

MCDOWELL, L. R. Recent Advances in Minerals and Vitamins on Nutrition of Lactating Cows. **Pakistan Journal of Nutrition**, v. 1, n. 1, p. 8–19, 2002.

MCFADDEN, M. Growing low potassium grass hay for close-up dry cows. , 2008. Michigan: Michigan Dairy Review. Disponível em: <www.msu.edu/~mdr/vol13no2/mcfadden.html>. .

MEGAHED, A. A.; HIEW, M. W. H.; EL BADAWY, S. A.; CONSTABLE, P. D. Plasma calcium concentrations are decreased at least 9 hours before parturition in multiparous Holstein-Friesian cattle in a herd fed an acidogenic diet during late gestation. **Journal of Dairy Science**, p. 1–14, 2018. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2017-13376>. .

MELENDEZ, P.; DONOVAN, A.; RISCO, C. A.; et al. Metabolic Responses of Transition Holstein Cows Fed Anionic Salts and Supplemented at Calving with Calcium and Energy 1. **Journal of Dairy Science**, v. 85, n. 5, p. 1085–1092, 2002. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(02)74169-6>. .

MELENDEZ, P.; DONOVAN, G. A.; RISCO, C. A.; LITTELL, R.; GOFF, J. P. Effect of calcium-energy supplements on calving-related disorders, fertility and milk yield during the transition period in cows fed anionic diets. **Theriogenology**, v. 60, n. 5, p. 843–854, 2003.

MIYAZAWA, M.; PAVAN, M. A.; MURAOKA, T. ET AL. **Manual de Análises Químicas de Solos, Plantas e Fertilizantes.** Brasília, DF, 1999.

MOORBY, J. M.; DEWHURST, R. J.; TWEED, J. K.; DHANOA, M. S.; BECK, N. F. Effects of altering the energy and protein supply to dairy cows during the dry period. 2. Metabolic and hormonal responses. **Journal of dairy science**, v. 83, n. 8, p. 1795–1805, 2000. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(00)75050-8>. .

MOORE, S. J.; VANDEHAAR, M. J.; SHARMA, B. K.; et al. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. **Journal of Dairy Science**, v. 83, n. 9, p. 2095–2104, 2000a. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(00)75091-0>. .

NAGY, O.; KOVÁC, G.; SEIDEL, H.; WEISSOVÁ, T. The effect of arterial blood sampling sites on blood gases and acid-base balance parameters in calves. **Acta. Vet. Hung**, v. 49, p. 331–340, 2001.

NEVES, R. C.; LENO, B. M.; STOKOL, T.; OVERTON, T. R.; MCART, J. A. A. Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. **Journal of Dairy Science**, v. 100, n. 5, p. 3796–3804, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021730231X>. .

NRC. NRC. 2001. **Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press , Washington, DC**, p. 1–333, 2001.

OETZEL, G. R. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. **J. Am. Vet. Med. Assoc.**, v. 209, p. 958–961, 1996.

OETZEL, G. R. Monitoring and testing dairy herds for metabolic disease. **Veterinary Clinics of North America - Food Animal Practice**, v. 20, n. 3 SPEC. ISS., p. 651–674, 2004.

OETZEL, G. R. Non-Infectious Diseases : Milk Fever. **In J. W. Fuquay & P. L. H. McSweeney (Eds.), Encyclopedia of Dairy Sciences**, v. 2, p. 239–245, 2011. Disponível em: <https://www.academia.edu/11909930/Non-Infectious_Diseases_Milk_Fever>. .

OETZEL, G. R.; MILLER, B. E. Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. **Journal of Dairy Science**, v. 95, n. 12, p. 7051–7065, 2012. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2012-5510>.
OETZEL, G. R. Oral calcium supplementation in peripartum dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 447–455, 2013. Elsevier Inc.

OETZEL, G. R. Minimizing hypocalcemia during early lactation. **Proceedings of the Tri-State Dairy Nutrition Conference**, , n. 608, p. 23–34, 2013. Disponível em: <https://www.cabdirect.org/cabdirect/abstract/20133232153>. .

OETZEL, G. R. Oral calcium supplementation in peripartum dairy cows. **Veterinary Clinics of North America - Food Animal Practice**, v. 29, n. 2, p. 447–455, 2013. Elsevier Inc.

OETZEL, G. R. Use of Acidifying Diets for Prevention of Milk Fever in Dairy Cattle. Disponível em: <http://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=9FAD99E5D0D2D760EBB915CEACD7B0C5?doi=10.1.1.538.9658&rep=rep1&type=pdf>. Acesso em: 1/2/2018.

OETZEL, G. R.; OLSON, J. D.; CURTIS, C. R.; FETTMAN, M. J. Ammonium Chloride and Ammonium Sulfate for Prevention of Parturient Paresis in Dairy Cows. **Journal of Dairy Science**, v. 71, n. 12, p. 3302–3309, 1988.

ORTOLANI, E. . Aspectos clínicos, epidemiológicos e terapêuticos da hipocalcemia de vacas leiteiras. **Arquivos Brasileiros de Medicina Veterinária e Zootecnia**, v. 47, p. 799–808, 1995.

OSPINA, P. A; NYDAM, D. V; STOKOL, T.; OVERTON, T. R. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. **Journal of dairy science**, v. 93, n. 2, p. 546–554, 2010. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2009-2277>. .

OSPINA, P. A; NYDAM, D. V; STOKOL, T.; OVERTON, T. R. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. **Journal of dairy science**, v. 93, n. 8, p. 3595–3601, 2010. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.2010-3074>. .

ØSTERGAARD, S.; SØRENSEN, J. T.; HOUE, H. A stochastic model simulating milk fever in a dairy herd. **Preventive Veterinary Medicine**, v. 58, n. 3–4, p. 125–143, 2003.

PEHRSON, B.; SVENSSON, C.; JONSSON, M. A comparative study of the effectiveness of calcium propionate and calcium chloride for the prevention of parturient paresis in dairy cows. **Journal of dairy science**, v. 81, n. 7, p. 2011–6, 1998. Elsevier. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0022030298757753>. .

RADOSTITS, O. M. Clinica veterinária: um tratado de doenças de bovinos, ovinos, suínos, caprinos e equinos. In: G. Koogan (Org.); **Clínica Veterinária**. 9 Ed ed., p.1278–1291, 2002. Rio de Janeiro.

RAJALA-SCHULTZ, P. J.; GRÖHN, Y. T.; MCCULLOCH, C. E.; GUARD, C. L. Effects of Clinical Mastitis on Milk Yield in Dairy Cows. **Journal of Dairy Science**, v. 82, n. 6, p. 1213–1220, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030299753440>. .

RAMOS-NIEVES, J. M.; THERING, B. J.; WALDRON, M. R.; JARDON, P. W.; OVERTON, T. R. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. **Journal of dairy science**, v. 92, n. 11, p. 5677–5691, 2009.

REID, I. M.; DEW, S. M.; COLLINS, R. A.; et al. The relationship between fatty liver and fertility in dairy cows: A farm investigation. **The Journal of Agricultural Science**, v. 101, n. 2, p. 499–502, 1983.

REINHARDT, T. A.; HORST, R. L.; GOFF, J. P. Calcium, Phosphorus, and Magnesium Homeostasis in Ruminants. **Veterinary Clinics of North America: Food Animal Practice**, v. 4, n. 2, p. 331–350, 1988. Elsevier Masson SAS. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0749072015310525>. .

REINHARDT, T. A.; LIPPOLIS, J. D.; MCCLUSKEY, B. J.; GOFF, J. P.; HORST, R. L. Prevalence of subclinical hypocalcemia in dairy herds. **Veterinary Journal**, v. 188, n. 1, p. 122–124, 2011. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.tvjl.2010.03.025>. .

REYNOLDS, C. K.; AIKMAN, P. C.; LUPOLI, B.; HUMPHRIES, D. J.; BEEVER, D. E. Splanchnic Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. **Journal of Dairy Science**, v. 86, n. 4, p. 1201–1217, 2003. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030203737047>. .

REZAMAND, P.; HOAGLAND, T. A.; MOYES, K. M.; SILBART, L. K.; ANDREW, S. M. Energy Status, Lipid-Soluble Vitamins, and Acute Phase Proteins in Periparturient Holstein and Jersey Dairy Cows With or Without Subclinical Mastitis. **Journal of Dairy Science**, v. 90, n. 11, p. 5097–5107, 2007. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030207719793>. .

RIBEIRO, E. S.; LIMA, F. S.; GRECO, L. F.; et al. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. **Journal of Dairy Science**, v. 96, n. 9, p. 5682–5697, 2013. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021300475X>. .

ROBERTS, T.; CHAPINAL, N.; LEBLANC, S. J.; et al. Metabolic parameters in transition cows as indicators for early-lactation culling risk. **Journal of Dairy Science**, v. 95, n. 6, p. 3057–3063, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030212002809>. .

ROCHE, J. R.; DALLEY, D.; MOATE, P.; et al. Dietary Cation-Anion Difference and the Health and Production of Pasture-Fed Dairy Cows 2. Nonlactating Periparturient Cows. **Journal of Dairy Science**, v. 86, n. 3, p. 979–987, 2003. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030203736819>. .

RODRÍGUEZ, E. M.; ARÍS, A.; BACH, A. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. , , n. 2012, p. 1–8, 2017. American Dairy Science Association.

ROSOL, T. J.; CAPEN, C. C. Pathophysiology of calcium, phosphorus, and magnesium metabolism in animals. **Veterinary Clinics of North America - Small Animal Practice**, v. 26, n. 5, p. 1155–1184, 1996. Elsevier. Disponível em: <http://dx.doi.org/10.1016/S0195-5616(96)50060-4>. .

SANTOS, J. E. . Distúrbios Metabólicos. **Nutrição de Ruminantes**. p.439–461, 2006. Jaboticabal:Funep.

SCHRÖDER, B.; WILKENS, M. R.; RICKEN, G. E.; et al. Calcium transport in bovine rumen epithelium as affected by luminal Ca concentrations and Ca sources. **Physiological Reports**, v. 3, n. 11, p. e12615–e12615, 2015. Disponível em: <http://physreports.physiology.org/cgi/doi/10.14814/phy2.12615>. .

SCOTT, D.; WIJK, N. VAN. Comparison in dairy cattle of mucosal toxicity of calcium formate and calcium chloride in oil. **New Zealand Veterinary Journal**, v. 48, n. 1, p. 24–26, 2000. Disponível em: <http://www.tandfonline.com/doi/abs/10.1080/00480169.2000.36153>.

SILVA, D.J.; QUEIROZ, A. . **Análises de alimentos (métodos químicos e biológicos)**. 3º ed. Viçosa, MG, Brasil, 2002. ed. Viçosa, MG, Brasil, 2002.

SOEST, P. J. V. S. Sumário. **Nutrition ecology of ruminants. Ithaca. Cornell University Press,** v. 2, p. 1–117, 1994.

STENGÄRDE, L.; TRÅVÉN, M.; EMANUELSON, U.; et al. Metabolic profiles in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. **Acta Veterinaria Scandinavica**, v. 50, n. 1, p. 1–11, 2008.

STOKOL, T.; NYDAM, D. V. Effect of Hemolysis on Nonesterified Fatty Acid and -Hydroxybutyrate Concentrations in Bovine Blood. **Journal of Veterinary Diagnostic Investigation**, v. 18, n. 5, p. 466–469, 2006. Disponível em: <http://vdi.sagepub.com/lookup/doi/10.1177/104063870601800507>. .

TAVERNE, M. A. M.; BEVERS, M. M.; VAN DER WEYDEN, G. C.; DIELEMAN, S. J.; FONTIJNE, P. Concentration of growth hormone, prolactin and cortisol in fetal and maternal blood and amniotic fluid during late pregnancy and parturition in cows with cannulated fetuses. **Animal Reproduction Science**, v. 17, n. 1–2, p. 51–59, 1988.

TAYLOR, M. S.; KNOWLTON, K. F.; MCGILLIARD, M. L.; SEYMOUR, W. M.; HERBEIN, J. H. Blood Mineral, Hormone, and Osteocalcin Responses of Multiparous Jersey Cows to an Oral Dose of 25-Hydroxyvitamin D3 or Vitamin D3 Before Parturition. **Journal of Dairy Science**, v. 91, n. 6, p. 2408–2416, 2008. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030208711913>. .

THILSING-HANSEN, T.; JØRGENSEN, R. J.; ØSTERGAARD, S. Milk fever control principles: a review. **Acta veterinaria Scandinavica**, v. 43, n. 1, p. 1–19, 2002. Disponível em: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1764183/>. .

ULFABELE, I.; EXAMINER, P.; HENLEY, R. United States Patent [191 Date of Patent : , p. 4–6, 1997. Disponível em: <https://patents.google.com/patent/US5631289A/en>. .

VIEIRA-NETO, A.; LIMA, I. R. P.; JR, F. L.; et al. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. **Journal of Dairy Science**, p. 1–19, 2017. American Dairy Science Association. Disponível em: <http://dx.doi.org/10.3168/jds.2016-12506>. .

WEISS, W. P.; AZEM, E.; STEINBERG, W.; REINHARDT, T. A. Effect of feeding 25-hydroxyvitamin D3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves. **Journal of Dairy Science**, v. 98, n. 8, p. 5588–5600, 2015. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S002203021500377X>. .

WIGGERS, K. D.; NELSON, D. K.; JACOBSON, N. L. Prevention of Parturient Paresis by a Low-Calcium Diet Prepartum : A Field Study. **Journal of Dairy Science**, v. 58, n. 3, p. 430–431. Elsevier. Disponível em: <http://dx.doi.org/10.3168/jds.S0022-0302(75)84584-X>. .

WILES, P. G.; GRAY, I. K.; KISSLING, R. C. Routine analysis of protein by Kjeldahl and Dumas methods: review and interlaboratory study dairy products. **. J. AOAC Int.**, v. 81, p. 620–632, 1998.

WILHELM, A. L.; MAQUIVAR, M. G.; BAS, S.; et al. Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. **Journal of Dairy Science**, p. 1–9, 2017. American Dairy Science Association. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030217301224>. .

WILKENS, M. R.; OBERHEIDE, I.; SCHRÖDER, B.; et al. Influence of the combination of 25-hydroxyvitamin D3 and a diet negative in cation-anion difference on peripartal calcium homeostasis of dairy cows. **Journal of Dairy Science**, v. 95, n. 1, p. 151–164, 2012. Elsevier. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0022030211006862>. .

XU, C.; WANG, Z. Comparative proteomic analysis of livers from ketotic cows. **Veterinary Research Communications**, v. 32, n. 3, p. 263–273, 2008.

YOUDEN, W. J. Index for rating diagnostic tests. **Cancer**, v. 3, n. 1, p. 32–35, 1950.

6. APÊNDICES

6.1 CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA)-2015

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

CERTIFICADO

Certificamos que o protocolo número 038/2015, referente ao projeto "**Efeito do uso de Formiato de Cálcio via oral no pós-parto imediato sobre a hipocalcemia em vacas leiteiras**", 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, em reunião de 17/06/2015

Vigência do projeto	01/07/2015 a 30/08/2015
Espécie/Linhagem	Bovinos da raça Holandesa
Número de animais	320 (trezentos e vinte)
Peso/Idade	Variado / Acima de 23 meses
Sexo	Fêmea
Origem	Propriedades comerciais de Castro, Paraná

CERTIFICATE

We certify that the protocol number 038/2015, regarding the project "**Effect of calcium formate given orally immediately postpartum on hypocalcemia of dairy cows**", under **Rodrigo de Almeira** 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 the State of Paraná, Brazil), in session of 06/17/2015.

Duration of the project	07.01.2015 to 08.30.2015
Specie/Line	Holstein cattle
Number of animals	320 (three hundred and twenty)
Wheight/Age	Miscellaneous / Above 23 months
Sex	female
Origin	Commercial Properties Castro, Paraná

Curitiba, 17 de Junho de 2015.

Ananda Portella Félix
Presidente CEUA-SCA

Simone Tostes de Oliveira Stedile
Vice-Presidente CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

6.2 CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) -2016

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

CERTIFICADO

Certificamos que o protocolo número 054/2016, referente ao projeto "**Aferição da curva do cálcio sérico com o uso de um suplemento mineral contendo formiato de cálcio via oral no pós-parto em vacas leiteiras**", 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 reunião de 10/08/2016.

Vigência do projeto	Agosto/2016 até Setembro/2016
Espécie/Linhagem	*Bos* sp. (bovino) / Holandesa
Número de animais	180
Peso/Idade	600 kg / 60 meses
Sexo	Fêmea
Origem	Fazenda localizada no distrito de Castrolanda em Castro – PR

CERTIFICATE

We certify that the protocol number 054/2016, regarding the project "**Calibration of the serum calcium curve with the use of a mineral supplement containing calcium formate orally in the postpartum period in dairy cows**" 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 the State of Paraná, Brazil), with degree 1 of invasiveness, in session of 10/08/2016.

Duration of the project	August/2016 until September/2017
Specie/Line	*Bos* sp. (bovine) / Holstein
Number of animals	180
Wheight/Age	600 kg / 60 months
Sex	Female
Origin	Farm located in the district of Castrolanda at Castro – PR

Curitiba, 10 de agosto de 2016.

Simone Tostes de Oliveira Stedile
Coordenadora CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR