# UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL FACULDADE DE AGRONOMIA PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

**DELANE RIBAS DA ROSA** 

RELAÇÃO ENTRE HORÁRIO DE ORDENHA, COMPORTAMENTO ALIMENTAR E DESEMPENHO DE VACAS LEITEIRAS

Porto Alegre 2023

## UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL FACULDADE DE AGRONOMIA PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

# RELAÇÃO ENTRE HORÁRIO DE ORDENHA, COMPORTAMENTO ALIMENTAR E DESEMPENHO DE VACAS LEITEIRAS

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Dissertação apresentada como um dos requisitos à obtenção do Grau de Mestre em Zootecnia, na Faculdade de Agronomia na Universidade Federal do Rio Grande do Sul.

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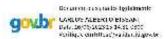
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#### **RESUMO**

# RELAÇÃO ENTRE HORÁRIO DE ORDENHA, COMPORTAMENTO ALIMENTAR E DESEMPENHO DE VACAS LEITEIRAS<sup>1</sup>

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Coorientador: Prof. Dr. Paulo César de Faccio Carvalho

Resumo: Os sistemas de criação de vacas leiteira a pasto possuem muitos benefícios, incluindo econômicos, ambientais e bem-estar animal e social. Porém variações na estrutura das pastagens, nas condições climáticas, estado fisiológico e sanitário do animal podem provocar oscilações na produção e composição do leite, afetando o retorno econômico da produção. Os bovinos seguem um ciclo circadiano, intensificando o pastejo ao amanhecer e entardecer. Neste sistema, as vacas não ficam o tempo todo na pastagem. Geralmente são retiradas do piquete para serem manejadas, principalmente para a ordenha e suplementação. Os horários de ordenha compreendidos entre 5h30 e 7h e 17h e 18h30 se sobrepõem aos momentos de maior pastejo. Pouco se tem descrito na literatura sobre este assunto, sendo os horários de ordenha determinados em função da disponibilidade de mão-de obra, intervalo entre ordenhas e recolhimento do leite. O objetivo deste estudo foi avaliar o efeito de alterar o horário das ordenhas do habitualmente realizado pelas propriedades leiteiras para às 8h e às 16h sobre o comportamento ingestivo e desempenho produtivo de vacas leiteiras mantidas em sistema pastoril. Trinta e seis vacas, de duas fazendas (A e B) foram avaliadas durante o manejo convencionalmente feito na propriedade e após a troca do horário de ordenha. Coletaram-se parâmetros referentes a produção e composição do leite, bem como os tempos em atividades do repertório alimentar. A produção de leite manteve-se inalterada em ambas as propriedades, porém o teor de gordura, sólidos totais e nitrogênio ureico do leite apresentou diferença entre os tratamentos na fazenda B. O tempo de pastejo total e vespertino na fazenda B aumentou após a troca. A mudança no horário de ordenha, sobretudo da tarde, pode beneficiar a atividade de pastejo nos momentos de maior motivação do animal em colher o alimento, mostrando-se uma alternativa de manejo em sistemas pastoris.

**Palavras-chave:** Pastejo, ruminação, ócio, composição do leite, produção de leite, estabilidade do leite, pastagem

<sup>&</sup>lt;sup>1</sup> Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (135p.), março de 2023.

#### **ABSTRACT**

# RELATIONSHIP BETWEEN MILKING TIME, FEEDING BEHAVIOR AND PERFORMANCE OF DAIRY COWS<sup>2</sup>

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Advisor: PhD Vivian Fischer

Co-advisor: PhD Paulo César de Faccio Carvalho

Abstract: Grazing systems for dairy cows have many benefits, including economic, environmental, social animal and welfare benefits. However, variations in pasture structure, weather conditions, physiological and health status of the animal can cause fluctuations in milk yield and composition that may affect the economic return. Cattle's activities follow a circadian cycle, with two intense grazing bouts at dawn and dusk. Generally, cows do not stay in the pasture all the time. They are usually taken out from the pasture to be managed, mainly for milking and supplementation few times a day. The conventional milking times overlap with the main grazing periods. Little has been described in the literature about this subject, and milking times are determined according to the availability of labor, milk collection and transport schedules and milking intervals. The aim of this study was to evaluate the effects of milking at 8:00 a.m. and 4:00 p.m. in contrast to the milking times usually used in dairy farms, on the ingestive behavior, productive performance and feed intake of grazing dairy cows. Thirty-six cows from two farms were evaluated in the conventional management and after changing milking times to 8 am and 4 pm. Milk yield and milk composition parameters were collected, as well as the time spent on feeding repertoire activities. Milk production remained unchanged on both farms, but the fat content, total solids, and urea nitrogen content of the milk increased between treatments on farm B. Total and afternoon grazing time on farm B increased after the change. Change in milking schedule especially anticipating milking at the afternoon can favor grazing activity at times of greater motivation of the animal to collect food, proving to be a management alternative to dairy farms in pasture systems.

**Keywords**: Grazing, rumination, idleness, milk composition, milk yield, milk stability, pasture

<sup>&</sup>lt;sup>2</sup> Master of Science dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (135p.), março de 2023.

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#### Lista de Abreviatura e Símbolos

ADF Acid detergent fiber

BE Before

CMS Consumo de matéria seca

CP Crude protein

DIM Days in milk

DM Dry matter

IA Immediately after

LA Late after

MAPA Ministério da Agricultura, Pecuária e Abastecimento

MUN Milk urea nitrogen

FDN Neutral detergent fiber

NUL Nitrogênio ureico do leite

RH Relative air humidity

SCC Somatic cell count

THI Temperature Humidity Index

TMR Total mixed ration (ração total misturada)

V:C Proporção volumoso:concentrado

CAPÍTULO I

### **INTRODUÇÃO**

Em sistemas de pastagem, as vacas estão mais susceptíveis às variações nas condições climáticas como vento, chuva, temperatura do ar, umidade, dentre outros. Os animais modificam seu comportamento para melhor se adequarem aos desafios do ambiente. Assim, em condições de calor, optam por pastejar nos períodos mais frescos, ou seja, ao amanhecer e entardecer, alocando as outras atividades nos demais horários do dia dentro de um ciclo circadiano. A criação de leiteira em pastagem não permite que as vacas permaneçam no pasto todo o tempo, pois os mesmos são retirados da pastagem durante os manejos de ordenha, suplementação, sanitário, reprodutivo, etc.

Os horários convencionais de ordenha, entre 5h30 e 7h da manhã e entre 17 e 18h da tarde, ocorrem em sobreposição aos momentos de maior intensidade de pastejo. Além disso, frequentemente os animais são suplementados antes ou após a ordenha, com volumosos fermentados ou alimentos concentrados com elevado valor energético e razoável teor de fibras, e palatáveis. Assim, quando a vaca retorna à pastagem após a ordenha, já recebeu parte considerável das suas necessidades nutricionais pelo suplemento, o que pode reduzir a sua motivação em ingerir o pasto.

Ao nosso conhecimento, após pesquisa em bases bibliográficas, soa muito escassas as informações sobre horários de ordenha em sistemas baseados em pastagem de modo a beneficiar a os momentos de maior motivação do animal em pastar. Geralmente, a escolha deste manejo é feita com base na rotina do produtor ou disponibilidade de mão-de-obra, horário de transporte do leite, entre outros. A identificação dos períodos do dia em que os animais preferem pastar e a liberação dos animais de outras atividades pode ser uma importante ferramenta para incrementar a ingestão de pasto. Além disso, foram selecionadas fazendas adotantes do "pastoreio rotatínuo", que se caracteriza por disponibilizar acesso das vacas aos piquetes considerando a estrutura de pasto mais favorável para a sua apreensão e consumo.

Objetivou-se alterar os horários de ordenha para reduzir a sobreposição com os períodos de maior intensidade pastejo, procurando manter os animais nos piquetes nestes momentos de maior motivação de pastejo, o que em hipótese, aumentará a

atividade de pastejo, e possivelmente o consumo de forragem sem afetar a produção e a composição do leite.

#### **REVISÃO BIBLIOGRÁFICA**

#### 1. Sistemas de Alimentação de Vacas Leiteiras

O sistema de produção de um rebanho é capaz de influenciar as condições nutricionais das vacas e modificar o perfil dos constituintes do leite (AULDIST et al., 2000; MACKLE et al., 1999). Em sistemas predominantemente pastoris, as mudanças na qualidade nutricional da pastagem ao longo do ano, as alterações fisiológicas dos animais de acordo com o estágio da lactação e as doenças do pós-parto são citadas por promover a sazonalidade da produção e composição do leite (AULDIST et al., 2000; O'CALLAGHAN et al., 2018).

Auldist et al. (2000) avaliaram a diferença entre os tipos de alimentação durante a lactação oferecidas às vacas da raça Holandesa criadas nos Estados Unidos e na Nova Zelândia com alimentação em total mixed ration (TMR) ou exclusivamente em pastagem. Em condições de pastejo, os animais apresentaram menores produções de leite e teores de ureia e sódio, mas maior concentração de gordura em comparação com vaca confinadas e alimentadas com TMR. Resultados semelhantes foram encontrados por Gulati et al. (2018) e O'Callaghan et al. (2018).

Gulati et al. (2018) investigaram as alterações na composição química do leite de vacas Holandesas submetidas a três tratamentos dietéticos: dois tipos de pastagens, azevém (*Lolium perenne L.*) e azevém associado com trevo branco (*Trifolium repens L.*), e a dieta TMR em confinamento. Maiores teores de sólidos totais, cálcio e fósforo foram encontradas no leite das vacas mantidas em pastagem de somente azevém e maiores teores de lactose foram encontrados no grupo TMR, assim como maior produção de leite (kg/vaca/dia) em comparação com os tratamentos a pasto.

Mudanças na perspectiva de valorização do leite fazem com que teores de sólidos, principalmente gordura e proteína, sejam importantes não apenas para a indústria, mas também para o retorno financeiro para o produtor de leite (RICE et al., 2019). Neste contexto, um estudo de Hanrahan et al. (2018) sobre os fatores associados à lucratividade em propriedades com produção de leite em pastagem, abordou diferentes parâmetros estruturais, produtivos e econômicos de propriedades leiteiras da Irlanda e correlacionou-os em análises estatísticas de regressão multivariada. Dentre os

resultados, os autores identificaram associação entre o uso de pastagem e menor custo de produção da tonelada de gordura e proteína lácteas entregue ao laticínio, o que pode ser uma possibilidade de aumento de lucro da propriedade por aumento da produção de gordura e proteína por vaca.

#### 2 Comportamento ingestivo em pastejo

Define-se como sistema de comportamento um conjunto de atitudes que são executadas em busca de um mesmo propósito, com destaque para a ingestão de água e alimento, que estão intrinsecamente relacionados com a nutrição e, consequentemente, com a produção (CURTIS, 1983). O comportamento animal é dependente de fatores bióticos (animais e sua fisiologia) e abióticos (luminosidade solar, temperatura do ar, umidade relativa do ar, radiação solar, pluviosidade, dentre outros) que, de acordo com o meio, estimulam a execução de ações imediatas. Estes, utilizando de sua liberdade de movimento para interagir e se adaptar com o meio (KILLEN et al., 2013).

Em sistemas de produção de ruminantes em pastejo, o consumo de forragem, essencial para determinar o desempenho produtivo, é controlado pelo comportamento ingestivo dos animais (SAMPAIO et al., 2016). O registro do comportamento e mensuração do consumo individual em pastejo são fundamentais para otimizar o manejo e identificar animais mais eficientes na conversão de alimento em produto animal (carne ou leite). Porém, essa mensuração é complexa, e é necessário que haja o entendimento dos fatores que influenciam no pastejo, assim como a interação na relação planta-animal-ambiente (CANGIANO et al., 2002). Por essa razão, estudos dessa natureza são de grande importância para o entendimento da resposta animal no ambiente de pastejo, como em pastejo rotacionado (ARAÚJO et al., 2017).

O repertório de comportamento alimentar do bovino é caracterizado por três principais atividades: pastejo, ruminação e descanso (ócio), as quais compõem 90% a 95% do tempo diário do animal. Os outros 5% a 10% são preenchidos com comportamentos sociais, caminhando, bebendo água (WALKER et al., 2008; KILGOUR, 2012), seguindo um ciclo circadiano.

O comportamento ingestivo de bovinos em pastejo é sensível ao ciclo natural claro-escuro, e os animais reagem distribuindo-os e padronizando-os nesses horários do dia (CURTIS, 1983), apresentando maiores períodos de pastejo nas horas próximas ao amanhecer e ao entardecer, sendo no restante do dia distribuído prioritariamente em descanso e ruminação. Em dias curtos, os eventos de pastejo se ficam mais próximos em decorrência da menor duração da fase diurna (GREGORINI, 2012).

O pastejo é definido como o processo em que o animal faz o uso dos seus sentidos, mandíbula e movimentos corporais para aprender, cortar a forragem do ambiente, com o auxílio da mandíbula para mastigá-lo e formar o bolo alimentar e, por fim, degluti-lo (EDWARDS et al., 1996). Em pastagens, os bovinos apresentam um longo e variável período de pastejo, podendo variar de 4 a 13 horas em 24 horas (KILGOUR, 2012; GUIMARÃES et al., 2020).

Na coletânea de Kilgour (2012) o autor observou que os bovinos possuem picos momentâneos de pastejo ao longo do dia variando na faixa das 5h às 8h e das 17h às 19h. Por outro lado, Pollock *et al.* (2022), constataram, em seu estudo com vacas em lactação durante o inverno da Irlanda, picos do pastejo matutino mais tardio, entre 7h e 9h. Ambos os estudos supracitados se enquadram nas faixas preferenciais de pastejo já descritas por Van Soest (1994) 5h e 9h e outra entre 17h e 22h.

O pastejo ao crepúsculo é o mais longo e intenso (GIBB, 1998). Segundo observações de Pires et al. (2001), durante o verão, bovinos reduzem os tempos de pastejo diurno em duas horas, acrescentando-as ao período noturno, devido ao maior frescor nos horários da noite. Durante a noite, ocorrem períodos de pastejo mais curtos, correspondente a 5% do tempo total de pastejo (KILGOUR, 2012). Phillips & Hecheimi (1989) relataram maiores taxas de bocados durante o entardecer em comparação com o pastejo ao amanhecer.

No estado de Goiás-GO, Zanine et al. (2006) avaliaram 30 vacas no terço inicial da lactação mestiças da raça Holandesa e Zebu mantidas em pastejo contínuo de *Brachiaria decumbens*. Esses autores observaram menor atividade de pastejo e intensificação da ruminação próximo às 13 horas. Com o entardecer, observaram aumento da atividade de pastejo após às 16 horas, o qual foi se reduzindo

gradativamente após às 19 horas. Os animais ficaram em ócio do final da madrugada ao amanhecer e intensificaram o pastejo ao raiar do sol. Assim, os autores sugerem que o manejo de ordenha um pouco antes do amanhecer seria o adequado para não atrapalhar os horários de maior pastejo.

Esta constatação foi testada por Mercês et al. (2012), no qual investigaram os efeitos do horário de ordenha tardio sobre o tempo de pastejo de vacas mestiças da raça Holandesa e Zebuína (grau de sangue entre ½ e ¾ H vs Z), na fase intermediária de lactação e produção de leite média de 3,7 kg/vaca/dia. Os autores observaram que a adoção do horário alternativo de ordenha (8h), em detrimento ao horário convencional (5h30min), beneficiou o consumo de pasto matutino em função de menores temperaturas, o que reduz o estresse térmico e aumenta o bem-estar durante o pastejo. Ou seja, o horário alternativo favorece os horários de maior ingestão de pasto antes da ordenha.

A ruminação é o ato de regurgitar, mastigar e insalivar o bolo alimentar realizada por ruminantes entre as refeições a partir de estímulos de parede celular de alimentos volumoso (VAN SOEST, 1994). Esta atividade apresenta variações de 1,4 a 6,9 horas de ruminação diurna, sendo 4,7 e 10,7 horas em observações de 24h com a maior parte realizado na posição deitado (KILGOUR, 2012) e durante a noite (POLLOCK et al., 2022). O tempo de ruminação é relacionado com a efetividade de fibra em detergente neutro (FDN) e é amplamente utilizado por influenciar em mais de 21 parâmetros ruminais, dentre eles pH e ácidos graxos de cadeia curta, ser sensível ao tamanho de partícula e mediar o consumo de matéria seca (CMS) (YANG & BEAUCHEMIN, 2009).

O tempo de ruminação é alterado pelas características físicas do alimento, como tamanho de partícula, químicas, como o teor e tipo de carboidrato (estruturais ou não-estruturais), consumo de matéria seca, proporção de FDN total da dieta. A mastigação durante a ruminação permite a redução do tamanho de partícula e a manutenção do pH ruminal (MAULFAIR et al., 2011). Alterações nesse tempo de ruminação provocam um desbalanço no fluxo de saliva afetando a fisiologia ruminal (SILVEIRA et al., 2021). Ajustes inadequados de tamanho de partícula aliado a baixa relação volumosos e concentrado (V:C) podem levar os animais à acidose ruminal. Assim, prefere-se dietas

com maior V:C para promover maior tempo de mastigação, mudança nos horários de refeição e diminuição da produção de ácidos de cadeia curta (YANG & BEAUCHEMIN, 2009).

As variações das características físico-químicas das forrageiras ao longo do dia, o fotoperíodo (PHILLIPS; SCHOFIELD, 1989) e o enchimento ruminal (DETMANN et al., 2014) influenciam na frequência, distribuição e no repertório comportamental durante o pastejo (GIBB, 1998; GREGORINI et al., 2006). Segundo Guimarães et al. (2020), o sistema de produção e, principalmente, as características físicas e bromatológicas dos ingredientes de uma dieta influenciam diretamente o comportamento ingestivo dos bovinos. Dentre essas características está a digestibilidade dos alimentos que influencia no padrão de ingestão e determina a composição nutricional total e o perfil dos nutrientes que serão absorvidos para suprir as exigências nutricionais do animal, os quais tendem a ser selecionados no momento do consumo (SILVA et al., 2010).

Considerando níveis de FDN e PB equivalentes, a digestibilidade do FDN das forragens será determinante para a ingestão de matéria seca, devido ao efeito físico dos volumosos sobre o enchimento do rúmen. Desta maneira, volumosos com maior digestibilidade proporcionam maior ingestão de matéria seca (ALLEN, 2000). Este fato fica evidente no estudo meta-analítico de Detmann et al. (2014), que avaliaram 10 experimentos brasileiros de bovinos em pastejo. Os autores observaram que a utilização de gramíneas tropicais gramíneas diminui o consumo de matéria seca (CMS) devido ao maior teor de FDN. De modo geral, admite-se que teores elevados de fibra limitam o CMS devido ao enchimento do rúmen-retículo. Dietas pobres em fibra tendem a reduzir o CMS, mas os níveis de energia podem compensados pela menor ingestão dessa fração menos fibrosa da dieta (MERTENS, 1997).

Dado e Allen (1995) apontam que, com o incremento da ingestão de volumoso, ocorre o aumento dos níveis de FDN da dieta, resultando em maior ocupação do espaço ruminal, o que leva ao aumento do tempo de mastigação, tornando mais eficiente o consumo de matéria seca ou FDN consumido, alterando também a taxa de passagem do rúmen devido à redução das partículas. Assim, os autores evidenciaram a importante correlação positiva entre a produção de leite e consumo de matéria seca, ocorrendo o

inverso para o tempo total de mastigação e ruminação por unidade de consumo, ou seja, maiores períodos de ruminação e mastigação levam à menor quantidade de leite produzida.

Kammes e Allen (2012) encontraram resultados similares na redução de CMS com o aumento de tamanho de partícula de silagem de *Dactylis glomerata*, porém o estudo não identificou uma relação entre maior tamanho de partícula e produção de leite e dos seus constituintes. Oliveira et al. (2017) concluíram que a ingestão de matéria seca pode ser influenciada positivamente e negativamente por fatores psicogênicos, físicos e químicos do alimento ou do ambiente, além de aspectos relativos a condições do animal, que irão modelar o repertório comportamental durante a alimentação. Assim, a alimentação é uma ação comportamental baseada na digestibilidade e a cinética da digestão, que aliado à taxa de passagem, determinam o comportamento ingestivo (NRC, 2001).

O momento da suplementação pode alterar o pastejo (SHEAHAN et al., 2013), podendo diminuir o consumo de matéria seca (CMS) de pastagem por meio do efeito de substituição (BARGO et al., 2003).

Ribeiro Filho et al. (2009) testaram duas ofertas de forragem de azevém: baixa (25 kg MS/vaca/dia) e alta (40 kg MS/vaca.dia) e constataram que a maior oferta de foragem aumentou a produção leiteira em 0,2Kg de leite/vaca/dia a cada Kg de matéria seca ou 0,8 Kg de leite/Kg matéria orgânica de pasto ingerido. Miguel et al. (2019), testaram dois níveis de suplementação de concentrado 0 e 4 kgMS/dia/dia em vacas em lactação e seus efeitos nas mesmas faixas de oferta de massa de forragem, 25 e 40 kg MS/dia. A suplementação de 4 Kg MS/dia/vaca teve efeito positivo sobre o consumo total de MS e a produção de leite quando a oferta de massa de forragem era menor, 25 Kg MS/dia, ou seja, a baixa oferta de forragem com uma suplementação de concentrado a base de milho e soja pode contribuir com o consumo total de matéria seca (MS) e aumentar a produção de leite.

Não apenas a produção de leite, mas sua composição pode ser alterada de acordo com a quantidade de massa de forragem oferecida e os momentos de acesso a ela. Kismul et al. (2018), buscaram compreender os efeitos na produção de leite,

comportamento e frequência de visitas ao robô de vacas de alta produção com acesso matutino e vespertino áreas de pastagem para apenas exercício (EX) e outra com 15 Kg MS/dia de massa de forragem (PROD) renovadas diariamente. Esses autores observaram que a produção de leite e os teores de gordura ficaram inalterados, mas os teores de proteína aumentaram, assim como maiores frequências de ordenha no tratamento EX. Em relação ao comportamento, observaram que o grupo PROD dedicou mais tempo ao ar livre, à atividade de pastejo e repouso do que o grupo EX. Por outro lado, na pesquisa de Zanine et al. (2019) não foi identificada alteração na composição do leite de vacas da raça Holandesa submetidas a 38,4, 30,3 e 26,8 kg MS/vaca/dia de oferta de forragem de pastagem mista durante o outono, porém a disponibilidade de forragem aumentou o tempo de pastejo e a produção de leite.

A avaliação das características quantitativas e qualitativas do(s) alimento(s) oferecido(s) em uma dieta e o entendimento dos fatores que motivam o comportamento ingestivo e o consumo voluntário são ferramentas para aprimorar manejos em prol do desempenho animal, atuando principalmente sobre a atividade de alimentação, ruminação e ócio (ALBRIGHT, 1993; CLARK et al., 2018). Variáveis como massa do bocado, taxa de bocado, tempo de pastejo e grau de seletividade são apontadas como determinantes do desempenho do animal em pastejo. A demanda nutricional de vacas de leite de alta produção é um desafio em sistemas pastoris devido à limitação energética das pastagens (WILKINSON & LEE, 2017). Desta maneira, os animais tendem a aumentar o tempo (500 a 700 min/dia) e a taxa de bocado (até 65 bocados/min) nos momentos de pastejo devido ao enchimento lendo do rúmen e à saciedade demorada (KNAUS, 2016).

Segundo o conceito de "Pastoreio Rotatinuo" a estrutura do pasto para a entrada dos animais na pastagem deve possibilitar a maximização da velocidade de ingestão de forragem. Quando a altura de entrada é adequada, essa velocidade se mantém com a redução de até 40% da altura de forragem inicial. A partir desta estrutura, inicia-se uma redução de ingestão devido à limitação na estrutura do pasto nas camadas mais baixas e aumento da exploração no pastejo, danificando estruturas essenciais para a recuperação da área para novo ciclo de pastejo (CARVALHO et al., 2016). Assim, este

manejo permite maior produção da pastagem e eficiência de colheita pelo animal (SCHONS et al., 2021).

#### 3 Intervalos e frequência de ordenha

Schmidt (1960) avaliou os efeitos dos intervalos entre ordenha de 4, 8, 12, 16 e 20 horas aplicados três vezes consecutivas em 15 vacas em lactação. Intervalos de 16 a 20 horas entre ordenhas nas condições experimentais diminuiu de forma quadrática a taxa de secreção do leite pela glândula mamária. Rémond et al. (2009) estudaram a influência de diferentes intervalos de ordenha sobre a produção de leite de vacas da raça Holandesa e Montbeliarde após o pico de lactação e nas fases ascendentes e descendentes na curva lactacional, com produção de 26,9 a 28,1 Kg/vaca/dia. Efeitos negativos expressivos sobre a produção de leite foram encontrados nos intervalos de 3-21h (-11%) e com uma ordenha ao dia (-28%). Os mesmos autores demonstraram também que intervalos entre ordenhas longos de 17h e 19h podem ser realizados sem perdas expressivas de rendimento leiteiro, desde que a próxima ordenha ocorra entre 7h e 5 h, respectivamente.

Com o objetivo de investigar o efeito do aumento repentino do intervalo de ordenha sobre a glândula mamária, Lakic et al. (2011) estudaram 27 vacas Swedish Red (SRB) saudáveis que foram ordenhadas duas vezes ao dia (manhã e tarde), exceto no dia 0 do experimento, o qual totalizou 24h de intervalo entre ordenhas. Os autores observaram aumento da produção de leite na primeira ordenha (manhã) e maiores contagens de células somáticas (CCS) na segunda ordenha (à tarde) após o intervalo prolongado, assim como mudanças no perfil de células somáticas e concentração de leucócitos polimorfonucleares. Esses resultados são justificados pelo aumento da permeabilidade dos capilares junto às células epiteliais mamárias decorrente do intervalo prolongado evidenciado por maiores concentrações de lactose no sangue e albumina sérica bovina no leite. Charton et al. (2016) demonstram que o evento único do aumento do intervalo entre ordenha de 12-14h para 24h resultou na diminuição de 0,75 kg/dia ao longo do estudo sem alterações significativas na composição do leite.

Os reflexos da variação no tempo entre as ordenhas sobre a produção e composição do leite dependem do estágio de lactação, podendo apresentar valores positivos com redução dos intervalos e aumento da frequência de ordenha no início da lactação (PHYN et al., 2014; PENRY et al., 2018).

Capelesso et al. (2019) submeteram 20 primíparas da raça Holandesa recém paridas a uma ou duas ordenhas por dia durante as oito primeiras semanas de lactação. Ao final do experimento, os animais ordenhados uma vez ao dia apresentaram menor produção de leite e teores de lactose, bem como maiores teores de gordura e proteína em uma ordenha, comparado ao tratamento com duas ordenhas diárias, sendo assim, os autores justificam como menor mobilização das reservas corporais em comparação com as primíparas do outro tratamento.

Mais recentemente, Hanling et al., (2021) estudaram os efeitos da frequência de vacas ordenhadas duas ou quatro vezes ao dia em intervalos regulares (6:6:6:6) e irregulares (9:3:9:3) no início de lactação com vacas multíparas e primíparas. Demostraram um aumento na produção diária de leite, gordura e proteína em multíparas com quatro ordenhas diárias em comparação a duas vezes ao dia. Os autores concluíram que o intervalo entre as ordenhas não influencia na produção e composição, mas sim a frequência de ordenha alterando a composição do leite.

#### HIPÓTESE E OBJETIVOS

#### 1.1. Hipótese

A realização de ordenhas em horários não coincidentes com os momentos de maior intensidade de pastejo favorecem a atividade de pastejo, reduzem o consumo de suplementos sem alteração expressiva da produção e composição do leite.

#### 1.2. Objetivo Principal:

Avaliar os efeitos da mudança dos horários convencionais da ordenha da manhã e da tarde sobre o comportamento ingestivo, consumo de suplementos e desempenho produtivo de vacas em lactação.

#### 1.3. Objetivos específicos:

- 1. Identificar se os horários alternativos de ordenha da manhã e da tarde favorecem o comportamento de pastejo;
- 2. Avaliar os efeitos da mudança dos horários das ordenhas da manhã e tarde sobre o consumo de suplementos de vacas em lactação;
- 4. Avaliar os efeitos da mudança dos horários das ordenhas da manhã e tarde sobre a produção de leite (kg/vaca/dia), teores de gordura, proteína e lactose no leite e características funcionais (acidez titulável e estabilidade do leite).

## **CAPÍTULO II**

## CHANGES IN MILKING TIME MODIFY BEHAVIOR OF GRAZING DAIRY COWS

This chapter is presented according to the publication standards of **LIVESTOCK SCIENCE** 

#### CHANGES IN MILKING TIME MODIFY BEHAVIOR OF GRAZING DAIRY COWS

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#### **Abstract**

Milking often coincides with the main grazing periods of dairy cows, at dawn and evening, and might impair grazing behavior and pasture consumption. This study aimed to evaluate the effects of changing the morning and evening milking time on the ingestive behavior and performance of lactating dairy cows. From March and April 2022, at the end of summer at the south hemisphere, 36 healthy multiparous Holstein and Jersey cows from two commercial farms (A and B) were evaluated during conventional milking time (between 6h and 7am and at 5pm for seven days. The same group of cows gradually switched to alternative milking time during 4 days, and after animals were kept under the alternative milking time (8 am

and 4 pm) for 28 days. Data on milk production, fat, protein, lactose, total solids, alcohol stability of milk, acidity and supplement intake were collected during the conventional milking time (days 1 to 7 of the trial) and at the last 14 days after the adoption of the alternative milking time (days 21 to 35). Also data of behavior was also collected on two days before and after milking time changes. Behavior activities consisted of diurnal ingestive behavior (time spent grazing, ruminating, idling and eating the supplement), position (standing up or lying down) and time in shade or in the outdoor part registered from 6:30 am to 6:30 pm. Behavior data were summarized for 12 hours of the diurnal period and two times intervals (Morning or MO: 6:30 am to 12:30 pm, Afternoon or AF: 12:31 pm to 6:30 pm) as well total diurnal time (MO+AF). Data were submitted to analysis of variance, and means between before (BE) and after change (AC) were compared using the mixed model at 5% probability level. Change in milking time for farm B increased fat, total solids and MUN contents (p<0.05), as well increased diurnal time spent grazing in B. Ruminating and idling time behave differently according to changes in milking time between farms. Cows spent more time standing up (P<0.001) on farm A and more time lying down (P<0.001) and outdoor (0.001) on farm B after change in milking time. In farm B, changes in milking time favored late afternoon grazing and reduced time spent feeding supplement without changes in milk yield, while increased milk solids contents of milk.

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**Keywords:** Grazing, rumination, idleness, fat content, solids content, milk yield, grazing systems

#### Introduction

Milking is a labor-intensive task performed usually at least twice a day, accounting for a expressive part of the time used on a farm (Culotta & Schmidt, 1988), representing 43 to 58% of a conventional 40-h work week (Edwards et al., 2020). Milking time are settled according to farm needs such time of milk collection, availability of labour and use of time in other farm's activities (Culotta & Schmidt, 1988). Milking times are also related to the beginning and end of the working day, which may extend the working days and/or allocate milking to undesirable or unusual times. Usual milking intervals are 10 (diurnal) and 14 hours, but shorter intervals as 8 allow to fit milking activities into conventional working time, turning more attractive for farm employees (Edwards et al., 2020).

On the other hand, cattle follow a circadian cycle of ingestive behavior spending more time grazing at dawn and dusk (Kilgour, 2012; Guimarães *et al.*, 2020; Pollock *et al.*, 2020). The hours of highest grazing activity occur two hours after dawn and in the last hours of the day and the first hours of the night (GREGORINI, 2012), due to the preference in performing these activities at times of mild air temperature (Legrand et al., 2009). Between these times, the animals usually seek natural or artificial shade (Schütz et al., 2014; Van Laer et al., 2015). However, usually these main grazing periods coincide with the conventional milking time, which can impair gazing and, consequently, pasture consumption.

Currently few papers demonstrate the achievements of different milking times in grazing systems (MERCÊS et al., 2012). However, no study has examined the

effects of milking time on main grazing periods or pasture intake of supplemented dairy cows, milked twice a day. Therefore, the hypothesis of this study was that changing milking times to not coincide with main grazing periods favor grazing and possibly pasture intake, and may alter supplement consumption, without negative effects on milk yield and composition. The present study aimed to evaluate the effects of changing milking times on ingestive behavior, supplement intake and milk yield and composition of lactating grazing cows.

#### **Material and Methods**

The experiment took place in two farms named A and B at Fagundes Varela-RS (Latitude: -28.8548, Longitude: -51.693, 28° 51′ 17″ South, 51° 41′ 35″ West), between the months of March and April 2022. The climate of the region is classified as humid subtropical Cfa (Köppen & Geiger, 1928).

All procedures were conducted according to welfare standards and approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul (n° 41758).

#### Management

Farms A and B are smallholder dairy farms with areas 10 and 22.5 ha respectively for dairy production. The production system is the rotatinuous grazing (SCHONS *et al.*, 2021), using Tifton-85 grass (*Cynodon spp.*) as the main pasture species, and corn and oat silage, concentrate and mineral salt as supplements.

The trial lasted 39 days. In the first seven days of the experiment on each farm, the animals were observed following the conventional management adopted on each farm. Between day 8 and 11 of the experiment, the groups of cows had their milking times changed by 20 minutes per day until they reached the alternative milking times at 8 am and 4 pm. Between days 12 and 39, cows were milked at the alternative milking times (Figures 1 and 2). Throughout the experiment, the animals received the same diet, free access to water and mineral salt, same sanitary management, facilities and contact with people. Thus, the treatments correspond to the milking times: before or BE (conventional time, days 1st to 7th of the experiment), and late after or LA (days 26th to 39th of the experiment) (Figure 1 and 2). The particularities of each farm in routine, feed management and milking, as well as structural details are described below.

#### Farm A

The infrastructure consisted of main family house, milking parlor in a herringbone design, Intermaq® pipeline milking machine with three clusters, which was connected to the waiting room and the feeding area, with headlock and troughs. The trough line was double with a length of 20 meters on each side, providing a spacing of 80 cm per cow. This whole area had a concrete floor, covered, without walls, with good natural ventilation, and no cooling system. Next door was a shed for the storage of concentrated feed and other ingredients, in which they mixed the feed according to the technician's instructions (Table 2).

The pasture area was managed by rotating tifton-85 (*Cynodon spp*) on the summer with oats (*Avena sativa sp*) crop during the winter. Each paddock averaged 2750 m<sup>2</sup> (Table 3). All the paddocks had drinking troughs, but no shade was available.

The conventional milking time adopted by the farm (before the study) was at 6:30 am and 5:00 pm. The usual milking routine consisted of *pre-dipping*, drying with individual paper towels, putting the clusters on and *pos-dipping*. Routine milking activities were maintained during the whole experiment.

At 06:00 am all cows were taken off the paddock and conducted to the milking parlour. After milking, cows were supplemented with approximately 15kg/cow/day of corn and oat silage and 4kg/cow/day of 14%CP concentrate (Table 2) after each milking. Then they were conducted to a new paddock, where they remained until 4:30 pm, when they were taken off the paddock and conducted to the supplementary barn, where they received silage + concentrate for 30 minutes, being milked at 5:00 pm (Figure 1). After milking, all cows were allocated to a new paddock until the next morning milking.

The lactation lot consisted of 20 primiparous and multiparous Holstein and Jersey x Holstein (Jersolanda), with body weight 598.6±87.50 Kg and 262.05± 119.28 days in milks, producing 26.91±2.57 kg of milk per day.

#### Farm B

The infrastructure consisted of family house, a calf shed, feeding shed with a waiting room with fan, milking parlour, with Sulinox® brand pipeline milking machine

with four clusters. The feeding area had a double trough line equipped with headlocks, totaling 48 meters length.

The pasture area is managed by alternating tifton-85 (*Cynodon spp*) at summer and annual ryegrass (*Lolium multiflorum*) pastures at winter without irrigation. Each paddock area averaged 6,850 m<sup>2</sup> (Table 3), with restricted shaded areas. Water troughs are located in the corridors between the paddocks and cows had free access.

The cows were milked (conventional management employed by the farm) at 7:00 am and 5:00 pm. The milking routine consisted of washing and drying the teats with individual paper towels, putting on the milking clusters and *pos-dipping*. After the milking, the cows were taken to the feeding area, where they received 6 kg/cow/day of commercial concentrate with 22% CP (Table 2). Following supplementation, the cows were driven to a new paddock, with exclusive access to pasture until 11 am. After this time, the cows had free access to the pasture and to the trough with 10 kg/cow of corn silage in the feeding shed. Natural shade was available at the corridor between paddocks and feeding. After afternoon milking, the cows received more 10 kg/cow of corn silage and then had free access to the pasture, shade, or feed shed (Figure 2).

There were 26 lactating primiparous and multiparous cows, Holstein and Jersey breeds, with body weight of 647.40± 276.04 kg, 201.00± 132.10 days in lactation and milk production of 25.11±5.88 Kg per day.

### Ingestive behavior

In each farm 18 primiparous and multiparous cows with more than 30 days in lactation and that would not calve during the experimental period were selected for behavior observation. The selected cows were not separated from their lot during the experiment but were identified.

The diurnal ingestive behavior of each animal was recorded weekly, totaling 5 days of observation. On each day, the behavior was evaluated for 12 hours (from 6:30 am to 6:30 pm). The animals were observed individually in a focal and intermittent manner at 10-minutes intervals (Thurow *et al.*, 2009). Activities were recorded as grazing, rumination, idling, supplement consumption and other activities, as well as posture (time spent standing or lying down), and the place at the time of observation (outdoors or without cover or in the shade) (Table 1). Time spent (in minutes) for each activity, the position, and the location were calculated multiplying the number of times activity. Further, behavior data were grouped per period of time: morning (6:30am to 12:30pm) and evening (12:31pm to 6:30pm). The milking time was measured on the same days of observation of ingestive behavior. It was considered as the time (in minutes) elapsed between the begging of milking of the first cow and the end of milking of the last cow of the lot.

#### Milk production and composition

Two milkings per day were performed on each farm. No routine milking activities were changed during the experiment. The milk production was recorded

once a week, at the morning and evening milking. The individual milk was collected and weighed by means of a meter in the milking machine at farm B. On farm A it was not possible to collect milk per cow, so some cows were sampled and values are shown only to characterize the farm and the herd. Two samples of 40 mL of milk were collected from each cow, in the morning milking at the farm A and the evening at the farm B, according to the availability of each property and logistics for sample collection and analysis. These samples were refrigerated at 4°C. One sample was used for acidity determination with Dornic solution (Vidal & Saran Netto, 2018) and milk stability to the alcohol test, using a solution of ethyl alcohol P.A., with ethanol concentration in the test solution ranging from 72% (Brasil, 2006, 2018) to 80%, with gradations increased 2% (v/v) (Zanela & Ribeiro, 2018). The second milk sample was sent to the Univates Milk Laboratory in the city of Lageado-RS for the determination of somatic cell count (SCC) by ISO 13366-2 method, fat, protein, lactose, total solids and urea nitrogen (MUN) contents by ISO 9622|IDF141:2013 method.

#### Feed consumption

It was not possible to measure the consumption of supplements individually for the selected cows, but the amounts of feed offered and the leftovers were weighed, and their difference was used to estimate the consumption of the lot. The mean values presented are expressed numerically as kg dry matter per animal per day (Table 3).

#### Feed composition

Concentrate composition was given by the manufacturer and samples of corn and oat silage, and pasture (Table 3) were collected on days 1st and 39th of the experiment on each farm and analyzed for dry matter by method 934.01, crude protein (CP) by method 954.01 and acid detergent fiber (ADF) by method 973.18, all according to Association of Official Analytical Chemists (AOAC, 2000). Neutral detergent fiber (NDF) was obtained by method of Van Soest (1967). The samples were analyzed by the Animal Nutrition Laboratory of the Federal University of Rio Grande do Sul, in the city of Porto Alegre, Rio Grande do Sul, Brazil.

#### Body weight evaluation

Body weight was recorded in the day 1st and 39th of experiment using cow weight measuring tape (Heinrichs et al., 1992).

#### Meteorological data

Bioclimatic data of air temperature (maximum, minimum and minimum), relative humidity (RH) and rainfall were collected from the Meteorological Station of the Diagnostic and Research in Fruit culture center (CEFRUTI) located in the city of Veranópolis – RS (Table 4) and Temperature Humidity Index (THI) was calculated

by the formula (Thom, 1959): THI= 46,4+(0,8 x TAVG)+(RH x (TAVG-1,4,4)/100, where: TAVG= Average temperature (°C), RH= Relative Humidity (%).

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## Statistical Analysis

Data of milk production, composition, alcohol stability, titratable acidity of milk, body weight and diurnal ingestive behavior data were averaged per cow, day, treatment (i.e. before and after milking time change). Further ingestive behavior was also averaged per time of day (morning and afternoon shifts). For farm A, we did not analyze milk yield and composition due to missing data. Individual cows (n = 18) were used as experimental units on each farm separately, according to a switch design. For Farm A, with 1 measurement day per period (before and after milking changes), the statistical model for diurnal ingestive behavior included treatment (milking time), cow and error as the random effects and day as repeated measures. The model used was  $Y_{ijk} = \mu + MT_i + E_{ijk}$ , where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the mean value of the dependent variable, MT is the is the fixed effect of the treatment (milking time, n=2, before and after the change in milking time, Eijk is the experimental error, considering as random effect animal and error and days as repeated measurement. For farm B, with 2 days of measurements per period, the statistical model for milk yield and composition, diurnal ingestive behavior included treatment (milking time), day of measurements, interaction between treatment and day as main effects, cow

and error as the random effects and day as repeated measures. The model used

was  $Y_{ijk} = \mu + MT_i + DT_j + MTDT_{ij} + E_{ijk}$ , where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the mean value of the dependent variable, MT is the is the fixed effect of the treatment (milking time, n=2, before and after the change in milking time), DT is measurement day (n = 2, per period), MTDT<sub>ij</sub> is interaction between measurement day and milking time,  $E_{ijk}$  is the experimental error, considering as random effect animal and error and days as repeated measurement.

For both farms, the ingestive behavior variables expressed as time per morning ou afternoon were submitted to statistical analysis considering milking time (n = 2, before and after milking time change), period of evaluation (n = 2, morning from 06:30 to 12:30 and afternoon from 12:30 to 18:30) and their interaction, as fixed effects, and animal and error as random effects, days of measurement as repeated measurement. The model used was  $Y_{ijk} = \mu + MT_i + DT_j + MTDT_{ij} + E_{ijk}$ , where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the mean value of the dependent variable, MT is the is the fixed effect of the treatment (milking time, n=2, before and after the change in milking time), DT is time of the day (n = 2, morning and afternoon), MTDT<sub>ij</sub> is interaction between time of the day and milking time,  $E_{ijk}$  is the experimental error, considering as random effect animal and error and days as repeated measurement.

All analysis were performed using the SAS® MIXED procedure, version 9.4. A structural selection test was performed using the Bayesian information criterion (BIC). Means were compared using the LSmeans option. A pairwise comparison of milk production, milk composition and feeding activity times was made between treatments (BE and AC) only on the farm B. For the production, composition, stability

and acidity of the milk from farm A, only a descriptive analysis was conducted, due to the small amount of data.

The correlation coefficients between variables were calculated using the CORR procedure. The power analysis of the sample size was calculated using the POWER procedure. The significant differences were declared when P < 0.05 and a trend considered to exist if 0.05 < P < 0.10.

Body weight values were compared by T-test when normal and homogeneous, and by Wilcoxon's test when non-parametric. A 5% significance level was considered for all tests.

## Results

Milk production, composition and stability

On farm A, due to missing data no statistical analysis was performed, and averages are shown at table 5 to characterize the herd. Milk production was moderate, 25 to 28 kg/day. Milk solids were considerably low as well SCC and ethanol stability, while acidity varied within the normal range.

On farm B, change of milking time did not affect milk production, concentrations of lactose and protein, as well acidity, ethanol stability (Table 6), while it increased the concentrations of fat, MUN and total solids in milk (P<0.0001).

## Feed consumption

After changing milk time, for farm A there was no change in the supplement consumption of the lot, while for farm B we noticed numerical reduction in the supplement consumption of the lot was observed after changing milking time.

## Body weight

Changing milking times did not change body weight of the cows on both farms, A (P=0.92) and B (P=0.95).

## Ingestive behavior

### Farm A

Change milking time did not affect diurnal time spent grazing, ruminating and idling (Table 7). Inspection of the time spent per hour (Figure 4) shows that there was delay in grazing bout from 9:30am in BE to 11:30am in AC after the first milking of the day as well an anticipation of the grazing bout after the second milking of the day in AC.

After milking time change supplementation time decreased, while time spent standing and in shade increased (P<0.0001) (Table 7).

For farm A, after changing milking time, grazing and ruminating times were reduced (P<0.001) between 06:30 am and 12:30 pm, while idling time increased (Table 8). In the morning and afternoon, time spent in supplementation, standing and in shade increased after milking time change (P<0.0001).

#### Farm B

Diurnal time spent grazing increased (P<0.0001) after changing milking time (Table 9). After milking time change, grazing bouts were noticed in the before morning milking and mostly after evening milking (Figure 5). Diurnal rumination and idling times were different between BE and AC according to the day of measurement (P<0.0001), i.e. after milking time change, rumination time decreased at day 1 but increased at day 2, with idling time showing the inverse pattern. The total supplementation time decreased after milking time change (P<0.001). Diurnal lying time was shorter in BE compared to AC on day 2, while opposite occurred in the total time spent standing (P<0.01) (Table 9). After milking time change, the diurnal time out of animal cover was greater (P<0.0001), with the opposite occurring with time in shade (Table 9).

Between 06:30 am and 12:30 pm, grazing time was similar in AC and BE, while between 12:31 pm and 6:30 pm, grazing time was greater (P<0.0001) in AC compared to BE. Between 06:30 am and 12:30 pm, rumination time was similar in AC compared to BE and was smaller (P<0.0001) in the second period of the day in AC compared to BE. From 06:30 am to 12:30 pm the idling time was similar in AC and BE, while between 12:31 pm and 6:30 pm the idling time was reduced after the milking time change (P<0.0001). In both periods of the day the supplementation time was smaller (P<0.0001) in AC. Animals reduced (P<0.0001) standing time in AC compared to BE at both times of the day and the opposite occurred for the lying time

(P<0.0001). After milking change, at morning and afternoon the time outdoors increased, while time spent in shade decreased (P<0.0001) (Table 10).

#### **Discussion**

To our knowledge this is the first study evaluating the effect of changing the milking time of grazing cows in order to avoid coinciding milking with the main grazing periods. Our hypothesis that changing milking times would increase grazing time and reduce supplement consumption, without deleterious changes on milk production and composition was accepted, at least for Farm B.

### Production variables and milk composition

The change in milking time from conventional (between 6 to 7 am and after 5 pm) to 8 am and 4 pm predictably shortened the diurnal time interval between milkings and increased the nocturnal time interval. It did not change the milk production in both farms, probably because these diurnal (8 hours) and nocturnal milking intervals (16 hours) did not expressively affect the intramammary pressure and the synthesis capacity, and thus, the health of the mammary gland (Lakic *et al.*, 2011). According to Rémond *et al.* (2009), intervals between milkings of 19-17 hours followed by intervals of 5-7 hours are feasible in order to maintain production without

loss of mammary gland health, the interval 17:7h being similar to the interval 11:13h, what is similar to our milking intervals

Different forage mass offers, as long as they provide the nutrient requirements, may not change the physicochemical characteristics of milk, as reported by Zanine et al. (2019), who offered 38.4 kg DM/cow/day, 30.3 kg DM/cow/day and 26.8 kg DM/cow/day of pasture in the fall period. Miguel et al. (2019) evaluated two supplementation levels (0 and 4 kg DM of 14% BW feed) at tow pasture offers: low 4.9 kg DM/cow/day and high 8.5 kg DM/cow/day, similar offers to the farms in this research (Table 3). These authors observed that supplementation at high forage supply had no effect on milk yield. However, at low supply, the supplementation with 4 kg DM feed/cow/day increased pasture intake and milk production.

The differences in fat, total solids and urea nitrogen concentration observed on farm B with the change in milking time may be explained by the fact that the reduced intake of supplement and the increased grazing time (used in this case as an indicator of increased pasture intake) increased fiber intake, explaining the increased fat and total solids content. The higher urea nitrogen values after the time change might be related to the higher concentration of crude protein observed in the composition of the pasture at the end of the study. Nevertheless, MUN value was higher than maximal threshold recommended by Onaciu et al. (2019), between 10 to 15 mg/dL of MUN.

The characteristics of the feed may influence the composition of the milk (Ametaj et al., 2010; gulati et al., 2018) as well as the volume of milk produced (Dineen et al., 2021). The allocation of animals in the paddock with pasture in different stages can influence behavior and milk production (Pollock et al., 2022). Clark et al. (2018) reported higher fat contents as the amount of concentrate was reduced in cows grazing during the morning and afternoon. Higher pasture consumption increased the total solids and urea content of milk (Torre-Santos et al., 2020). However, we recognize that in the present study pasture intake was not measured, and we made some inferences about pasture intake based grazing time. The similarity of the ethanol stability may be related to same nutrient intake (Gabbi et al., 2018) and mild weather conditions, without causing heat stress (Abreu et al., 2020). Also the similarity in acidity values may be explained by the similar values of crude protein and SCC of milk (ZANELA & RIBEIRO, 2018).

#### Behavioral variables

The time of access to the paddock is important to combine the highest consumption with the best pasture quality (KISMUL *et al.*, 2018). Thus, the variation in the grazing routine on farm B, before and after milking time change affected the ingestive behavior, increasing grazing time especially during the afternoon, what probably augmented the consumption of pasture, promoting fat and total solids contents (Leiber et al., 2022). Moreover, supplementation influences grazing behavior (Ribeiro Filho et al., 2009). The combination of increased grazing time with

reduced supplementation time and supplement consumption after the milking time change confirmed our hypothesis, that access to pasture during main grazing bouts induced by changing milking time increase grazing time and thus, pasture intake, without impair milk yield and its physical and chemical characteristics.

Furthermore, in grazing systems, cows are more susceptible to variations in weather conditions such as wind, rain, air temperature, humidity, radiation compared with confined systems. The animals modify their behavior to better adapt to the challenges of the environment and, thus, in hot conditions they choose to graze during the coolest periods, i.e., at dawn and dusk (Pires et al., 2001), allocating the other activities to the other times of the day (Gregorini, 2012).

The meteorological data registered in this study show that the animals were not subjected to challenging conditions in terms of heat stress, as can be seen by the minimum and maximum temperatures as well by THI values below 68 (Silanikove, 2000), despite the significant variation detected in the climatic variables. Martello et al. (2013) consider 4 and 24°C as ideal for lactating cows, but because of solar radiation and relative humidity, this confort temperature range can vary between 7 and 21°C. THI values below 70 have been proposed as adequate for high yielding dairy cows (Polsky & Von Keyserlingk, 2017).

On both farms, the total grazing times were shorter than those reported by Guimarães *et al.* (2020), average of 520.13 min/day (8.66 h/day) for feeding, probably because of fact cows were observed only during the diurnal phase, for 12

hours. Nevertheless, the review by Kilgour (2012) presents a variation in grazing time between 6.8 to 13 hours in 24 hours of observation.

There was no change in total grazing time on farm A between BE and AC on farm A. From this perspective, the results found on the first farm reject the hypothesis of this paper that the proposed alternative management could increase grazing and pasture consumption. On the other hand, results on farm B support our hypothesis.

The animal's decision to graze is related to several factors that include the chemical characteristics of the pasture and its physiological state, and thus, reflects the evaluation of contribution of the pasture to attain nutrient requirements of the animals (GREGORINI, 2012). According to Carvalho et al. (2016), grazing time and the chemical attributes of the forage are directly related, and grazing time reflects physicochemical limitation of the available forage.

The proposed alternative management aimed to allow more time for the animal to ingest pasture at the main grazing periods. However, when analyzing activity by time of day, after milking time change, on farm A the grazing activity decreased from 06:30 am to 12:00 pm, while on the farm B the grazing time was similar during the morning. If we take into account the time of the main grazing periods, from 5:00 to 9:00 am, changing morning milking from 6:30 or 7:00 am to 8:00 am (Kilgour, 2012; Pollock et al., 2022) still allowed partial overlapping of grazing period with milking, that might explain the reason grazing time did not increase in farm B. Similarly, Mercês et al. (2012) did not reported difference for the time spent grazing when changing the morning milking time from 5:30 am to 8:00

am. Also, it might be worth to consider that in the south of Brazil, at the end of summer when the trial was run, sunrise was delayed and consequently morning main grazing occurred later (7 am to 9 am) as reported by Pollock et al. (2022) compared with other studies (Kilgour, 2012; Guimarães et al., 2020). Therefore the 8:00 am milking time did not favor grazing at the early hours of the day, restricting access to the pasture at times when greater grazing intensity would occur, similar to the results of Pollock et al. (2020), when milking between 5:30 am and 7:30 am in summer in Ireland.

The second milking of the day, at 4:00 pm was more efficient to favor grazing, maybe because the main grazing period occurs after 5:00 pm (KILGOUR, 2012), so it did not coincide with afternoon milking. The decision to fix the morning miking at 8:00 am was made to keep the milking interval of 8 and 16 hours in order to not impair milk synthesis in the mammary gland (Lakic et al., 2011; Rémond et al., 2009).

Differences between farms for the time spent in behavioral activities were probably related to the management such as supplement delivery times and frequencies and shade allowance. On farm A, as described in methods and materials, the animals were free to roam in the enclosure, and fed exclusively on pasture. Farm B provided the animals with a mid-day supplementation of corn silage at the trough, where they had access to shade. Corn silage supplementation for grazing dairy cows may lead to substitution when supply is low, but does not change milk yield and composition (Miguel et al., 2022).

In the second half of the diurnal period, the alternative milking time favored grazing activity on farm B. These results corroborate the hypothesis raised in this study. This phenomenon is related to natural seeking behavior for higher nutrient intake before the night period, and its consequent slow release through rumen fermentation. Thus, the intake rate at the end of the day tends to be higher compared to dawn (Gregorini, 2012), mainly after 4:00 pm with gradual reduction after 7:00 pm (Zanine et al., 2006).

The similarity of rumination times independent of milking time may be explained by the similarity of the FDN values of the pasture and the supplement between periods (Yang & Beauchemin, 2009; Guimarães et al., 2020). The total diurnal rumination time was lower than the values reported by Stone *et al.* (2017), 6.4 hours/day of activity, probably related to the observation period (diurnal) as rumination occurs more frequently during the night (Souza et al., 2007; Mercês et al., 2012; Clark et al., 2018). The increase in rumination time on farm B after the change in milking time may be related to the increase in fat and solids and the reduction in the amount of supplement intake, similar to results of Miguel et al. (2022).

The variations in idling time, defined as the absence of chewing activity are probably derived from the combination of the variations in grazing and rumination times, as these three behaviors are mutually exclusive.

The variation in diurnal time spent standing up after the change in milking time, increasing on Farm A and reducing on Farm B, may be associated with

differences in the variations of grazing and shade times observed and with meteorological differences (lower temperature values at Farm B compared to Farm A) and management (i.e. supplement delivery). On farm B, cows could choose where to stay (barn or pasture) from 11 am until 4 pm.

In this study in both farms and periods the total time the animals were lying down during observations was below the values described in the literature, on average 10.9 h/day (Thompson et al., 2019), mainly because our observation was restricted to the diurnal period. Also, cows raised in grazing systems show shorter lying time than confined cows (Tucker et al, 2021; Kismul et al., 2018). Lying time is considered an indicator of comfort and welfare and can easily be changed in unfavorable conditions (Fregonesi & Leaver, 2001). Changes in the environment such as air temperature, incidence of solar radiation or other stressors and changes in management can influence the timing of this behavior (Tucker et al., 2021).

The increased time outdoors and less time in the shade during the day after the change in milking time on both farms may have occurred due to the variation in grazing time (Farm A) and lower values of THI for both farms. The change of milking time favored grazing in the cooler moments of the day, seeking shade in the hotter moments to mitigate energy expenditure to maintain homeostasis (Mercês et al., 2012). When animals have access to good forage availability they stay more time outdoors (Kismul et al., 2018). The need for shade is related to weather conditions, mainly temperature and solar radiation. The solar radiation was not measured, but the temperature did not reach extreme levels (Schütz et al., 2009). Another factor

that may have interfered was the access to the shed with the supplementation. On farm A the type of shade available was in or around the feeding shed, and access was allowed only before and after milking. On the paddock there was no shade. On the other hand, on farm B, the animals had the choice of staying on the pasture, under the trees or in the shade of the shed. Lack of shade or restrict shade area affect time spent grazing, ruminating as well lying and standing (Vizzotto et al., 2015; Stivanin et al., 2019; Reis et al., 2021).

#### Conclusion

Changing milking times from 6:30-7:00 am to 8:00 am and from 5:00 pm to 4:00 pm affects dairy cow behavior, without adversely effects on milk production and most milk characteristics.

The alternative milking times increased the afternoon grazing time, and the time spent outdoor. Other behaviors such as rumination and idling occurred to a small extent and varied with little consistently between farms as well as standing and lying times.

On farm B, changing milking time reduced time spent feeding supplement and numerically supplement intake, while increased fat, total solids, MUN with out changing acidity and ethanol stability. Finally, besides milking management, the routine and infrastructure offered to the cows may also influence the ingestive behavior and effectiveness of pasture milk production systems.

Repeat trials of this experiment in other seasons or other milking times may elucidate the effects of these factors on ingestive behavior, production and management in pasture dairy cattle.

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#### **Ethical statement**

This study was approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul, protocol number 41758.

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## References

- Abreu AS, Fischer V, Stumpf MT, McManus CM, González FHD, da Silva JBS,
- Heisler G. Natural tree shade increases milk stability of lactating dairy cows during
- the summer in the subtropics. Journal of Dairy Research, v.87, n.4, p.444-447.
- https://doi.org/10.1017/S0022029920000916. Epub 2020 Oct 30. PMID: 33121555.
- Ametaj BN, Zebeli Q, Saleem F, Psychogios N, Lewis MJ, Dunn SM, Xia J, Wishart
- DS. Metabolomics reveals unhealthy alterations in rumen metabolism with increased
- proportion of cereal grain in the diet of dairy cows. Metabolomics, v. 6, n. 4, p. 583–
- 545 594, 2010.https://doi.org/10.1007/s11306-010-0227-6.
- AOAC. Official Methods of Analysis. 17. ed. Washington, DC: AOAC, 2000.
- 547 BRASIL. Instrução Normativa no 68, de 12 dezembro de 2006. 2006.
- http://www.cidasc.sc.gov.br/inspecao/files/2020/09/IN-MAPA-no-68-de-12-de-
- 549 dezembro-2006.pdf.
- 550 BRASIL. Instrução Normativa no 76, de 26 de novembro de 2018. 2018.
- 551 https://www.in.gov.br/materia/-
- /asset publisher/Kujrw0TZC2Mb/content/id/52750141/do1-2018-11-30-instrucao-
- 553 normativa-n-77-de-26-de-novembro-de-2018-52749887.
- 554 Carvalho PCF, Bremm C, Bonnet OJF, Savian JV, Schons RMT, Szymczak LS,
- Baggio T, Moojen FG, Silva DFF, Marin A, Gandara L, Bolzan AMS, Neto GFS,
- Moraes A, Monteiro ALG, Santos DT, Laca EA. Como a estrutura do pasto influencia
- o animal em pastejo? Exemplificando as interações planta-animal sob as bases e

- fundamentos do Pastoreio "Rotatínuo". VIII Simpósio sobre manejo estratégico da
- pastagem, October, p. 21, 2016.
- Clark CEF, Kaur R, Millapan LO, Golder HM, Thomson PC, Horadagoda A, Islam
- MR, Kerrisk KL, Garcia SC. The effect of temperate or tropical pasture grazing state
- and grain-based concentrate allocation on dairy cattle production and behavior.
- 563 Journal of Dairy Science, v. 101, n. 6, p. 5454–5465, 2018.
- 564 https://doi.org/10.3168/jds.2017-13388.
- 565 Culotta CP, Schmidt GH. An Economic Evaluation of Three Times Daily Milking of
- 566 Dairy Cows. Journal of Dairy Science, v. 71, n. 7, p. 1960-1966, 1988.
- 567 https://doi.org/10.3168/jds.S0022-0302(88)79767-2.
- Demski JB, Junior IA, Gimenes FMA, Toledo LM, Miranda MS, Giacomini AA, Silva
- 569 GA. Milk production and ingestive behavior of COWS grazing on marandu and
- 570 mulato in pastures under rotational stocking. Revista Brasileira de Zootecnia, v. 48,
- 2019. https://doi.org/10.1590/RBZ4820180231.
- Dineen M, McCarthy B, Dillon P, Coughlan F, Galvin N, Van Amburgh ME. The effect
- of concentrate supplement type on milk production, nutrient intake, and total-tract
- nutrient digestion in mid-lactation, spring-calving dairy cows grazing perennial
- ryegrass (Lolium perenne L.) pasture. Journal of Dairy Science, v. 104, n. 11, p.
- 576 11593–11608, 2021. https://doi.org/10.3168/jds.2021-20148.
- Edwards JP, Kuhn-Sherlock B, Dela Rue BT, Eastwood CR. Short communication:

- Technologies and milking practices that reduce hours of work and increase flexibility
- through milking efficiency in pasture-based dairy farm systems. Journal of Dairy
- 580 Science, Journal of Animal Feed Science, v. 103, n. 8, p. 7172-7179, 2020.
- 581 https://doi.org/10.3168/jds.2019-17941.
- Different levels of supplied energy for lactating cows affect physicochemical
- attributes of milk, v.28, n.1, 2018. https://doi.org/10.22358/jafs/83703/2018.
- Gibb MJ. Animal Grazing/Intake Terminology and Definitions. Pasture Ecology and
- 585 Animal Intake, v. 3, n. 3, p. 20–35, 1998.
- 586 Gregorini P. Diurnal grazing pattern: Its physiological basis and strategic
- management. Animal Production Science, v. 52, n. 7, p. 416-430, 2012.
- 588 https://doi.org/10.1071/AN11250.
- Guimarães YLF, Debortoli EC, Santos J, Gopinger E. Comportamento ingestivo de
- 590 bovinos em diferentes sistemas de produção uma revisão sistemática de estudos
- científicos. Research, Society and Development, v. 9, n. 10, p. 1–14, 2020.
- 592 https://doi.org/http://dx.doi.org/10.33448/rsd-v9i10.8705.
- 593 Gulati A, Galvin N, Lewis E, Hennessy D, McManus JJ, Fenelon MA, Guinee TP.
- Outdoor grazing of dairy cows on pasture versus indoor feeding on total mixed ration:
- 595 Effects on gross composition and mineral content of milk during lactation. Journal of
- 596 Dairy Science, v. 101, p. 2710–2723, 2018. https://doi.org/10.3168/jds.2017-13338.
- Heinrichs AJ, Rogers GW, Cooper JB. Predicting Body Weight and Wither Height in

- Holstein Heifers Using Body Measurements. Journal of Dairy Science, v. 75, n. 12,
- p. 3576–3581, 1992. https://doi.org/10.3168/JDS.S0022-0302(92)78134-X.
- 600 Kilgour RJ. In pursuit of "normal": A review of the behaviour of cattle at pasture.
- 601 Applied Animal Behaviour Science, v. 138, n1-2, p. 1-11, 2012.
- 602 https://doi.org/10.1016/j.applanim.2011.12.002.
- Kismul H, Spörndly E, Höglind M, Næss G, Eriksson T. Morning and evening pasture
- access comparing the effect of production pasture and exercise pasture on milk
- production and cow behaviour in an automatic milking system. Livestock Science, v.
- 217, n. September, p. 44–54, 2018. https://doi.org/10.1016/j.livsci.2018.09.013.
- Köppen W, Geiger R. Klimate der Erde. Gotha: Verlag Justus Perthes. 1928.
- Lakic B, Svennersten Sjaunja K, Norell L, Dernfalk J, Östensson K. The effect of a
- single prolonged milking interval on inflammatory parameters, milk composition and
- yield in dairy cows. Veterinary Immunology and Immunopathology, v. 140, n. 1–2, p.
- 110–118, 2011. https://doi.org/10.1016/j.vetimm.2010.11.022.
- 612 Legrand AL, Von Keyserlingk MAG, Weary DM. Preference and usage of pasture
- versus free-stall housing by lactating dairy cattle. Journal of Dairy Science, v. 92, n.
- 8, p. 3651–3658, 2009. https://doi.org/10.3168/jds.2008-1733.
- 615 Leiber F, Moser FN, Ammer S, Probst JK, Baki C, Spengler AN, Bieber A.
- Relationships between Dairy Cows' Chewing Behavior with Forage Quality,
- Progress of Lactation and Efficiency Estimates under Zero-Concentrate Feeding

- 618 Systems. Agriculture, v. 12, n. 10, p. 1570, 2022.
- 619 https://doi.org/10.3390/agriculture12101570.
- 620 Martello LS, Junior HS, Silva SL, Titto EAL. Respostas fisiológicas e produtivas de
- vacas holandesas em lactação submetidas a diferentes ambientes. Revista
- Brasileira de Saúde e Produção Animal, v. 14, n. 3, p. 406-414, 2013.
- https://doi.org/10.1590/S1519-99402013000300016.
- 624 Mercês LM, Marques JA, Barbosa LP, Brandão TO, Garcia MP, Costa AKA. Horário
- alternativo de ordenha e o comportamento ingestivo de vacas mestiças leiteiras em
- sistema de produção a pasto. Acta Scientiarum Animal Sciences, v. 34, n. 2, p.
- 627 197–202, 2012. https://doi.org/10.4025/actascianimsci.v34i2.12476
- Mezzalira JC, Carvalho PCF, Fonseca L, Bremm C, Reffatti MV, Poli CHEC,
- Trindade JK. Aspectos metodológicos do comportamento ingestivo de bovinos em
- pastejo. Revista Brasileira de Zootecnia, v. 40, n. 5, p. 1114-1120, 2011.
- 631 https://doi.org/10.1590/S1516-35982011000500024.
- 632 Miguel MF, Delagarde R., Ribeiro-Filho HMN. Corn silage supplementation for dairy
- cows grazing annual ryegrass at two pasture allowances. Arquivo Brasileiro de
- 634 Medicina Veterinaria e Zootecnia, v. 71, n. 3, p. 1037–1046, 2019.
- 635 https://doi.org/10.1590/1678-4162-9795.
- 636 Miguel MF, Ribeiro-Filho HMN, Delagarde R. Effects of corn silage supplementation
- strategy and grazing intensity on herbage intake, milk production, and behavior of

- dairy cows. Journal of Dairy Science, 2022. https://doi.org/10.3168/jds.2021-21649.
- Onaciu G, Jurco E, Jurco S, Maciuc V, Ognean L. Influence of varying ranges milk
- urea nitrogen on chemical, hygienic and physical quality traits of cow milk. Romanian
- 641 Biotechnological Letters, v. 24, n. 5, p. 866–873, 2019.
- 642 https://doi.org/10.25083/rbl/24.5/866.873.
- Pires MFA, Verneque RS, Vilela D. Ambiente e comportamento animal na produção
- do leite. Informe Agropecuário, v. 22, n. 211, p. 11–21, 2001.
- Pollock JG, Gordon AW, Huson KM, McConnell DA. The effect of frequency of fresh
- pasture allocation on pasture utilisation and the performance of high yielding dairy
- cows. Animals, v. 10, n. 11, p. 1–13, 2020. https://doi.org/10.3390/ani10112176.
- Pollock JG, Gordon AW, Huson KM, McConnell DA. The Effect of Frequency of
- Fresh Pasture Allocation on the Feeding Behaviour of High Production Dairy Cows.
- 650 Animals, v. 12, n. 3, 2022. https://doi.org/10.3390/ani12030243.
- 651 Polsky L, Von Keyserlingk MAG. Invited review: Effects of heat stress on dairy cattle
- welfare. Journal of Dairy Science, v. 100, n. 11, p. 8645–8657, 2017.
- 653 https://doi.org/10.3168/jds.2017-12651.
- Reis NS, Ferreira IC, Mazocco LA, Souza ACB, Pinho GAS, Fonseca Neto AM,
- 655 Malaquias JV, Macena FA, Muller AG, Martins CF, Balbino LC. Shade Modifies
- 656 Behavioral and Physiological Responses of Low to Medium Production Dairy Cows
- at Pasture in an Integrated Crop-Livestock-Forest System. Animal, 2021.

- 658 https://doi.org/10.3390/ani11082411.
- Rémond B, Pomiés D, Julien C, Guinard-Flament J. Performance of dairy cows
- milked twice daily at contrasting intervals. Animal, v. 3, n. 10, p. 1463–1471, 2009.
- https://doi.org/10.1017/S1751731109990371.
- Ribeiro Filho HMN, Heydt MS, Baade EAS, Thaler Neto A. Consumo de forragem e
- produção de leite de vacas em pastagem de azevém-anual com duas ofertas de
- 664 forragem. Revista Brasileira de Zootecnia, v. 38, n. 10, p. 2038-2044, 2009.
- https://doi.org/10.1590/S1516-35982009001000026.
- Schons RMT, Laca EA, Savian JV, Mezzalira JC, Schneider EAN, Caetano LAM,
- Zubieta AS, Benvenutti MA, Carvalho PCF. 'Rotatinuous' stocking: An innovation in
- 668 grazing management to foster both herbage and animal production. Livestock
- 669 Science, v. 245, n. April 2019, p. 104406, 2021.
- 670 https://doi.org/10.1016/j.livsci.2021.104406.
- Schütz KE, Rogers AR, Cox NR, Tucker CB. Dairy cows prefer shade that offers
- greater protection against solar radiation in summer: Shade use, behaviour, and
- body temperature. Applied Animal Behaviour Science, v. 116, n. 1, p. 28–34, 2009.
- 674 https://doi.org/10.1016/j.applanim.2008.07.005.
- Schütz KE, Cox NR, Tucker CB. A field study of the behavioral and physiological
- effects of varying amounts of shade for lactating cows at pasture. Journal of Dairy
- Science, v. 97, n. 6, p. 3599–3605, 2014. https://doi.org/10.3168/jds.2013-7649.

- 678 Sheahan AJ, Gibbs SJ, Roche JR. Timing of supplementation alters grazing
- behavior and milk production response in dairy cows. Journal of Dairy Science, v.
- 96, n. 1, p. 477–483, 2013. https://doi.org/10.3168/jds.2012-5781.
- Silanikove N. Effects of heat stress on the welfare of extensively managed domestic
- ruminants. Livestock Production Science, v. 67, n. 1-2, p. 1-18, 2000.
- 683 https://doi.org/10.1016/S0301-6226(00)00162-7.
- Souza SRMBO, Ítavo LCV, Rímoli J, Ítavo CCBF, Dias AM. Comportamento
- Ingestivo Diurno de Bovinos em Confinamento e em Pastagens. Archivos de
- Zootecnia, v. 56, p. 67–70, 2007. http://www.redalyc.org/articulo.oa?id=49556009.
- Stivanin SCB, Werncke D, Vizzotto EF, Stumpf MT, Thaler Neto A, Fischer V.
- Variation in available shaded area changes behaviour parameters in grazing dairy
- cows during the warm season, v.48, 2019. https://doi.org/10.1590/rbz4820180316.
- Stone AE, Jones BW, Becker CA, Bewley JM. Influence of breed, milk yield, and
- temperature-humidity index on dairy cow lying time, neck activity, reticulorumen
- temperature, and rumination behavior. Journal of Dairy Science, v. 100, n. 3, p.
- 693 2395–2403, 2017. https://doi.org/10.3168/jds.2016-11607.
- Thompson AJ, Weary DM, Bran JA, Daros RR, Hötzel MJ, Von Keyserlingk MAG.
- 695 Lameness and lying behavior in grazing dairy cows. Journal of Dairy Science, v. 102,
- 696 n. 7, p. 6373–6382, 2019. https://doi.org/10.3168/jds.2018-15717.
- Thurow JM, Nabinger C, Castilhos ZMS, Carvalho PCF, Medeiros CMO, Machado

- 698 MD. Estrutura da vegetação e comportamento ingestivo de novilhos em pastagem
- natural do Rio Grande do Sul. Revista Brasileira de Zootecnia, v. 38, n. 5, p. 818-
- 700 826, 2009. https://doi.org/10.1590/S1516-35982009000500006.
- Torre-Santos S, Royo LJ, Martínez-Fernández A, Chocarro C, Vicente F. The mode
- of grass supply to dairy cows impacts on fatty acid and antioxidant profile of milk.
- Foods, v. 9, n. 9, 2020. https://doi.org/10.3390/foods9091256.
- Tucker CB, Jensen MB, Passillé AM, Hänninen L, Rushen J. Invited review: Lying
- time and the welfare of dairy cows. Journal of Dairy Science, v. 104, n. 1, p. 20–46,
- 706 2021. https://doi.org/10.3168/jds.2019-18074.
- Van Laer E, Moons CPH, Ampe B, Sonck B, Vandaele L, Campeneere S, Tuyttens
- FAM. Effect of summer conditions and shade on the production and metabolism of
- Holstein dairy cows on pasture in temperate climate. Animal, v. 9, n. 9, p. 1547–
- 710 1558, 2015. https://doi.org/10.1017/S1751731115000816.
- Van Soest PJ. Development of a comprehensive system of feed analyses and
- application to forage. Journal of Animal Science, v. 26, n. 1, p. 119–128, 1967.
- Vidal AMC, Saran Netto A. Obtenção e processamento do leite e derivados. 2018.
- 714 https://doi.org/10.11606/9788566404173.
- Vizzotto EF, Fischer V, Thaler Neto A, Abreu AS, Stumpf MT, Werncke D, Schmidt
- FA, McManus CM. Access to shade changes behavioral and physiological attributes
- of dairy cows during the hot season in the subtropics. Animal, v.9, n. 9, 2015, p.

- 718 1559-1566. https://doi.org/10.1017/S1751731115000877.
- Yang WZ, Beauchemin KA. Increasing physically effective fiber content of dairy cow
- diets through forage proportion versus forage chop length: Chewing and ruminal pH.
- 721 Journal of Dairy Science, v. 92, n. 4, p. 1603-1615, 2009.
- 722 https://doi.org/10.3168/jds.2008-1379.
- Zanela MB, Ribeiro MER. LINA Leite Instável Não Ácido Qualidade do leite.
- 724 Embrapa Clima Temperado, n. 356, p. 19, 2018.
- Zanine AM, Santos EM, Parente HN, Ferreira DJ, Cecon PR. Comportamento da
- Ingestão em Bovinos (Ruminantes) em Pastagem de Capim Brachiaria decumbens
- na Região Centro-Oeste do Brasil. Archives of Veterinary Science, v. 11, n. 2, p. 17–
- 24, 2006. https://doi.org/10.5380/avs.v11i2.6765.
- Zanine AM, Motta GPR, Ferreira DJ, Souza AL, Ribeiro MD, Geron LJV, Fajardo M,
- Sprunk M, Pinho RMA.. Milk performance and grazing behaviour of dairy cows in
- response to pasture allowance. Animal Production Science, v. 59, n. 4, p. 749–756,
- 732 2019. https://doi.org/10.1071/AN17513.

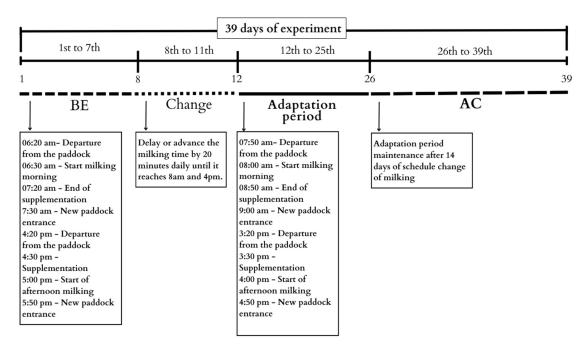


Figure 1 - Experiment timeline Farm A

BE= Before, AC= After change

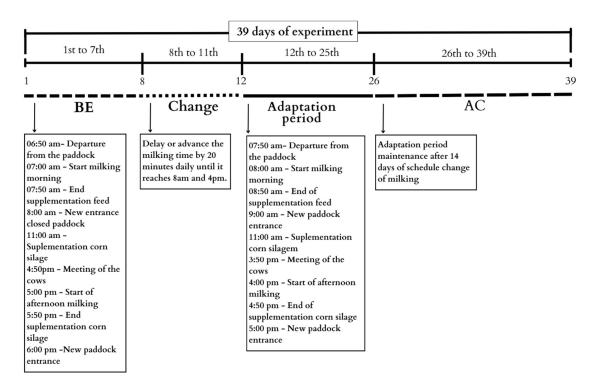


Figure 2 - Experiment timeline Farm B

BE= Before, AC= After change

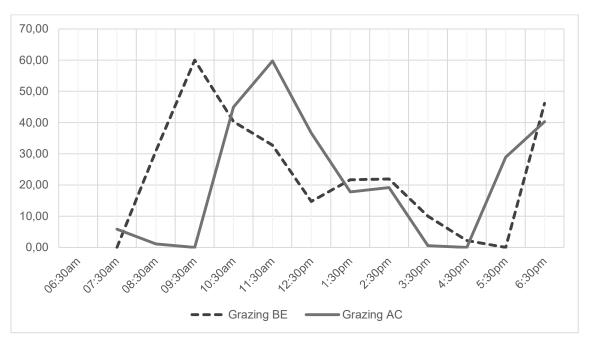


Figure 3 - Minutes of grazing activity per hour within the observation period at farm A

BE= before; AC= After the change.

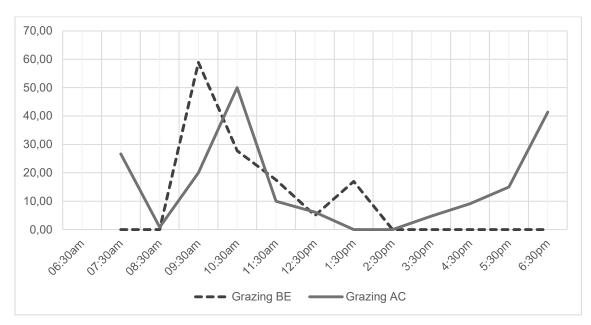


Figure 4 - Minutes of grazing activity per hour within the observation period at farm B

BE= before; AC= After the change.

Table 1 - Ethogram describing the evaluated behavioral activities

Behavioural					
Categories	Description of activity				
Ingestive					
Grazing	Jaw movements to apprehend, cut and swallow the				
	forage on pasture				
Rumination	Regurgitation, chewing again previously swallowed				
	food				
Idle	Standing or lying down without jaw movements.				
Other activities	Activities other than those previously mentioned,				
	such as locomotion, social interactions, drinking				
	water				
Posture					
Standing	Positioned with all four feet on the ground				
Lying down	Positioned with either flank in contact with the ground				
Local					
Outdoor	Staying in a place with no cover				

Shade	Staying with majority of the body under the shaded
	area

Table 2 - Ingredients and proportion of feed components on farms A and B

Farm	Ingredient	Proportion in feed
A	Ground corn	82%
	Soybean meal	14%
	Sodium bicarbonate	1%
	Mineral mix	3%
	Ground corn	67%
В	Croana com	0170
	Soybean meal	22%
	Wheat bran	7%
	Mineral mix	4%

Table 3 - Chemical composition of bulk feeds of Farms A and B

Farm	Food	DM (%)	CP (%)	NFD (%)	AFD (%)	PS
						(KgDM/cow/day)
Α	Tifton-85 grazing (BE)	23.87	20.96	66.56	25.55	3.81
	Corn + oat Silage (BE)	27.28	8.29	69.57	38.18	-
	Tifton-85 grazing (AC)	22.86	22.86	64.81	31.51	3.47
	Corn + oat Silage (AC)	32.75	10.31	53.81	32.58	-
В	Tifton-85 grazing (BE)	19.54	19.57	64.74	28.23	7.41
	Corn Silage (BE)	32.14	9.20	52.95	24.17	-
	Tifton-85 grazing (AC)	25.34	22.28	68.06	29.70	9.08
	Corn Silage (AC)	31.45	10.96	47.50	27.04	-

DM= Dry Matter; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; PS = Pasture supply. BE= before; AC= After the change.

Table 4 - Average values of air temperature (°C), precipitation (mm), relative air humidity (%) (RH) and THI registered at the observation days by the meteorological station in the city of Veranópolis-RS

Form	Dava	Air temperature (°C)			Precipitation	RH	THI
Farm	Days	Average	Max	Min	(mm)	(%)	IIII
	BE	16.8	19.4	14.1	1.8	95.54	62.13
A	BE	19.6	21.9	17.2	78.6	93.79	66.96
^	AC	13.6	18.4	8.8	0	86.91	56.58
	AC	17.7	24.1	11.3	0	78.94	63.17
В	BE	16.2	20	12.4	0	88.23	60.95
	BE	17.6	19.6	15.7	21.8	90.13	63.36
	AC	11.6	16.4	6.7	0	84.02	53.45
	AC	14.9	20.7	9.2	0	79.48	58.72

RH= Relative humidity; THI = Temperature humidity Index; BE= before; AC= After the change.

Table 5 - Average descriptive values of composition and physical-chemical parameters of milk and supplement consumption of Farms A in before (BE) and after the change (AC)

Variable	Treatment	Average
Milk Yield (Kg/cow/day)	BE AC	28.87 25.25
SCC (cel/mL)¹	BE AC	68.00 97.25
Fat content (%)	BE AC	2.07 2.68
Protein content (%)	BE AC	2.71 2.95
Lactose content (%)	BE AC	4.34 4.47
Total solids content (%)	BE AC	9.93 11.01
MUN (mg/dL) (%)	BE AC	11.68 10.52
Stability to alcohol test (°GL)²	BE AC	69.00 74.00
Acidity (°D)	BE AC	15.50 17.50

BE= before; AC= After the change.  $^1$ SCC values corrected on baseline  $\log_{10}$ .  $^2$ Reference value according to normative instruction 62, IN62 (Brasil, 2011)

Table 6 - P-value of effects and average values of composition and physical-chemical parameters of milk and supplement consumption of Farms B in before (BE) and after the change (AC)

Variable	Treatment	Day	treat*day	BE	AC
Milk Yield (Kg/cow/day)	0.6259	0.7044	0.0621	24.25	25.00
SCC (cel/mL)¹	0.5173	0.2273	0.3777	5.41	5.54
Fat contente (%)	0.0133	0.4312	0.0717	4.19 b	4.77 a
Protein contente (%)	0.3730	0.3726	0.1310	3.40	3.45
Lactose contente (%)	0.8028	0.0091	0.8410	4.42	4.43
Total solids contente (%)	0.0034	0.2839	0.0722	12.97 b	13.63 a
MUN (mg/dL)	<0.0001	<0.0001	0.8831	11.93 b	15.35 a
Stability to alcohol test (°GL)²	0.9750	0.0605	0.9252	74.45	74.14
Acidity (°D)	0.8406	0.2061	0.4619	18.45	18.53

BE= before; AC= After the change. Representative means of the two observation days, because there was no significant interaction treatment\*day. a, b - means in the same row followed by different letters are significantly different (Ismeans;  $P \le 0.05$ ). ¹SCC values corrected on baseline  $log_{10}$ . ²Reference value according to normative instruction 62, IN62 (Brasil, 2011)

Table 7 - P-value and average of effects times of ingestive behavioral activities position and place of lactating cows with during grazing access in the treatment before (BE) and after the change (AC) on Farm A

Behavior	Treatment	tment BE	
Grazing time (min)	0.5899	267.27	255.45
Rumination time (min)	0.1452	139.09	108.18
Idling time (min)	0.3279	130.00	112.73
Supplementation time (min)	<0.0001	108.18 b	158.18 a
Other activities times (min)	0.5189	86.36	82.73
Standing time (min)	<0.0001	578.18 b	710.91 a
Lying down time (min)	<0.0001	150.91	19.10
Outdoor time (min)	<0.0001	615.45 a	473.64 b
Shade time (min)	<0.0001	113.64 b	256.36 a

BE= before; AC= After the change. a, b - means in the same row followed by different letters are significantly different (Ismeans; P≤0.05).

Table 8 - P-value of effects and average times of ingestive behavioral activities position and place of lactating cows with during grazing access in relation to the period of the day in the treatment before (BE) and after the change (AC) on Farm A

	Day	Treatmen	Chiff	treat*shif		
Behavior	shift	t	Shift	t	BE	AC
			<0.000		186.11	145.56
Grazing time (min)	MO	0.0702	1	0.0003	а	b
	AF				76.11	90.56
			<0.000			
Rumination time (min)	MO	0.0645	1	<0.0001	64.44 a	23.89 b
	AF				72.22	88.89
			<0.000			
Idlin a time o (min)	МО	0.3307	1	<0.0001	29.44 b	72.22 a
Idling time (min)					111.67	
	AF				а	57.22 b
Supplementation time	МО	<0.0001	0.7547	0.9170	49.44 b	78.89 a
(min)	AF				50.00 b	80.00 a
Other activities times (min)	МО	0.0956	0.0956	0.0617	40.56	41.11
Other activites times (min)	AF				50.00 a	40.56 b
			<0.000		301.67	361.67
	MO	<0.0001	1	0.1546	b	а
Standing time (min)					264.44	341.11
	AF				b	а
	MO	<0.0001	0.0019	0.1301	67.78 a	8.33 b
Lying down time (min)	AF				95.56 a	18.33 b
			<0.000		320.00	271.67
Outdoor time (min)	МО	<0.0001	1	<0.0001	а	b
					310.00	197.78
	AF				а	b
			<0.000			
Shade time (min)	МО	<0.0001	1	<0.0001	49.44 b	98.33 a
						161.67
	AF				50.00 b	а

BE= before; AC= After the change, MO= First half of the day, AF= Second half of the day. a, b - means in the same row followed by different letters are significantly different (Ismeans; P≤0.05). For significant treatment\*shift interactions the means were compared BE and AC within each shift.

Table 9 - P-value of effects and average times of ingestive behavioral activities position and place of lactating cows with during grazing access in the treatment before (BE) and after the change (AC) on Farm B

	Experiment					
Behavior	day	Treatment	Day	treat*day	BE	AC
Grazing time (min)	-	<0.0001	0.8641	0.1645	125.28¹ b	183.89¹ a
	1	0.1523	0.6539	<0.0001	178.33 a	81.11 b
Rumination time (min)	2				102.22 b	167.22 a
Idling time (min)	1	0.0593	1.000	<0.0001	162.78 b	210.00 a
	2				231.11 a	141.67 b
Supplementation time (min)	-	<0.0001	0.0005	0.7888	181.94¹ a	152.78¹ b
Others activites times (min)	-	0.0606	0.0018	0.5934	83.05¹ b	90.00¹ a
Stading time (min)	1	<0.0001	0.0177	0.0005	669.44	640.00
	2				684.44 a	567.22 b
Lying down time (min)	1	<0.0001	0.0177	0.0005	60.56	90.00
	2				45.56 b	162.78 a
Outdoor time (min)	1	<0.0001	0.0019	0.0016	331.67 b	421.11 a
	2				392.22 b	420.56 a
Shade time (min)	1	<0.0001	0.0024	0.0020	365.56 a	308.33 b
	2				336.67 a	308.89 b

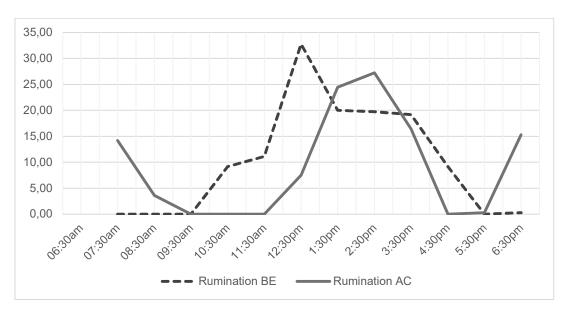
BE= before; AC= After the change. a, b - means in the same row followed by different letters are significantly different (Ismeans; P≤0.05). ¹Representative means of the two observation days, because there was no significant interaction treatment\*shift. For significant treatment\*day interactions the means were compared BE and AC within each day.

Table 10 - P-value of effects and average times of ingestive behavioral activities position and place of lactating cows with during grazing access in relation to the period of the day in the treatment before (BE) and after the change (AC) on Farm B

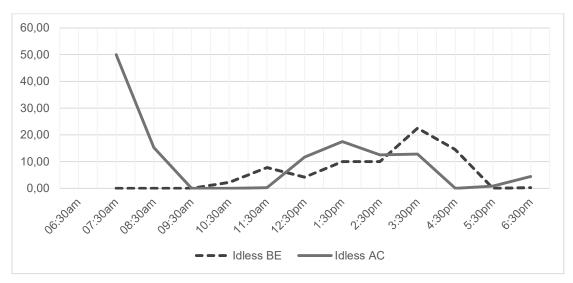
Behavior	Day shift	Treatment	Shift	treat*shift	BE	AC
Grazing time (min)	MO AF	<0.0001	<0.0001	<0.0001	108.33 16.94 b	113.61 70.28 a
Rumination time (min)	MO AF	0.0110	<0.0001	0.0001	30.28 74.44 a	37.50 40.28 b
Idling time (min)	MO AF	0.1231	<0.0001	<0.0001	71.95 65.55 a	84.17 38.33 b
Supplementation time (min)	MO AF	<0.0001	<0.0001	0.5328	118.06 a 60.83 a	94.17 b 40.00 b
Other activites times (min)	MO AF	0.0755	0.0025	0.0396	40.56 42.56 b	40.00 50.00 a
Standing time (min)	MO	<0.0001	<0.0001	0.7499	333.89 a	299.44 b
Lying down time (min)	AF MO AF	<0.0001	<0.0001	0.5371	228.06 a 36.11 b 15.28 b	196.94 b 70.56 a 43.06 a
Outdoor time (min)	MO AF	<0.0001	<0.0001	0.4804	239.17 b 98.06 b	275.00 a 140.28 a
Shade time (min)	MO AF	<0.0001	0.0299	0.2443	128.89 a 145.28 a	94.72 b 99.72 b

BE= before; AC= After the change, MO= First half of the day, AF= Second half of the day. Representative means of the two observation days, because there was no significant interaction treatment\*shift. a, b - means in the same row followed by different letters are significantly different (Ismeans; P≤0.05). For significant treatment\*shift interactions the means were compared BE and AC within each shift.

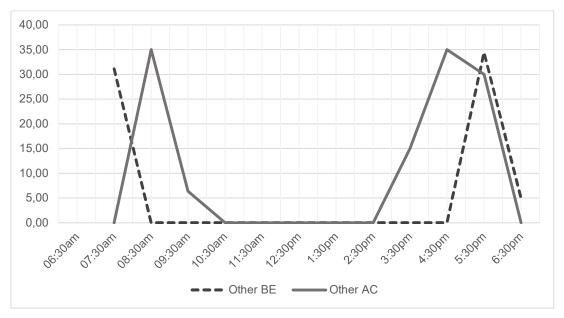
## Supplementary files



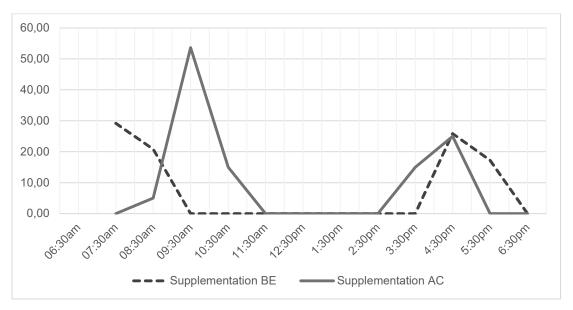
Supplementary Figure S1 - Minutes of rumination activity per hour within the observation period at farm A



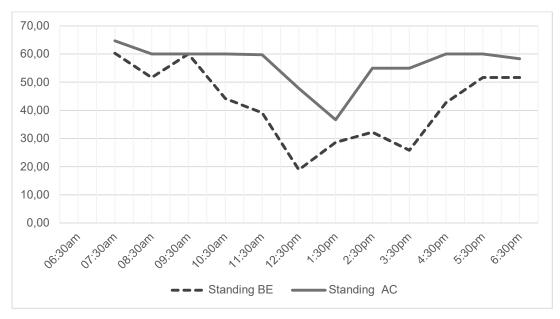
Supplementary Figure S2 - Minutes of idling activity per hour within the observation period at farm A



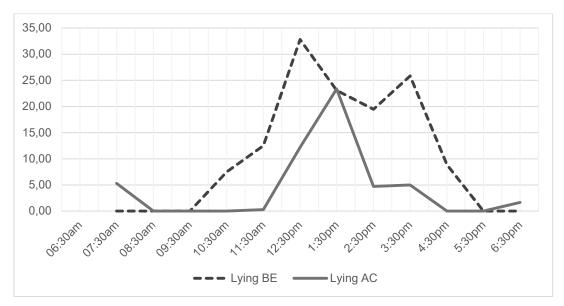
Supplementary Figure S3 - Minutes of others activities per hour within the observation period at farm A



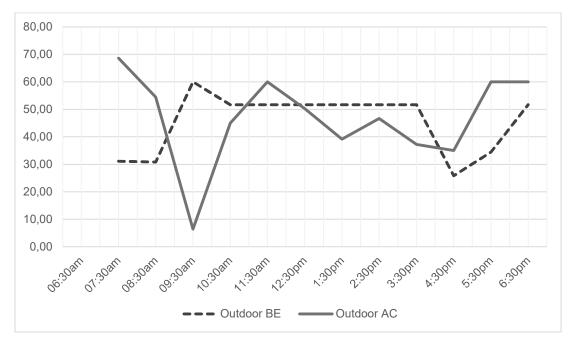
Supplementary Figure S4 - Minutes of supplementation activity per hour within the observation period at farm A



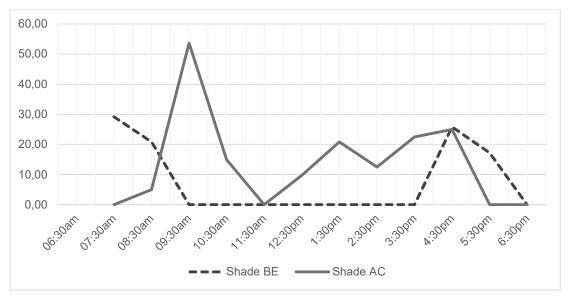
Supplementary Figure S5 - Minutes in standing position per hour within the observation period at farm A



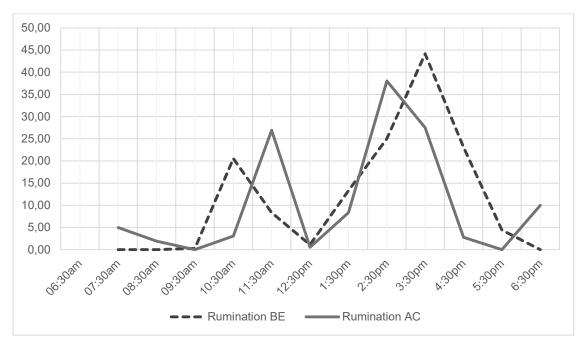
Supplementary Figure S6 - Minutes in lying position per hour within the observation period at farm A



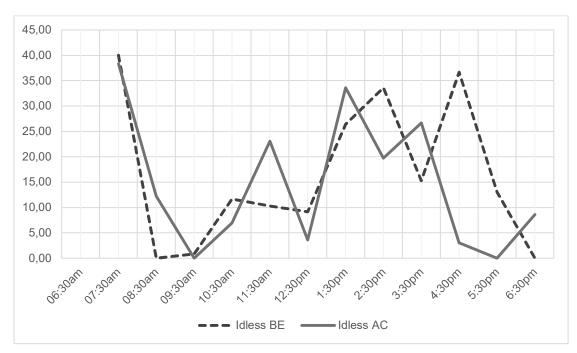
Supplementary Figure S7 - Minutes outdoors per hour within the observation period at farm A



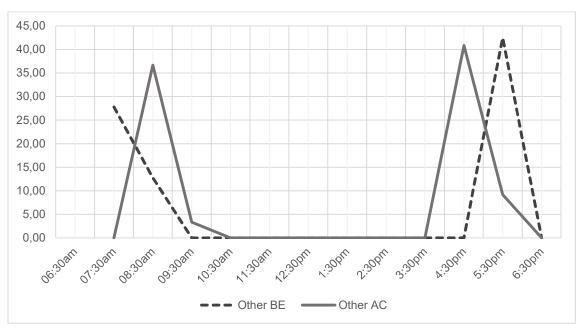
Supplementary Figure S8 - Minutes shade per hour within the observation period at farm A



Supplementary Figure S9 - Minutes of rumination activity per hour within the observation period at farm  ${\sf B}$ 

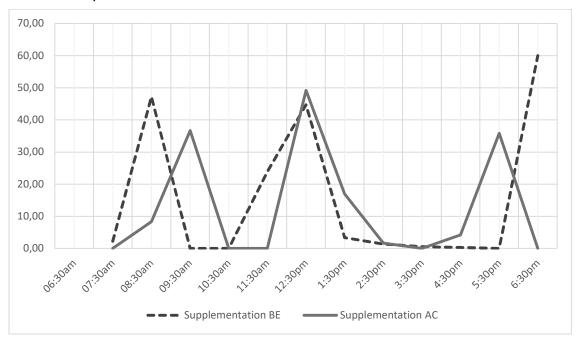


Supplementary Figure S10 - Minutes of idling activity per hour within the observation period at farm B

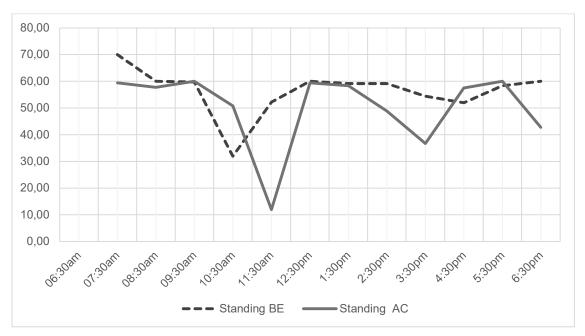


Supplementary Figure S11 - Minutes of others activities per hour within the observation period at farm  ${\sf B}$ 

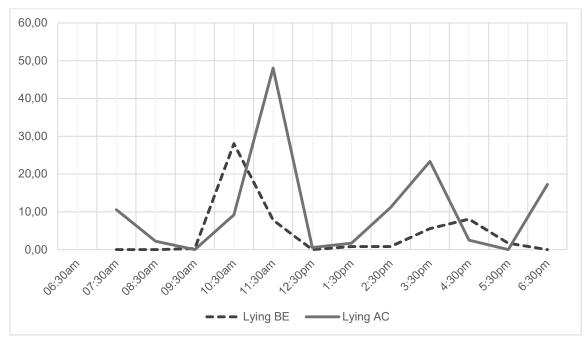
# Supplementary figure S12 - Minutes of supplementation activity per hour within the observation period at farm B



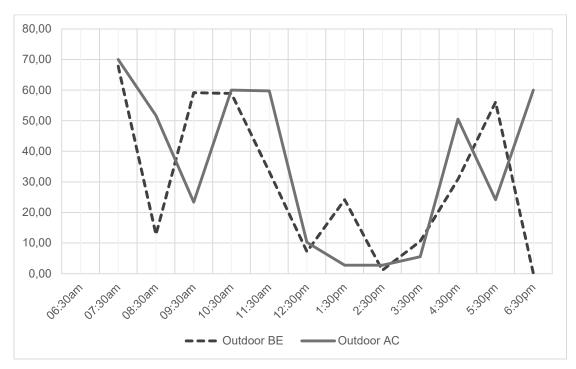
Supplementary Figure S12 - Minutes of supplementation activity per hour within the observation period at farm B



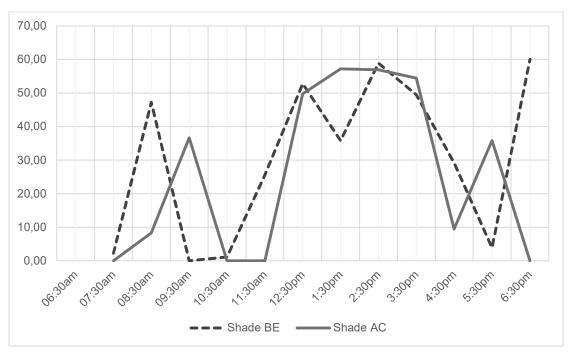
Supplementary Figure S13 - Minutes in standing position per hour within the observation period at farm  ${\sf B}$ 



Supplementary Figure S14 - Minutes in lying position per hour within the observation period at farm B



Supplementary Figure S15 - Minutes outdoors per hour within the observation period at farm B



Supplementary Figure S16 - Minutes shade per hour within the observation period at farm B

CAPÍTULO III

## **CONSIDERAÇÕES FINAIS**

Este estudo mostrou-se inovador por não haver detalhamento científico de uma prática realizada no campo explorando os efeitos da troca ou até mesmo de diferentes horários de ordenha.

Durante a execução desta pesquisa, deparei-me com várias limitações, a primeira delas foi o distanciamento físico dos colegas e professores do programa de pós-graduação devido a questões sanitárias mundiais.

A escolha em trabalhar com sistema pastoril deixou-se totalmente dependente das condições climática e desempenho das plantas, as quais tiveram seu desenvolvimento atrasado em função de uma estiagem no ano do experimento, no entanto, não inviabilizou o estudo. A execução deste experimento ocorreu no final do verão e início do outono de 2022. Em outras épocas do ano os resultados poderiam ser diferentes devidos a maior efeito da temperatura do ar e incidência solar sob os animais.

Diante dos dados e argumentações apresentadas podemos constatar que o horário de ordenha da tarde às 16h beneficia o aumento da ingestão de pastagem, por maior conforto térmico, mostraram-se uma alternativa de manejo para os produtores de leite de sistemas pastoris. No entanto, outros horário de ordenha em sistemas de produção de leite pastoris podem ser estudados de modo a investigar e definir um melhor horário para a ordenha.

Além da eficiência produtiva, podemos pensar neste manejo de modo a enquadrar a ordenha dentro dos horários comerciais de trabalho em casos de mão de obra contratada.

#### REFERENCIAS BIBLIOGRÁFICAS

ALBRIGHT, J. L. Feeding behavior of dairy cattle. **Journal of Dairy Science**, Champaign, v. 76, n. 2, p. 485-498, 1993. Disponível em: https://doi.org/10.3168/jds.S0022-0302(93)77369-5. Acesso em: 1° set. 2021.

ALLEN, M. S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. **Journal of Dairy Science**, Champaign, v. 83, n. 7, p. 1598-1624, 2000. Disponível em: https://doi.org/10.3168/jds.S0022-0302(00)75030-2. Acesso em: 7 set. 2021.

AMETAJ, B. N. *et al.* Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. **Metabolomics**, New York, v. 6, n. 4, p. 583-594, 2010. Disponível em: https://doi.org/10.1007/s11306-010-0227-6. Acesso em: 18 ago. 2021.

AOAC. Official methods of analysis. 17th ed. Rockville: AOAC, 2000.

ARAÚJO, R. A. *et al.* Grazing behavior and spatial distribution of feces of Young bulls in silvopastoral systems and Marandu monoculture in the Pre-Amazon region. **Acta Scientiarum**, Maringá, v. 39, n. 1, p. 83-90, 2017. Disponível em: https://doi.org/10.4025/actascianimsci.v39i1.33085. Acesso em: 4 ago. 2021.

AULDIST, M. J.; NAPPER, A. R.; KOLVER, E. S. Contribution of nutrition to seasonal variation of milk composition in New Zealand Friesian and US Holstein dairy cows. **Asian-Australasian Journal of Animal Sciences**, Seoul, v. 13, p. 513-515, 2000. Acesso em: 4 ago. 2021.

BARGO, F. *et al.* Invited review: production and digestion of supplemented dairy cows on pasture. **Journal of Dairy Science**, Champaign, v. 86, n. 1, p. 1-42, 2003. Disponível em: https://doi.org/10.3168/jds.S0022-0302(03)73581-4. Acesso em: 1º set. 2021.

BONDAN, C. *et al.* Variation of cow's milk composition across different daily milking sessions and feasibility of using a composite sampling. **Ciência Rural**, Santa Maria, v. 49, n. 6, p. 1-6, 2019. Disponível em: https://doi.org/10.1590/0103-8478cr20181004. Acesso em: 8 jan. 2023.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa nº 68, de 12 dezembro de 2006. **Diário Oficial da União: Seção 1**, Brasília, DF, 14 dez. 2006. Disponível em: http://www.cidasc.sc.gov.br/inspecao/files/2020/09/IN-MAPA-no-68-de-12-de-dezembro-2006.pdf. Acesso em: 21 set. 2021.

- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Gabinete do Ministro. Instrução Normativa nº 76, de 26 de novembro de 2018. **Diário Oficial da União: Seção 1**, Brasília, DF, n. 230, p. 9, 30 nov. 2018. Disponível em: https://www.in.gov.br/materia/-
- /asset\_publisher/Kujrw0TZC2Mb/content/id/52750141/do1-2018-11-30-instrucao-normativa-n-77-de-26-de-novembro-de-2018-52749887. Acesso em: 21 set. 2021.
- CANGIANO, C. A. *et al.* Effect of liveweight and pasture height on cattle bite dimensions during progressive defoliation. **Australian Journal of Agricultural Research**, East Melbourne, v. 53, n. 5, p. 541-549, 2002. Disponível em: https://doi.org/10.1071/AR99105. Acesso em: 1° set. 2021.
- CAPELESSO, A. *et al.* Reducing milking frequency in early lactation improved the energy status but reduced milk yield during the whole lactation of primiparous Holstein cows consuming a total mixed ration and pasture. **Journal of Dairy Science**, Champaign, v.102, p. 8919-8930, 2019. Disponível em: https://doi.org/10.3168/jds.2019-16629. Acesso em: 20 set. 2021
- CARVALHO, P. C. F. *et al.* Como a estrutura do pasto influencia o animal em pastejo? Exemplificando as interações planta-animal sob as bases e fundamentos do pastoreio "Rotatínuo". *In*: SIMPÓSIO SOBRE MANEJO ESTRATÉGICO DA PASTAGEM, 8., 2016, Viçosa, MG. [Anais...]. [Viçosa, MG: Simfor], 2016. p. 21. Acesso em: 19 jan. 2023.
- CHARTON, C. *et al.* Individual responses of dairy cows to a 24-hour milking interval. **Journal of Dairy Science**, Champaign, v. 99, n. 4, p. 3103-3112, 2016. Disponível em: https://doi.org/10.3168/jds.2015-9782. Acesso em: 8 set. 2021.
- CLARK, C. E. F. *et al.* The effect of temperate or tropical pasture grazing state and grain-based concentrate allocation on dairy cattle production and behavior. **Journal of Dairy Science**, Champaign, v. 101, n. 6, p. 5454-5465, 2018. Disponível em: https://doi.org/10.3168/jds.2017-13388. Acesso em: 8 set. 2021.
- CULOTTA, C. P.; SCHMIDT, G. H. An economic evaluation of three times daily milking of dairy cows. **Journal of Dairy Science**, Champaign, v. 71, n. 7, p. 1960-1966, 1988. Disponível em: https://doi.org/10.3168/jds.S0022-0302(88)79767-2. Acesso em: 5 jan. 2023.
- CURTIS, S. E. Environmental aspects of housing for animal production. Ames: The Iowa University Press, 1983.
- DADO, R. G.; ALLEN, M. S. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. **Journal of Dairy Science**, Champaign, v. 78, n. 1, p. 118-133, 1995. Disponível em: https://doi.org/10.3168/jds.S0022-0302(95)76622-X. Acesso em: 1° set. 2021.

- DEMSKI, J. B. *et al.* Milk production and ingestive behavior of COWS grazing on marandu and mulato ii pastures under rotational stocking. **Revista Brasileira de Zootecnia**, Viçosa, MG, [s. l.], v. 48, [art.] e20180231, 2019. Disponível em: https://doi.org/10.1590/RBZ4820180231. Acesso em: 22 fev. 2022.
- DETMANN, E. *et al.* An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. **Livestock Science**, Amsterdam, v. 162, n. 1, p. 141-153, 2014. Disponível em: https://doi.org/10.1016/j.livsci.2014.01.029. Acesso em: 7 set. 2021.
- DINEEN, M. *et al.* The effect of concentrate supplement type on milk production, nutrient intake, and total-tract nutrient digestion in mid-lactation, spring-calving dairy cows grazing perennial ryegrass (Lolium perenne L.) pasture. **Journal of Dairy Science**, Champaign, v. 104, n. 11, p. 11593-11608, 2021. Disponível em: https://doi.org/10.3168/jds.2021-20148. Acesso em: 8 jan. 2023.
- EDWARDS, A. G. R. *et al.* The use of spatial memory by grazing animals to locate food patches in spatially heterogeneous environments: an example with sheep. **Applied Animal Behaviour Science**, Amsterdam, v.50, p. 147-160 1996. Acesso em: 8 set. 2021.
- EDWARDS, J. P. *et al.* Short communication: Technologies and milking practices that reduce hours of work and increase flexibility through milking efficiency in pasture-based dairy farm systems. **Journal of Dairy Science**, Champaign, v. 103, n. 8, p. 7172-7179, 2020. Disponível em: https://doi.org/10.3168/jds.2019-17941. Acesso em: 5 jan. 2023.
- GIBB, M. J. Animal grazing/intake terminology and definitions. **Pasture Ecology and Animal Intake**, [s. l.], v. 3, n. 3, p. 20-35, 1998. Acesso em: 8 set. 2021.
- GREGORINI, P. Diurnal grazing pattern: Its physiological basis and strategic management. **Animal Production Science**, Melbourne, v. 52, n. 7, p. 416-430, 2012. Disponível em: https://doi.org/10.1071/AN11250. Acesso em: 8 set. 2021.
- GREGORINI, P.; TAMMINGA, S.; GUNTER, S. A. Review: Behavior and daily grazing patterns of cattle. **Professional Animal Scientist**, Champaign, v. 22, n. 3, p. 201-209, 2006. Disponível em: https://doi.org/10.15232/S1080-7446(15)31095-0. Acesso em: 14 jul. 2022.
- GUIMARÃES, Y. L. F. *et al.* Comportamento ingestivo de bovinos em diferentes sistemas de produção uma revisão sistemática de estudos científicos. **Research, Society and Development**, Vargem Grande Paulista, v. 9, n. 10, p. 1-14, 2020. Disponível em: https://doi.org/http://dx.doi.org/10.33448/rsd-v9i10.8705. Acesso em: 4 set. 2021.

GULATI, A. *et al.* Outdoor grazing of dairy cows on pasture versus indoor feeding on total mixed ration: effects on gross composition and mineral content of milk during lactation. **Journal of Dairy Science**, Champaign, v. 101, p. 2710-2723, 2018. Disponível em: https://doi.org/10.3168/jds.2017-13338. Acesso em: 4 ago. 2021.

HANLING, H. H.; MCGILLIARD, M. L.; CORL, B. A. Uneven milking intervals are adequate to achieve the benefits of increased milking frequency in early lactation. **Journal of Dairy Science**, Champaign, v. 104, n. 8, p. 9355-9361, 2021. Disponível em: https://doi.org/10.3168/jds.2020-20100. Acesso em: 8 dez. 2022.

HANRAHAN, L. *et al.* Factors associated with profitability in pasture-based systems of milk production. **Journal of Dairy Science**, Champaign, v. 101, n. 6, p. 5474-5485, 2018. Disponível em: https://doi.org/10.3168/jds.2017-13223. Acesso em: 12 ago. 2021.

HEINRICHS, A. J.; ROGERS, G. W.; COOPER, J. B. Predicting Body Weight and Wither Height in Holstein Heifers Using Body Measurements. **Journal of Dairy Science**, Champaign, v. 75, n. 12, p. 3576-3581, 1992. Disponível em: https://doi.org/10.3168/JDS.S0022-0302(92)78134-X. Acesso em: 23 fev. 2023.

KAMMES, K. L.; ALLEN, M. S. Nutrient demand interacts with grass particle length to affect digestion responses and chewing activity in dairy cows. **Journal of Dairy Science**, Champaign, v. 95, n. 2, p. 807-823, 2012. Disponível em: https://doi.org/10.3168/jds.2011-4588. Acesso em: 8 set. 2021.

KILGOUR, R. J. In pursuit of "normal": a review of the behaviour of cattle at pasture. **Applied Animal Behaviour Science**, Amsterdam, v. 138, n. 1/2, p. 1-11, 2012. Disponível em: https://doi.org/10.1016/j.applanim.2011.12.002. Acesso em: 8 set. 2021.

KILLEN, S. S. *et al.* Environmental stressors alter relationships between physiology and behaviour. **Trends in Ecology and Evolution**, Amsterdam, v. 28, n. 11, p. 651-658, 2013. Disponível em: https://doi.org/10.1016/j.tree.2013.05.005. Acesso em: 1º set. 2021.

KISMUL, H. *et al.* Morning and evening pasture access - comparing the effect of production pasture and exercise pasture on milk production and cow behaviour in an automatic milking system. **Livestock Science**, Amsterdam, v. 217, p. 44-54, 2018. Disponível em: https://doi.org/10.1016/j.livsci.2018.09.013. Acesso em: 8 dez. 2022.

- KNAUS, W. Perspectives on pasture versus indoor feeding of dairy cows. **Journal of the Science of Food and Agriculture**, London, v. 96, n. 1, p. 9-17, 2016. Disponível em: https://doi.org/10.1002/jsfa.7273. Acesso em: 9 mar. 2021.
- KÖPPEN, W.; GEIGER, R. **Klimate der Erde**. Gotha: Verlag Justus Perthes, 1928.
- LAKIC, B. *et al.* The effect of a single prolonged milking interval on inflammatory parameters, milk composition and yield in dairy cows. **Veterinary Immunology and Immunopathology**, Amsterdam, v. 140, n. 1/2, p. 110-118, 2011. Disponível em: https://doi.org/10.1016/j.vetimm.2010.11.022. Acesso em: 26 out. 2021.
- LEGRAND, A. L.; VON KEYSERLINGK, M. A.G.; WEARY, D. M. Preference and usage of pasture versus free-stall housing by lactating dairy cattle. **Journal of Dairy Science**, Champaign, v. 92, n. 8, p. 3651-3658, 2009. Disponível em: https://doi.org/10.3168/jds.2008-1733. Acesso em: 2 mar. 2023.
- LEIBER, F. *et al.* Relationships between dairy cows' chewing behavior with forage quality, progress of lactation and efficiency estimates under zero-concentrate feeding systems. **Agriculture**, Basel, v. 12, n. 10, p. 1570. 2022. Disponível em: https://doi.org/10.3390/agriculture12101570. Acesso em: 8 jan. 2023.
- LOURENÇO, J. C. S. *et al.* Variabilidade dos componentes do leite de vacas da raça holandês em diferentes horários de ordenha. *In*: MEDEIROS, J. A.; NIRO, C. M.; MEDEIROS, J. M. P. (org.). **Produção animal e vegetal:** inovações e atualidades. Jardim do Seridó: Agron Food Academy, 2021. p. 1305-1312. *E-book*. Disponível em: https://doi.org/10.53934/9786599539633-146. Acesso em: 8 jan. 2023.
- LOVEDAY, S. M. *et al.* Type A and B bovine milks: Heat stability is driven by different physicochemical parameters. **Journal of Dairy Science**, Champaign, v. 104, n. 11, p. 11413-11421, 2021. Disponível em: https://doi.org/10.3168/jds.2021-20201. Acesso em: 8 jan. 2023.
- MACKLE, T. R. *et al.* Variation in the composition of milk protein from pasture-fed dairy cows in late lactation and the effect of grain and silage supplementation. **New Zealand Journal of Agricultural Research**, Wellington, v. 42, n. 2, p. 147-154, 1999. Disponível em: https://doi.org/10.1080/00288233.1999.9513364. Acesso em: 4 ago. 2021.
- MARTELLO, L. S. *et al.* Respostas fisiológicas e produtivas de vacas holandesas em lactação submetidas a diferentes ambientes. **Revista Brasileira de Saúde e Produção Animal**, Salvador, v. 14, n. 3, p. 406-414, 2013. Disponível em: https://doi.org/10.1590/S1519-99402013000300016. Acesso em: 6 fev. 2023.

- MARTINS, C. M. M. R. *et al.* Effect of dietary cation-anion difference on performance of lactating dairy cows and stability of milk proteins. **Journal of Dairy Science**, Champaign, v. 98, n. 4, p. 2650-2661, 2015. Disponível em: https://doi.org/10.3168/jds.2014-8926. Acesso em: 1º mar. 2023.
- MAULFAIR, D. D.; FUSTINI, M.; HEINRICHS, A. J. Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows. **Journal of Dairy Science**, Champaign, v. 94, n. 7, p. 3527-3536, 2011. Disponível em: https://doi.org/10.3168/jds.2010-3718. Acesso em: 7 set. 2021.
- MERCÊS, L. M. *et al.* Horário alternativo de ordenha e o comportamento ingestivo de vacas mestiças leiteiras em sistema de produção a pasto. **Acta Scientiarum Animal Sciences**, Maringá, v. 34, n. 2, p. 197-202, 2012. Disponível em: https://doi.org/10.4025/actascianimsci.v34i2.12476. Acesso em: 3 mar. 2021.
- MERTENS, D. R. Creating a system for meeting the fiber requirements of dairy cows. **Journal of Dairy Science**, Champaign, v. 80, n. 7, p. 1463-1481, 1997. Disponível em: https://doi.org/10.3168/jds.S0022-0302(97)76075-2. Acesso em: 8 set. 2021.
- MEZZALIRA, J. C. *et al.* Aspectos metodológicos do comportamento ingestivo de bovinos em pastejo. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 40, n. 5, p. 1114-1120, 2011. Disponível em: https://doi.org/10.1590/S1516-35982011000500024. Acesso em: 8 mar. 2021.
- MIGUEL, M. F.; DELAGARDE, R.; RIBEIRO-FILHO, H. M.N. Corn silage supplementation for dairy cows grazing annual ryegrass at two pasture allowances. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 71, n. 3, p. 1037-1046, 2019. Disponível em: https://doi.org/10.1590/1678-4162-9795. Acesso em: 7 jan. 2023.
- MIGUEL, M. F.; RIBEIRO-FILHO, H. M.N.; DELAGARDE, R. Effects of corn silage supplementation strategy and grazing intensity on herbage intake, milk production, and behavior of dairy cows. **Journal of Dairy Science**, Champaign, v. 106, n. 2, p. 1013-1025, Feb. 2023. Disponível em: https://doi.org/10.3168/jds.2021-21649. Acesso em: 8 jan. 2023.
- NATIONAL RESEARCH COUNCIL. **Nutrient requirements of dairy cattle**. 7th ed. Washington, DC: The National Academies, 2001. Disponível em: https://doi.org/10.17226/9825. Acesso em: 8 abr. 2021.
- O'CALLAGHAN, T. F. *et al.* Pasture feeding changes the bovine rumen and milk metabolome. **Metabolites**, Basel, v. 8, n. 2, [art.] 27, 2018. Disponível em: https://doi.org/10.3390/metabo8020027. Acesso em: 4 ago. 2021.

- OLIVEIRA, B. C. *et al.* Mecanismos reguladores de consumo em bovinos de corte. **Nutritime Revista Eletrônica**, Viçosa, MG, v. 14, n. 4, p. 6066-6075, 2017. Acesso em: 7 set. 2021.
- ONACIU, G. *et al.* Influence of varying ranges milk urea nitrogen on chemical, hygienic and physical quality traits of cow milk. **Romanian Biotechnological Letters**, Bucharest, v. 24, n. 5, p. 866-873, 2019. Disponível em: https://doi.org/10.25083/rbl/24.5/866.873. Acesso em: 11 jan. 2023.
- PENRY, J. F. *et al.* Association of milking interval and milk production rate in an automatic milking system. **Journal of Dairy Science**, Champaign, v. 101, n. 2, p. 1616-1625, 2018. Disponível em: https://doi.org/10.3168/jds.2016-12196. Acesso em: 3 ago. 2021.
- PHILLIPS, C. J. C.; HECHEIMI, K. The effect of forage supplementation, herbage height and season on the ingestive behaviour of dairy cows. **Applied Animal Behaviour Science**, Amsterdam, v. 24, n. 3, p. 203-216, 1989. Disponível em: https://doi.org/10.1016/0168-1591(89)90067-1. Acesso em: 9 set. 2021.
- PHILLIPS, C. J. C.; SCHOFIELD, S. A. The effect of supplementary light on the production and behaviour of dairy cows. **Animal Production**, [s. I.], v. 48, n. 2, p. 293-303, 1989. Disponível em: https://doi.org/10.1017/S0003356100040290. Acesso em: 9 set. 2021.
- PHYN, C. V. C. *et al.* Temporary alterations to postpartum milking frequency affect whole-lactation milk production and the energy status of pasture-grazed dairy cows. **Journal of Dairy Science**, Champaign, v. 97, n. 11, p. 6850-6868, 2014. Disponível em: https://doi.org/10.3168/jds.2013-7836. Acesso em: 20 set. 2021.
- PIRES, M. F. A.; VERNEQUE, R. S.; VILELA, D. Ambiente e comportamento animal na produção do leite. **Informe Agropecuário**, Belo Horizonte, v. 22, n. 211, p. 11-21, 2001. Acesso em: 4 set. 2021.
- POLLOCK, J. G *et al.* The effect of frequency of fresh pasture allocation on the feeding behaviour of high production dairy cows. **Animals**, Basel, v. 12, n. 3, [art.] 243, 2022. Disponível em: https://doi.org/10.3390/ani12030243. Acesso em: 3 ago. 2021.
- POLLOCK, J. G. *et al.* The effect of frequency of fresh pasture allocation on pasture utilisation and the performance of high yielding dairy cows. **Animals**, Basel, v. 10, n. 11, [art.] 2176, [p. 1-13], 2020. Disponível em: https://doi.org/10.3390/ani10112176. Acesso em: 12 jul. 2022.

- POLSKY, L.; VON KEYSERLINGK, M. A.G. Invited review: Effects of heat stress on dairy cattle welfare. **Journal of Dairy Science**, Champaign, v. 100, n. 11, p. 8645-8657, 2017. Disponível em: https://doi.org/10.3168/jds.2017-12651. Acesso em: 6 fev. 2023.
- REIS, N. S. *et al.* Shade modifies behavioral and physiological responses of low to medium production dairy cows at pasture in an integrated crop-livestock-forest system. **Animals**. Basel, v. 11, n. 8, [art.] 2411, 2021. Disponível em: https://doi.org/10.3390/ani11082411. Acesso em: 12 jul. 2022.
- RÉMOND, B. *et al.* Performance of dairy cows milked twice daily at contrasting intervals. **Animal**, Cambridge, v. 3, n. 10, p. 1463-1471, 2009. Disponível em: https://doi.org/10.1017/S1751731109990371. Acesso em: 26 jul. 2021.
- RIBEIRO FILHO, H. M. N. *et al.* Consumo de forragem e produção de leite de vacas em pastagem de azevém-anual com duas ofertas de forragem. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 38, n. 10, p. 2038-2044, 2009. Disponível em: https://doi.org/10.1590/S1516-35982009001000026. Acesso em: 4 set. 2021.
- RICE, P. *et al.* Defining a functional unit for dairy production LCA that reflects the transaction between the farmer and the dairy processor. **International Journal of Life Cycle Assessment**, Landsberg, v. 24, n. 4, p. 642-653, 2019. Disponível em: https://doi.org/10.1007/s11367-018-1486-0. Acesso em: 12 ago. 2021.
- SAMPAIO, A. F. *et al.* Correlação entre comportamento ingestivo e consumo de nutrientes em vacas a pasto. **Revista Científica de Produção Animal**, Areia, PB, v. 18, n. 2, p. 110-120, 2016. Disponível em: https://periodicos.ufpb.br/ojs2/index.php/rcpa/article/view/42605. Acesso em: 10 mar. 2021.
- SCHMIDT, G. H. Effect of milking intervals on the rate of milk and fat secretion. **Journal of Dairy Science**, Champaign, v. 43, n. 2, p. 213-219, 1960. Disponível em: https://doi.org/10.3168/jds.S0022-0302(60)90143-0. Acesso em: 6 maio 2021.
- SCHONS, R. M. T. *et al.* 'Rotatinuous' stocking: an innovation in grazing management to foster both herbage and animal production. **Livestock Science**, Amsterdam, v. 245, [art.] 104406, 2021. Disponível em: https://doi.org/10.1016/j.livsci.2021.104406. Acesso em: 7 jan. 2023.
- SCHÜTZ, K. E. *et al.* Dairy cows prefer shade that offers greater protection against solar radiation in summer: shade use, behaviour, and body temperature. **Applied Animal Behaviour Science**, Amsterdam, v. 116, n. 1, p. 28-34, 2009. Disponível em: https://doi.org/10.1016/j.applanim.2008.07.005. Acesso em: 6 fev. 2023.

- SCHÜTZ, K. E.; COX, N. R.; TUCKER, C. B. A field study of the behavioral and physiological effects of varying amounts of shade for lactating cows at pasture. **Journal of Dairy Science**, Champaign, v. 97, n. 6, p. 3599-3605, 2014. Disponível em: https://doi.org/10.3168/jds.2013-7649. Acesso em: 6 jul. 2021.
- SHEAHAN, A. J.; GIBBS, S. J.; ROCHE, J. R. Timing of supplementation alters grazing behavior and milk production response in dairy cows. **Journal of Dairy Science**, Champaign, v. 96, n. 1, p. 477-483, 2013. Disponível em: https://doi.org/10.3168/jds.2012-5781. Acesso em: 30 ago. 2021.
- SILANIKOVE, N. Effects of heat stress on the welfare of extensively managed domestic ruminants. **Livestock Production Science**, Amsterdam, v. 67, n. 1/2, p. 1-18, 2000. Disponível em: https://doi.org/10.1016/S0301-6226(00)00162-7. Acesso em: 1º mar. 2023.
- SILVA, R. R. *et al.* Comportamento ingestivo diurno de novilhos Nelore recebendo níveis crescentes de suplementação em pastejo de capim-braquiária Diurnal ingestive behavior of Nellore steers receiving increasing levels of supplement in brachiaria pasture. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 39, n. 9, p. 2073-2080, 2010. Acesso em: 6 fev. 2023.
- SILVEIRA, R. K.; TEIXEIRA, U. H. G.; SILVEIRA, A. P. Alimentos processados para bovinos: Riscos relacionados aos teores de fibra. **Revista Brasileira de Nutrição Animal**, Fortaleza, v. 15, n. 1, p. 1-11, 2021. Acesso em: 8 set. 2021.
- SOUZA, S. R. M. B. O. *et al.* Comportamento ingestivo diurno de bovinos em confinamento e em pastagens. **Archivos de Zootecnia**, Córdoba, v. 56, p. 67-70, 2007.Disponível em: http://www.redalyc.org/articulo.oa?id=49556009. Acesso em: 8 fev. 2023.
- STONE, A. E. *et al.* Influence of breed, milk yield, and temperature-humidity index on dairy cow lying time, neck activity, reticulorumen temperature, and rumination behavior. **Journal of Dairy Science**, Champaign, v. 100, n. 3, p. 2395-2403, 2017. Disponível em: https://doi.org/10.3168/jds.2016-11607. Acesso em: 13 jul. 2022.
- THOMPSON, A. J. *et al.* Lameness and lying behavior in grazing dairy cows. **Journal of Dairy Science**, Champaign, v. 102, n. 7, p. 6373-6382, 2019. Disponível em: https://doi.org/10.3168/jds.2018-15717. Acesso em: 7 mar. 2023.
- THUROW, J. M. *et al.* Estrutura da vegetação e comportamento ingestivo de novilhos em pastagem natural do Rio Grande do Sul. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 38, n. 5, p. 818-826, 2009. Disponível em: https://doi.org/10.1590/S1516-35982009000500006. Acesso em: 23 set. 2021.

- TORRE-SANTOS, S. *et al.* The mode of grass supply to dairy cows impacts on fatty acid and antioxidant profile of milk. **Foods**, Basel, v. 9, n. 9, [art.] 1256, 2020. Disponível em: https://doi.org/10.3390/foods9091256. Acesso em: 12 jul. 2022.
- TUCKER, C. B. *et al.* Invited review: Lying time and the welfare of dairy cows. **Journal of Dairy Science**, Champaign, v. 104, n. 1, p. 20-46, 2021. Disponível em: https://doi.org/10.3168/jds.2019-18074 . Acesso em: 13 jul. 2022.
- VAN LAER, E. *et al.* Effect of summer conditions and shade on the production and metabolism of Holstein dairy cows on pasture in temperate climate. **Animal**, Cambridge, v. 9, n. 9, p. 1547-1558, 2015. Disponível em: https://doi.org/10.1017/S1751731115000816. Acesso em: 6 jul. 2021.
- VAN SOEST, P. J. Development of a comprehensive system of feed analyses and application to forage. **Journal of Animal Science**, Champaign, v. 26, n. 1, p. 119-128, 1967. Acesso em: 2 mar. 2023.
- VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca, NY: Cornell University Press, 1994.
- VIDAL, A. M. C.; SARAN NETTO, A. (org.). **Obtenção e processamento do leite e derivados**. Pirassununga: FZEA-USP, 2018. Disponível em: https://doi.org/10.11606/9788566404173. Acesso em: 21 set. 2021.
- WALKER, S. L. *et al.* Lameness, activity time-budgets, and estrus expression in dairy cattle. **Journal of Dairy Science**, Champaign, v. 91, n. 12, p. 4552-4559, 2008. Disponível em: https://doi.org/10.3168/jds.2008-1048. Acesso em: 4 set. 2021.
- WILKINSON, J. M.; LEE, M. R.F. Review: Use of human-edible animal feeds by ruminant livestock. **Animal**, Cambridge, v. 12, n. 8, p. 1735-1743, 2017. Disponível em: https://doi.org/10.1017/S175173111700218X. Acesso em: 4 ago. 2021.
- YANG, W. Z.; BEAUCHEMIN, K. A. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. **Journal of Dairy Science**, Champaign, v. 92, n. 4, p. 1603-1615, 2009. Disponível em: https://doi.org/10.3168/jds.2008-1379. Acesso em: 8 set. 2021.
- ZANELA, M. B.; RIBEIRO, M. E. R.: **LINA Leite Instável Não Ácido Qualidade do leite**. Pelotas: Embrapa Clima Temperado, 2018. 19 p. (Comunicado técnico, 356). Acesso em: 7 jan. 2023.
- ZANINE, A. M. *et al.* Milk performance and grazing behaviour of dairy cows in response to pasture allowance. **Animal Production Science**, Melbourne, v. 59, n.

4, p. 749-756, 2019. Disponível em: https://doi.org/10.1071/AN17513. Acesso em: 8 dez. 2022.

ZANINE, A.M. *et al.* Comportamento da Ingestão em Bovinos (Ruminantes) Em Pastagem de Capim *Brachiaria decumbens* na Região Centro-Oeste do Brasil. **Archives of Veterinary Science**, Curitiba, v. 11, n. 2, p. 17-24, 2006. Disponível em: https://doi.org/10.5380/avs.v11i2.6765. Acesso em: 8 set. 2021.

### **APÊNDICE I**



## PRÓ-REITORIA DE PESQUISA



Comissão De Ética No Uso De Animais

## CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 41758

Título:

HORARIO DE ORDENHA, COMPORTAMENTO ALIMENTAR, CONSUMO E DESEMPENHO

PRODUTIVO DE VACAS LEITEIRAS

Vigência: 15/01/2022 à 15/03/2023

Pesquisadores:

#### **Equipe UFRGS:**

VIVIAN FISCHER - coordenador desde 15/01/2022 Delane Ribas Da Rosa - desde 15/01/2022 PAULO CESAR DE FACCIO CARVALHO - pesquisador desde 15/01/2022

#### **Equipe Externa:**

Leandro Ebert - pesquisador desde 15/01/2022

Comissão De Ética No Uso De Animais aprovou o mesmo, em reunião realizada em 04/07/2022 - Reunião por webconferência - Mconf UFRGS, em seus aspectos éticos e metodológicos, para a utilização de 40 vacas leiteiras, Bos taurus, adultas e em lactação provenientes das fazendas dos proprietários: Leinor Zandoná, CPF n°559.090.350-53 e Ivânia M.G. Binda, CPF n° 720.532.320-72, onde ocorrerão os experimentos com animais de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Quinta-Feira, 14 de Julho de 2022

MAITE DE MORAES VIEIRA Coordenador da comissão de ética

loité de el Vierra

APÊNDICE II - Normas utilizadas para a preparação do capítulo II Guide for Authors: Livestock Science - Feb 2023

#### INTRODUCTION

Types of article

- 1. Original Research Articles (Regular Papers)
- 2. Review Articles
- 3. Short Communications
- 4. Position Papers
- 5. Technical Notes
- 6. Book Reviews

Original Research Articles should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form. They should not occupy more than 12 Journal pages.

Review Articles should cover subjects falling within the scope of the journal which are of active current interest. Reviews will often be invited, but submitted reviews will also be considered for publication.

All reviews will be subject to the same peer review process as applies for original papers. They should not occupy more than 12 Journal pages. (Occasionally the Editor will invite an author to submit a review; such commissioned reviews should be submitted using the Invited Reviews article category in the e-submission process.)

A Short Communication is a concise but complete description of a limited investigation, which will not be included in a later paper. Short Communications may be submitted to the journal as such, or may result from a request to condense a regular paper, during the peer review process. They should not occupy more than 5 journal pages (approximately 10 manuscript pages) including figures, tables and references.

Opinion Papers are informative and thought-provoking articles on key issues, often dealing with matters of public concern. These will usually be invited, but a submitted paper may also be considered for publication. They should not occupy more than 12 Journal pages.

A Technical Note is a report on a new method, technique or procedure falling within the scope of Livestock Science. It may involve a new algorithm, computer program (e.g. for statistical analysis or for simulation), or testing method for example. The Technical Note should be used for information that cannot adequately incorporated into an Original Research Article, but that is of sufficient value to be brought to the attention of the readers of Livestock Science. The note should describe the nature of the new method, technique or procedure and clarify how it differs from those currently in use if cannot be incorporated. They should not occupy more than 5 Journal pages.

Book Reviews will be included in the journal on a range of relevant books which are not more than two years old.

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- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

## Further considerations

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**BEFORE YOU BEGIN** 

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advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

## **Definitions**

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is importante for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in

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PREPARATION

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- Title should be clear, descriptive and not too long
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- Keywords (indexing terms)
- Introduction
- Material studied, area descriptions, methods, techniques
- Results
- Discussion
- Conclusion

- Acknowledgment and any additional information concerning research grants, and so on
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Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

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## VITA

Delane Ribas da Rosa é brasileira, natural de Curitiba-PR, nascida em 19 de fevereiro de 1996, filha de Elizângela S. R. Rosa e Wagner M. da Rosa. Realizou seu ensino fundamental e médio próximo à sua residência, em Pinhais-PR.

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