# 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

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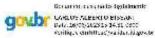
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#### RESUMO

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

Autor(a): Zoot. Delane Ribas da Rosa

Orientadora: Prof.<sup>a</sup> Dra. Vívian Fischer

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>

Author: Zoot. Delane Ribas da Rosa

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                                  |
| ТНІ  | 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 nãoestruturais), 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

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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                                       |

CHANGES IN MILKING TIME MODIFY BEHAVIOR OF GRAZING DAIRY COWS

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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, 22 alcohol stability of milk, acidity and supplement intake were collected during the 23 conventional milking time (days 1 to 7 of the trial) and at the last 14 days after the 24 adoption of the alternative milking time (days 21 to 35). Also data of behavior was 25 also collected on two days before and after milking time changes. Behavior activities 26 consisted of diurnal ingestive behavior (time spent grazing, ruminating, idling and 27 eating the supplement), position (standing up or lying down) and time in shade or in 28 the outdoor part registered from 6:30 am to 6:30 pm. Behavior data were 29 summarized for 12 hours of the diurnal period and two times intervals (Morning or 30 MO: 6:30 am to 12:30 pm, Afternoon or AF: 12:31 pm to 6:30 pm) as well total diurnal 31 time (MO+AF). Data were submitted to analysis of variance, and means between 32 before (BE) and after change (AC) were compared using the mixed model at 5% 33 probability level. Change in milking time for farm B increased fat, total solids and 34 MUN contents (p<0.05), as well increased diurnal time spent grazing in B. 35 Ruminating and idling time behave differently according to changes in milking time 36 between farms. Cows spent more time standing up (P<0.001) on farm A and more 37 time lying down (P<0.001) and outdoor (0.001) on farm B after change in milking 38 time. In farm B, changes in milking time favored late afternoon grazing and reduced 39 time spent feeding supplement without changes in milk yield, while increased milk 40 solids contents of milk. 41

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

44 Introduction

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Milking is a labor-intensive task performed usually at least twice a day, 46 accounting for a expressive part of the time used on a farm (Culotta & Schmidt, 47 1988), representing 43 to 58% of a conventional 40-h work week (Edwards et al., 48 2020). Milking time are settled according to farm needs such time of milk collection, 49 availability of labour and use of time in other farm's activities (Culotta & Schmidt, 50 1988). Milking times are also related to the beginning and end of the working day, 51 which may extend the working days and/or allocate milking to undesirable or unusual 52 times. Usual milking intervals are 10 (diurnal) and 14 hours, but shorter intervals as 53 8 allow to fit milking activities into conventional working time, turning more attractive 54 for farm employees (Edwards et al., 2020). 55

On the other hand, cattle follow a circadian cycle of ingestive behavior 56 spending more time grazing at dawn and dusk (Kilgour, 2012; Guimarães et al., 57 2020; Pollock et al., 2020). The hours of highest grazing activity occur two hours 58 after dawn and in the last hours of the day and the first hours of the night 59 (GREGORINI, 2012), due to the preference in performing these activities at times of 60 mild air temperature (Legrand et al., 2009). Between these times, the animals usually 61 seek natural or artificial shade (Schütz et al., 2014; Van Laer et al., 2015). However, 62 usually these main grazing periods coincide with the conventional milking time, 63 which can impair gazing and, consequently, pasture consumption. 64

<sup>65</sup> Currently few papers demonstrate the achievements of different milking times <sup>66</sup> 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.

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### 75 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).

#### 83 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 88 farm, the animals were observed following the conventional management adopted 89 on each farm. Between day 8 and 11 of the experiment, the groups of cows had their 90 milking times changed by 20 minutes per day until they reached the alternative 91 milking times at 8 am and 4 pm. Between days 12 and 39, cows were milked at the 92 alternative milking times (Figures 1 and 2). Throughout the experiment, the animals 93 received the same diet, free access to water and mineral salt, same sanitary 94 management, facilities and contact with people. Thus, the treatments correspond to 95 the milking times: before or BE (conventional time, days 1st to 7th of the experiment), 96 and late after or LA (days 26th to 39th of the experiment) (Figure 1 and 2). The 97 particularities of each farm in routine, feed management and milking, as well as 98 structural details are described below. 99

100 Farm A

101 The infrastructure consisted of main family house, milking parlor in a herringbone design, Intermag<sup>®</sup> pipeline milking machine with three clusters, which 102 was connected to the waiting room and the feeding area, with headlock and troughs. 103 The trough line was double with a length of 20 meters on each side, providing a 104 spacing of 80 cm per cow. This whole area had a concrete floor, covered, without 105 walls, with good natural ventilation, and no cooling system. Next door was a shed 106 for the storage of concentrated feed and other ingredients, in which they mixed the 107 108 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.

113 The conventional milking time adopted by the farm (before the study) was at 114 6:30 am and 5:00 pm. The usual milking routine consisted of *pre-dipping*, drying with 115 individual paper towels, putting the clusters on and *pos-dipping*. Routine milking 116 activities were maintained during the whole experiment.

At 06:00 am all cows were taken off the paddock and conducted to the milking 117 parlour. After milking, cows were supplemented with approximately 15kg/cow/day of 118 119 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 120 4:30 pm, when they were taken off the paddock and conducted to the supplementary 121 barn, where they received silage + concentrate for 30 minutes, being milked at 5:00 122 pm (Figure 1). After milking, all cows were allocated to a new paddock until the next 123 morning milking. 124

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.

128 Farm B

The infrastructure consisted of family house, a calf shed, feeding shed with a waiting room with fan, milking parlour, with Sulinox<sup>®</sup> 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 138 7:00 am and 5:00 pm. The milking routine consisted of washing and drying the teats 139 with individual paper towels, putting on the milking clusters and pos-dipping. After 140 141 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 142 supplementation, the cows were driven to a new paddock, with exclusive access to 143 pasture until 11 am. After this time, the cows had free access to the pasture and to 144 the trough with 10 kg/cow of corn silage in the feeding shed. Natural shade was 145 available at the corridor between paddocks and feeding. After afternoon milking, the 146 cows received more 10 kg/cow of corn silage and then had free access to the 147 pasture, shade, or feed shed (Figure 2). 148

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.

#### 153 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 158 5 days of observation. On each day, the behavior was evaluated for 12 hours (from 159 6:30 am to 6:30 pm). The animals were observed individually in a focal and 160 intermittent manner at 10-minutes intervals (Thurow et al., 2009). Activities were 161 recorded as grazing, rumination, idling, supplement consumption and other 162 activities, as well as posture (time spent standing or lying down), and the place at 163 the time of observation (outdoors or without cover or in the shade) (Table 1). Time 164 spent (in minutes) for each activity, the position, and the location were calculated 165 166 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 167 milking time was measured on the same days of observation of ingestive behavior. 168 It was considered as the time (in minutes) elapsed between the begging of milking 169 of the first cow and the end of milking of the last cow of the lot. 170

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#### 172 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 175 and weighed by means of a meter in the milking machine at farm B. On farm A it was 176 not possible to collect milk per cow, so some cows were sampled and values are 177 shown only to characterize the farm and the herd. Two samples of 40 mL of milk 178 were collected from each cow, in the morning milking at the farm A and the evening 179 at the farm B, according to the availability of each property and logistics for sample 180 collection and analysis. These samples were refrigerated at 4°C. One sample was 181 used for acidity determination with Dornic solution (Vidal & Saran Netto, 2018) and 182 milk stability to the alcohol test, using a solution of ethyl alcohol P.A., with ethanol 183 concentration in the test solution ranging from 72% (Brasil, 2006, 2018) to 80%, with 184 gradations increased 2% (v/v) (Zanela & Ribeiro, 2018). The second milk sample 185 was sent to the Univates Milk Laboratory in the city of Lageado-RS for the 186 determination of somatic cell count (SCC) by ISO 13366-2 method, fat, protein, 187 lactose, total solids and urea nitrogen (MUN) contents by ISO 9622/IDF141:2013 188 method. 189

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#### 191 *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). 197

#### 198 Feed composition

Concentrate composition was given by the manufacturer and samples of corn 199 and oat silage, and pasture (Table 3) were collected on days 1st and 39th of the 200 experiment on each farm and analyzed for dry matter by method 934.01, crude 201 protein (CP) by method 954.01 and acid detergent fiber (ADF) by method 973.18, all 202 according to Association of Official Analytical Chemists (AOAC, 2000). Neutral 203 detergent fiber (NDF) was obtained by method of Van Soest (1967). The samples 204 were analyzed by the Animal Nutrition Laboratory of the Federal University of Rio 205 Grande do Sul, in the city of Porto Alegre, Rio Grande do Sul, Brazil. 206

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### 208 Body weight evaluation

209 Body weight was recorded in the day 1st and 39th of experiment using cow 210 weight measuring tape (Heinrichs et al., 1992).

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#### 212 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 (%).

219

#### 220 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), 228 the statistical model for diurnal ingestive behavior included treatment (milking time), 229 cow and error as the random effects and day as repeated measures. The model 230 used was  $Y_{ijk} = \mu + MT_i + E_{ijk}$ , where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the mean 231 value of the dependent variable, MT is the is the fixed effect of the treatment (milking 232 time, n=2, before and after the change in milking time, Eijk is the experimental error, 233 considering as random effect animal and error and days as repeated measurement. 234 235 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), 236 day of measurements, interaction between treatment and day as main effects, cow 237 and error as the random effects and day as repeated measures. The model used 238

was Y<sub>ijk</sub> =  $\mu$  + MT<sub>i</sub> + DT<sub>j</sub> + MTDT <sub>ij</sub> + E<sub>ijk</sub>, where Y<sub>ijk</sub> 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<sub>ijk</sub> 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 245 afternoon were submitted to statistical analysis considering milking time (n = 2, 246 before and after milking time change), period of evaluation (n = 2, morning from 247 248 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 249 measurement. The model used was  $Y_{ijk} = \mu + MT_i + DT_j + MTDT_{ij} + E_{ijk}$ , where  $Y_{ijk}$ 250 is the dependent variable,  $\mu$  is the mean value of the dependent variable, MT is the 251 is the fixed effect of the treatment (milking time, n=2, before and after the change in 252 milking time), DT is time of the day (n = 2, morning and afternoon), MTDT<sub>ij</sub> is 253 interaction between time of the day and milking time, Eijk is the experimental error, 254 considering as random effect animal and error and days as repeated measurement. 255

All analysis were performed using the SAS<sup>®</sup> 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.

270

# 271 **Results**

#### 272 *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).

280

281 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.

285

286 Body weight

<sup>287</sup> Changing milking times did not change body weight of the cows on both <sup>288</sup> farms, A (P=0.92) and B (P=0.95).

289

290 Ingestive behavior

291 Farm A

292 Change milking time did not affect diurnal time spent grazing, ruminating and 293 idling (Table 7). Inspection of the time spent per hour (Figure 4) shows that there 294 was delay in grazing bout from 9:30am in BE to 11:30am in AC after the first milking 295 of the day as well an anticipation of the grazing bout after the second milking of the 296 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). 303 Farm B

Diurnal time spent grazing increased (P<0.0001) after changing milking time 304 (Table 9). After milking time change, grazing bouts were noticed in the before 305 morning milking and mostly after evening milking (Figure 5). Diurnal rumination and 306 idling times were different between BE and AC according to the day of measurement 307 (P≤0.0001), i.e. after milking time change, rumination time decreased at day 1 but 308 increased at day 2, with idling time showing the inverse pattern. The total 309 supplementation time decreased after milking time change (P<0.001). Diurnal lying 310 time was shorter in BE compared to AC on day 2, while opposite occurred in the total 311 time spent standing (P<0.01) (Table 9). After milking time change, the diurnal time 312 out of animal cover was greater (P<0.0001), with the opposite occurring with time in 313 shade (Table 9). 314

Between 06:30 am and 12:30 pm, grazing time was similar in AC and BE, 315 while between 12:31 pm and 6:30 pm, grazing time was greater (P<0.0001) in AC 316 compared to BE. Between 06:30 am and 12:30 pm, rumination time was similar in 317 AC compared to BE and was smaller (P<0.0001) in the second period of the day in 318 AC compared to BE. From 06:30 am to 12:30 pm the idling time was similar in AC 319 and BE, while between 12:31 pm and 6:30 pm the idling time was reduced after the 320 milking time change (P<0.0001). In both periods of the day the supplementation time 321 was smaller (P<0.0001) in AC. Animals reduced (P<0.0001) standing time in AC 322 compared to BE at both times of the day and the opposite occurred for the lying time 323

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

326

# 327 Discussion

328

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.

334

# 335 *Production variables and milk composition*

The change in milking time from conventional (between 6 to 7 am and after 5 336 pm) to 8 am and 4 pm predictably shortened the diurnal time interval between 337 milkings and increased the nocturnal time interval. It did not change the milk 338 339 production in both farms, probably because these diurnal (8 hours) and nocturnal milking intervals (16 hours) did not expressively affect the intramammary pressure 340 and the synthesis capacity, and thus, the health of the mammary gland (Lakic et al., 341 2011). According to Rémond et al. (2009), intervals between milkings of 19-17 hours 342 followed by intervals of 5-7 hours are feasible in order to maintain production without 343

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 346 requirements, may not change the physicochemical characteristics of milk, as 347 reported by Zanine et al. (2019), who offered 38.4 kg DM/cow/day, 30.3 kg 348 DM/cow/day and 26.8 kg DM/cow/day of pasture in the fall period. Miguel et al. 349 (2019) evaluated two supplementation levels (0 and 4 kg DM of 14% BW feed) at 350 tow pasture offers: low 4.9 kg DM/cow/day and high 8.5 kg DM/cow/day, similar 351 offers to the farms in this research (Table 3). These authors observed that 352 353 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 354 and milk production. 355

The differences in fat, total solids and urea nitrogen concentration observed 356 on farm B with the change in milking time may be explained by the fact that the 357 reduced intake of supplement and the increased grazing time (used in this case as 358 an indicator of increased pasture intake) increased fiber intake, explaining the 359 increased fat and total solids content. The higher urea nitrogen values after the time 360 change might be related to the higher concentration of crude protein observed in the 361 composition of the pasture at the end of the study. Nevertheless, MUN value was 362 higher than maximal threshold recommended by Onaciu et al. (2019), between 10 363 to 15 mg/dL of MUN. 364

The characteristics of the feed may influence the composition of the milk 365 (Ametaj et al., 2010; gulati et al., 2018) as well as the volume of milk produced 366 (Dineen et al., 2021). The allocation of animals in the paddock with pasture in 367 different stages can influence behavior and milk production (Pollock et al., 2022). 368 Clark et al. (2018) reported higher fat contents as the amount of concentrate was 369 reduced in cows grazing during the morning and afternoon. Higher pasture 370 consumption increased the total solids and urea content of milk (Torre-Santos et al., 371 2020). However, we recognize that in the present study pasture intake was not 372 measured, and we made some inferences about pasture intake based grazing time. 373 The similarity of the ethanol stability may be related to same nutrient intake (Gabbi 374 et al., 2018) and mild weather conditions, without causing heat stress (Abreu et al., 375 2020). Also the similarity in acidity values may be explained by the similar values of 376 crude protein and SCC of milk (ZANELA & RIBEIRO, 2018). 377

378

#### 379 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).

397 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 398 the minimum and maximum temperatures as well by THI values below 68 399 (Silanikove, 2000), despite the significant variation detected in the climatic variables. 400 Martello et al. (2013) consider 4 and 24°C as ideal for lactating cows, but because 401 of solar radiation and relative humidity, this confort temperature range can vary 402 between 7 and 21°C. THI values below 70 have been proposed as adequate for high 403 yielding dairy cows (Polsky & Von Keyserlingk, 2017). 404

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

45

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 420 animal to ingest pasture at the main grazing periods. However, when analyzing 421 activity by time of day, after milking time change, on farm A the grazing activity 422 decreased from 06:30 am to 12:00 pm, while on the farm B the grazing time was 423 similar during the morning. If we take into account the time of the main grazing 424 periods, from 5:00 to 9:00 am, changing morning milking from 6:30 or 7:00 am to 425 8:00 am (Kilgour, 2012; Pollock et al., 2022) still allowed partial overlapping of 426 grazing period with milking, that might explain the reason grazing time did not 427 increase in farm B. Similarly, Mercês et al. (2012) did not reported difference for the 428 time spent grazing when changing the morning milking time from 5:30 am to 8:00 429

am. Also, it might be worth to consider that in the south of Brazil, at the end of 430 summer when the trial was run, sunrise was delayed and consequently morning 431 main grazing occurred later (7 am to 9 am) as reported by Pollock et al. (2022) 432 compared with other studies (Kilgour, 2012; Guimarães et al., 2020). Therefore the 433 8:00 am milking time did not favor grazing at the early hours of the day, restricting 434 access to the pasture at times when greater grazing intensity would occur, similar to 435 the results of Pollock et al. (2020), when milking between 5:30 am and 7:30 am in 436 summer in Ireland. 437

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 443 probably related to the management such as supplement delivery times and 444 frequencies and shade allowance. On farm A, as described in methods and 445 materials, the animals were free to roam in the enclosure, and fed exclusively on 446 pasture. Farm B provided the animals with a mid-day supplementation of corn silage 447 at the trough, where they had access to shade. Corn silage supplementation for 448 grazing dairy cows may lead to substitution when supply is low, but does not change 449 milk yield and composition (Miguel et al., 2022). 450

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 458 explained by the similarity of the FDN values of the pasture and the supplement 459 460 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), 461 6.4 hours/day of activity, probably related to the observation period (diurnal) as 462 rumination occurs more frequently during the night (Souza et al., 2007; Mercês et 463 al., 2012; Clark et al., 2018). The increase in rumination time on farm B after the 464 change in milking time may be related to the increase in fat and solids and the 465 reduction in the amount of supplement intake, similar to results of Miguel et al. 466 (2022). 467

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 477 down during observations was below the values described in the literature, on 478 average 10.9 h/day (Thompson et al., 2019), mainly because our observation was 479 restricted to the diurnal period. Also, cows raised in grazing systems show shorter 480 lying time than confined cows (Tucker et al, 2021; Kismul et al., 2018). Lying time is 481 482 considered an indicator of comfort and welfare and can easily be changed in unfavorable conditions (Fregonesi & Leaver, 2001). Changes in the environment 483 such as air temperature, incidence of solar radiation or other stressors and changes 484 in management can influence the timing of this behavior (Tucker et al., 2021). 485

The increased time outdoors and less time in the shade during the day after 486 the change in milking time on both farms may have occurred due to the variation in 487 grazing time (Farm A) and lower values of THI for both farms. The change of milking 488 time favored grazing in the cooler moments of the day, seeking shade in the hotter 489 moments to mitigate energy expenditure to maintain homeostasis (Mercês et al., 490 2012). When animals have access to good forage availability they stay more time 491 outdoors (Kismul et al., 2018). The need for shade is related to weather conditions, 492 mainly temperature and solar radiation. The solar radiation was not measured, but 493 the temperature did not reach extreme levels (Schütz et al., 2009). Another factor 494

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).

502

# 503 Conclusion

504 Changing milking times from 6:30-7:00 am to 8:00 am and from 5:00 pm to 505 4:00 pm affects dairy cow behavior, without adversely effects on milk production and 506 most milk characteristics.

507 The alternative milking times increased the afternoon grazing time, and the 508 time spent outdoor. Other behaviors such as rumination and idling occurred to a 509 small extent and varied with little consistently between farms as well as standing and 510 lying times.

511 On farm B, changing milking time reduced time spent feeding supplement and 512 numerically supplement intake, while increased fat, total solids, MUN with out 513 changing acidity and ethanol stability. Finally, besides milking management, the 514 routine and infrastructure offered to the cows may also influence the ingestive 515 behavior and effectiveness of pasture milk production systems. 516 Repeat trials of this experiment in other seasons or other milking times may 517 elucidate the effects of these factors on ingestive behavior, production and 518 management in pasture dairy cattle.

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522

#### 523 **Ethical statement**

524 This study was approved by the Ethics Committee on Animal Use of the 525 Federal University of Rio Grande do Sul, protocol number 41758.

526

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534

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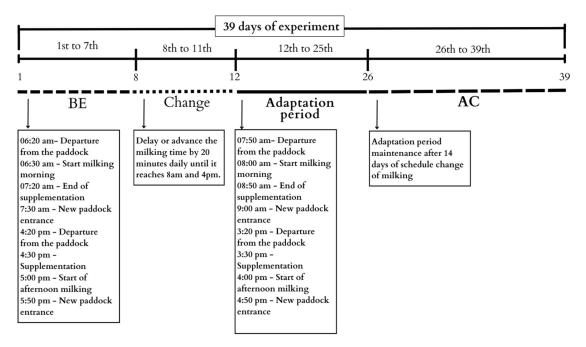


Figure 1 - Experiment timeline Farm A

BE= Before, AC= After change

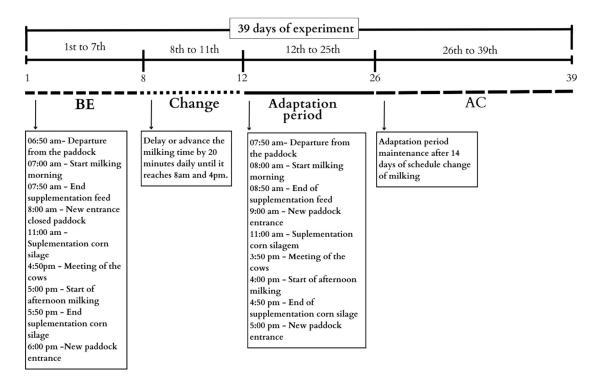
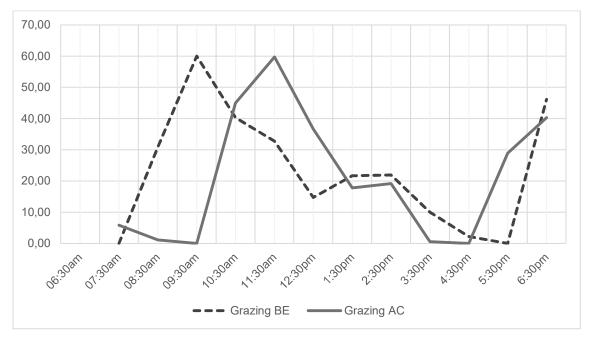


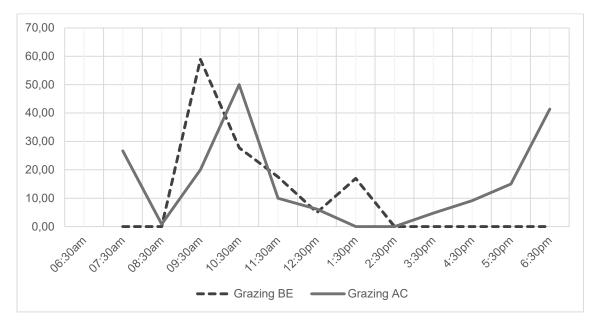
Figure 2 - Experiment timeline Farm B

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.

| 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                        |  |  |  |
|                  |   |  |  |  |

Table 1 - Ethogram describing the evaluated behavioral activities

 Shade
 Staying with majority of the body under the shaded

 area

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

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

| Farm | Food                                       | DM (%)         | CP (%)        | NFD (%)        | AFD (%)        | PS<br>(KgDM/cow/day) |
|------|--|----------------|---------------|----------------|----------------|----------------------|
|      | 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          | -                    |
| A    | 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          | -                    |
| В    | Corn Silage (BE)<br>Tifton-85 grazing (AC) | 32.14<br>25.34 | 9.20<br>22.28 | 52.95<br>68.06 | 24.17<br>29.70 | -                    |

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

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

| Farm | Days | Air temperature (°C) |      |      | Precipitation | RH       | THI   |
|------|------|----------------------|------|------|---------------|----------|-------|
|      |      | Average              | Max  | Min  | (mm)          | (mm) (%) |       |
| A    | BE   | 16.8                 | 19.4 | 14.1 | 1.8           | 95.54    | 62.13 |
|      | 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 |

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

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<br>AC  | 28.87<br>25.25 |
| SCC (cel/mL) <sup>1</sup>        | BE<br>AC  | 68.00<br>97.25 |
| Fat content (%)                  | BE<br>AC  | 2.07<br>2.68   |
| Protein content (%)              | BE<br>AC  | 2.71<br>2.95   |
| Lactose content (%)              | BE<br>AC  | 4.34<br>4.47   |
| Total solids content (%)         | BE<br>AC  | 9.93<br>11.01  |
| MUN (mg/dL) (%)                  | BE<br>AC  | 11.68<br>10.52 |
| Stability to alcohol test (°GL)² | BE<br>AC  | 69.00<br>74.00 |
| Acidity (°D)                     | BE<br>AC  | 15.50<br>17.50 |

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

Table 6 - P-value of effects and average values of composition and physicalchemical 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) <sup>1</sup>                    | 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) <sup>2</sup> | 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≤0.05). <sup>1</sup>SCC values corrected on baseline  $log_{10}$ . <sup>2</sup>Reference 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 | BE       | AC       |  |
|------------------------------|-----------|----------|----------|--|
| 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).

|                             | Day Treatme | Treatmen | Shift  | treat*shif |         |         |
|-----------------------------|-------------|----------|--------|------------|---------|---------|
| Behavior                    | shift       | t        |        | t          | BE      | AC      |
|                             |             |          | <0.000 |            | 186.11  | 145.56  |
| Grazing time (min)          | МО          | 0.0702   | 1      | 0.0003     | а       | b       |
|                             | AF          |          |        |            | 76.11   | 90.56   |
|                             |             |          | <0.000 |            |         |         |
| Rumination time (min)       | МО          | 0.0645   | 1      | <0.0001    | 64.44 a | 23.89 b |
|                             | AF          |          |        |            | 72.22   | 88.89   |
|                             |             |          | <0.000 |            |         |         |
| Lillion (the second         | 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 activites times (min) | МО          | 0.0956   | 0.0956 | 0.0617     | 40.56   | 41.11   |
|                             | AF          |          |        |            | 50.00 a | 40.56 b |
|                             |             |          | <0.000 |            | 301.67  | 361.67  |
| <b>-</b>                    | МО          | <0.0001  | 1      | 0.1546     | b       | а       |
| Standing time (min)         |             |          |        |            | 264.44  | 341.11  |
|                             | AF          |          |        |            | b       | а       |
| Lation data the control (   | МО          | <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       |

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

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;  $P \le 0.05$ ). For significant treatment\*shift interactions the means were compared BE and AC within each shift.

|                              | Experiment |           |        |           |                      |                       |
|------------------------------|------------|-----------|--------|-----------|----------------------|-----------------------|
| Behavior                     | day        | Treatment | Day    | treat*day | BE                   | AC                    |
| Grazing time (min)           | -          | <0.0001   | 0.8641 | 0.1645    | 125.281 b            | 183.89 <sup>1</sup> a |
| Rumination time (min)        | 1          | 0.1523    | 0.6539 | <0.0001   | 178.33 a             | 81.11 b               |
|                              | 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    | 83.05 <sup>1</sup> 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              |

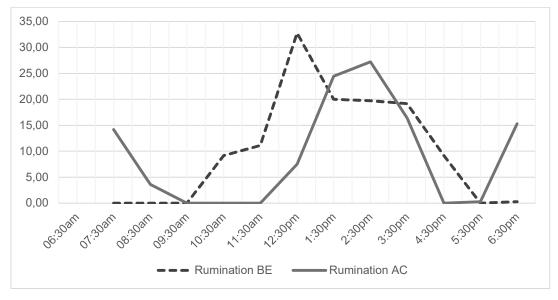
Table 9 - P-value of effects and average times of ingestive behavioral activities position and place of lactating cows with during grazing access in the treatment before (BE) and after the change (AC) on Farm B

BE= before; AC= After the change. a, b - means in the same row followed by different letters are significantly different (Ismeans; P $\leq$ 0.05). <sup>1</sup>Representative 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

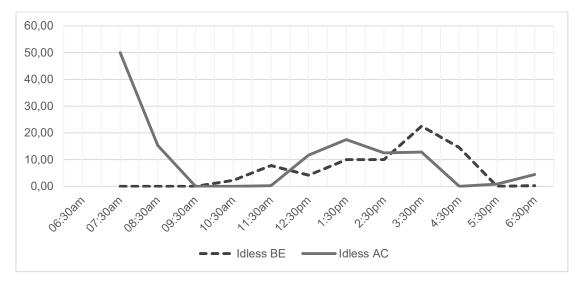
| Behavior                    | Day shift | Treatment | Shift   | treat*shift | BE       | AC       |
|-----------------------------|-----------|-----------|---------|-------------|----------|----------|
| Grazing time (min)          | MO        | <0.0001   | <0.0001 | <0.0001     | 108.33   | 113.61   |
|                             | AF        |           |         |             | 16.94 b  | 70.28 a  |
| Rumination time (min)       | МО        | 0.0110    | <0.0001 | 0.0001      | 30.28    | 37.50    |
|                             | AF        |           |         |             | 74.44 a  | 40.28 b  |
| Idling time (min)           | МО        | 0.1231    | <0.0001 | <0.0001     | 71.95    | 84.17    |
|                             | AF        |           |         |             | 65.55 a  | 38.33 b  |
| Supplementation time (min)  | МО        | <0.0001   | <0.0001 | 0.5328      | 118.06 a | 94.17 b  |
|                             | AF        |           |         |             | 60.83 a  | 40.00 b  |
| Other activites times (min) | МО        | 0.0755    | 0.0025  | 0.0396      | 40.56    | 40.00    |
|                             | AF        |           |         |             | 42.56 b  | 50.00 a  |
|                             | МО        | <0.0001   | <0.0001 | 0.7499      | 333.89 a | 299.44 b |
| Standing time (min)         |           |           |         |             |          |          |
|                             | AF        |           |         |             | 228.06 a | 196.94 b |
| Lying down time (min)       | MO        | <0.0001   | <0.0001 | 0.5371      | 36.11 b  | 70.56 a  |
|                             | AF        |           |         |             | 15.28 b  | 43.06 a  |
| Outdoor time (min)          | МО        | <0.0001   | <0.0001 | 0.4804      | 239.17 b | 275.00 a |
|                             | AF        |           |         |             | 98.06 b  | 140.28 a |
| Shade time (min)            | МО        | <0.0001   | 0.0299  | 0.2443      | 128.89 a | 94.72 b  |
|                             | AF        |           |         |             | 145.28 a | 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 $\leq$ 0.05). For significant treatment\*shift interactions the means were compared BE and AC within each shift.

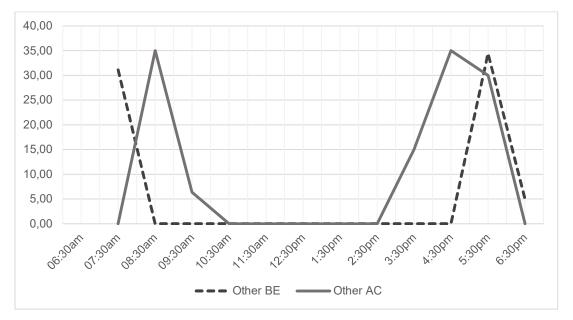


# Supplementary files

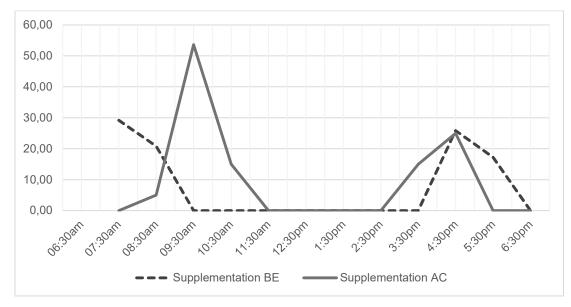
Supplementary Figure S1 - Minutes of rumination activity per hour within the observation period at farm A



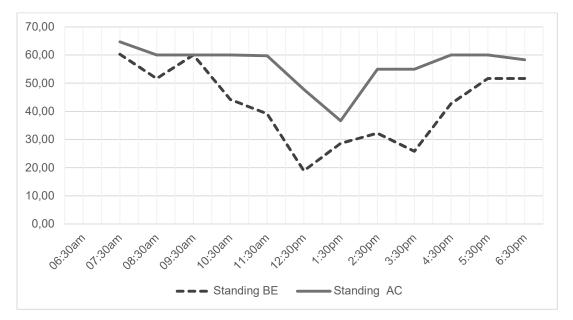
Supplementary Figure S2 - Minutes of idling activity per hour within the observation period at farm A



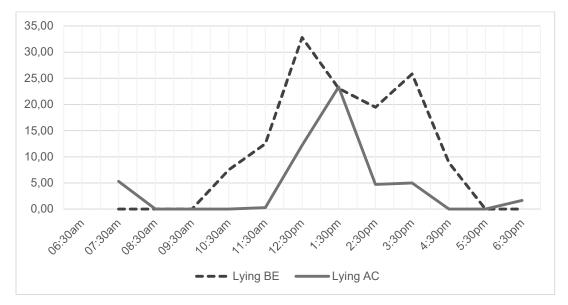
Supplementary Figure S3 - Minutes of others activities per hour within the observation period at farm A



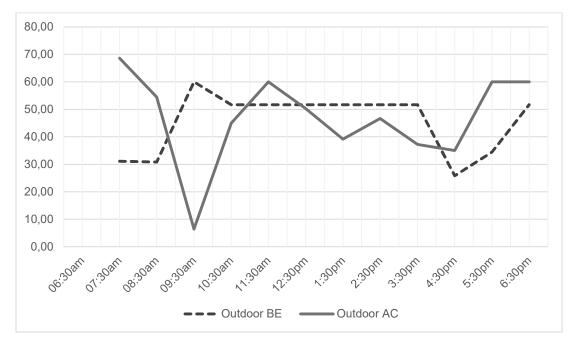
Supplementary Figure S4 - Minutes of supplementation activity per hour within the observation period at farm A



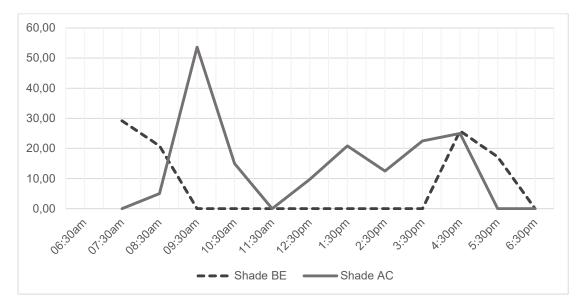
Supplementary Figure S5 - Minutes in standing position per hour within the observation period at farm A



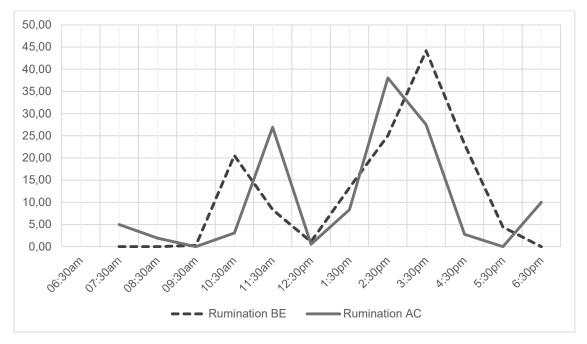
Supplementary Figure S6 - Minutes in lying position per hour within the observation period at farm A



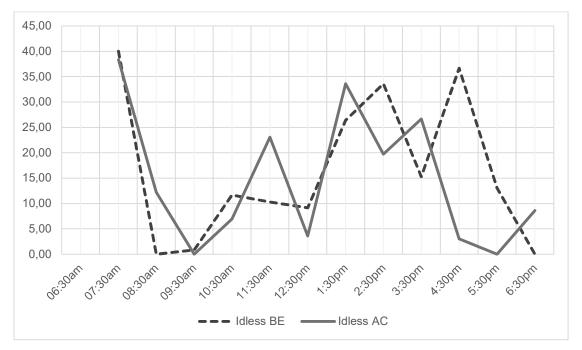
Supplementary Figure S7 - Minutes outdoors per hour within the observation period at farm A



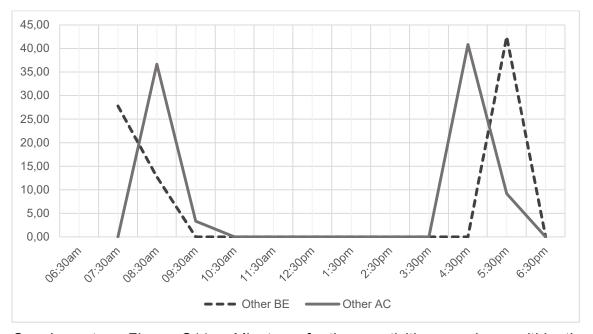
Supplementary Figure S8 - Minutes shade per hour within the observation period at farm A



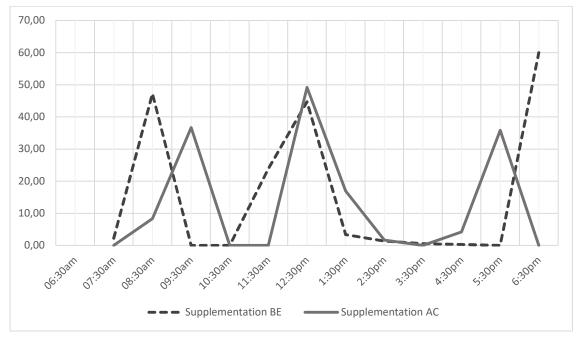
Supplementary Figure S9 - Minutes of rumination activity per hour within the observation period at farm B



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

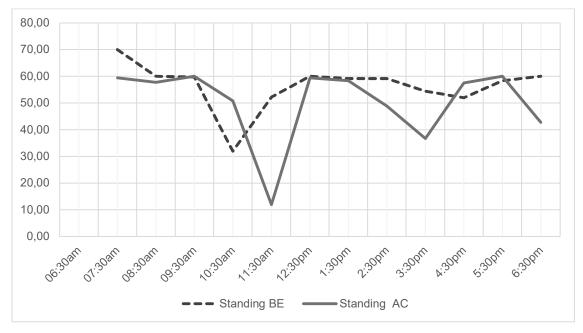


Supplementary Figure S11 - Minutes of others activities per hour within the observation period at farm B

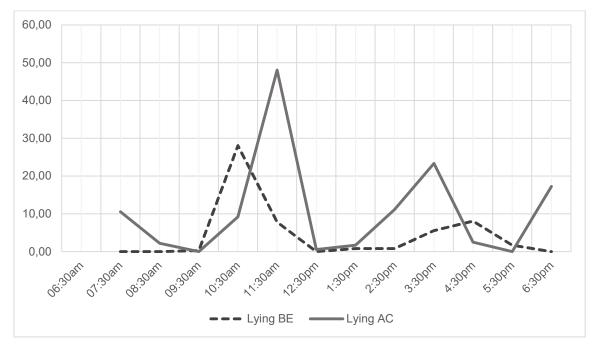


Supplementary figure S12 - Minutes of supplementation activity per hour within the observation period at farm B

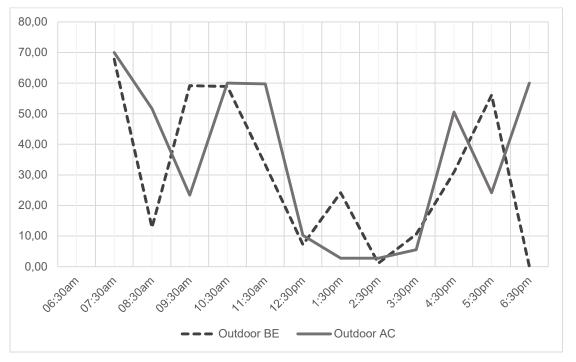
Supplementary Figure S12 - Minutes of supplementation activity per hour within the observation period at farm B



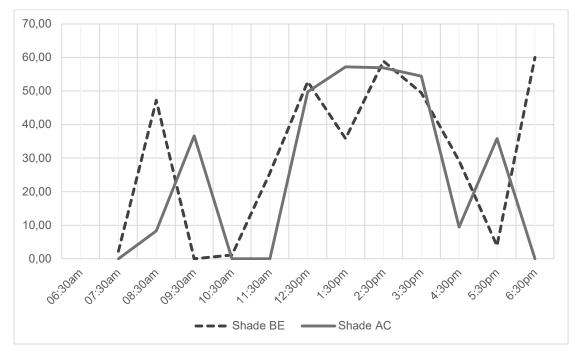
Supplementary Figure S13 - Minutes in standing position per hour within the observation period at farm B



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.

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## **APÊNDICE I**



# UFRGS

# PRÓ-REITORIA DE PESQUISA



UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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

loite de l'Vieira

MAITE DE MORAES VIEIRA Coordenador da comissão de ética

## 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.

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### PREPARATION

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- 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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# 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.

Em 2015, ingressou no curso de Bacharelado em Zootecnia na Universidade Federal do Paraná (UFPR)). No ano de 2021, concluiu sua graduação em Zootecnia e iniciou Mestrado acadêmico no Programa de Pós-Graduação em Zootecnia vinculado à Universidade Federal do Rio Grande do Sul (UFRGS), sob orientação da Prof<sup>a</sup> Dr<sup>a</sup> Vivian Fischer, sendo a área de concentração em Produção Animal e linha de pesquisa em Sistemas de Produção de Ruminantes.