UNIVERSIDADE FEDERAL DE LAVRAS

DEPARTAMENTO DE ZOOTECNIA

JOÃO PEDRO ANDRADE REZENDE

FATORES NUTRICIONAIS E COMPORTAMENTAIS ASSOCIADOS À DINÂMICA DO pH RUMINAL EM VACAS LEITEIRAS

LAVRAS – MG

2026

JOÃO PEDRO ANDRADE REZENDE

FATORES NUTRICIONAIS E COMPORTAMENTAIS ASSOCIADOS À DINÂMICA DO pH RUMINAL EM VACAS LEITEIRAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para a obtenção do título de Doutor.

Profa. Dra. Marina de Arruda Camargo Danés

Orientadora

LAVRAS – MG

2026

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Rezende, João Pedro Andrade.
Fatores nutricionais e comportamentais associados à dinâmica do pH ruminal em vacas leiteiras / João Pedro Andrade Rezende. - 2026.
148 p.

Orientadora: Marina de Arruda Camargo Danés

Tese (Doutorado) - Universidade Federal de Lavras, 2026.
Bibliografia.

1. Acidose subclínica. 2. pH ruminal. 3. Atividade mastigatória. 4. Ruminação. 5. Magnésio. I. Danés, Marina de Arruda Camargo. II. Universidade Federal de Lavras. III. Título.

JOÃO PEDRO ANDRADE REZENDE

FATORES NUTRICIONAIS E COMPORTAMENTAIS ASSOCIADOS À DINÂMICA DO pH RUMINAL EM VACAS LEITEIRAS

NUTRITIONAL AND BEHAVIORAL FACTORS ASSOCIATED WITH RUMINAL pH DYNAMICS IN DAIRY COWS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para a obtenção do título de Doutor.

APROVADA em 26 de março de 2026.

Dra. Marina de Arruda Camargo Danés, Universidade Federal de Lavras
Dr. Marcos Neves Pereira, Universidade Federal de Lavras
Dra. Polyana Pizzi Rotta, Universidade Federal de Viçosa
Dr. João Luiz Pratti Daniel, Universidade Estadual de Maringá
Dr. Jorge Henrique Carneiro, Dairy Inside Consultoria

gov.br
Documento assinado digitalmente
MARINA DE ARRUDA CAMARGO DANES
Data: 18/08/2026 13:17:31-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. Marina de Arruda Camargo Danés
Orientadora

LAVRAS – MG
2026

AGRADECIMENTOS

Primeiramente, agradeço a Deus pela vida e pela saúde para trabalhar.

Agradeço aos meus pais, Kleber e Adriana, que desde sempre me deram todo o apoio e incentivo possível para buscar meus sonhos.

Ao meu irmão Francisco, pela parceria e por compartilhar comigo o gosto por vacas leiteiras.

À minha noiva Aline, por confiar em mim até quando eu mesmo não confiava, ter me dado forças e me feito melhor desde que a conheci.

À toda minha família, que dá sentido para meus dias. Todo esse esforço é por vocês.

Agradeço à Professora Marina, por ser esse exemplo tão grande de profissional e pessoa. Muito obrigado por todo direcionamento, aprendizado, confiança, e amizade durante todos esses anos de pós-graduação.

Agradeço ao Professor Marcos, pelo exemplo e pela quantidade imensurável de conhecimento transmitida em todos esses anos de UFLA, seja em sala de aula ou na convivência em fazenda.

Agradeço ao Jorge, pelo exemplo profissional, parcerias e amizade durante esses últimos anos.

Agradeço aos demais membros da banca, Professora Polyana, Professor João Daniel, Professor Mateus e Júlia, por terem dedicado tempo para ler o trabalho e participar desse momento tão importante em minha vida, além de serem referências profissionais.

Agradeço aos grupos de estudo INPPAR, UFLALEITE e Grupo do Leite, pela convivência e por toda a ajuda durante as muitas escalas e coletas do doutorado. Em especial, agradeço ao Luiz Carlos, Maria Clara e Daniel por toda ajuda na condução do primeiro experimento dessa tese. Ao Wesley, por toda a ajuda no início do doutorado e pela amizade.

À República Chumbo Quente, que por muitas vezes deixou a vida em Lavras mais divertida.

À Universidade Federal de Lavras e ao Programa de Pós-Graduação em Zootecnia, pela excelente oportunidade.

O presente trabalho foi realizado com o apoio da Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG). Agradeço também à FAPEMIG pelo apoio financeiro concedido à rede PROTEIOS: Nutrição Proteica de Animais de Produção (RED-00052-23).

RESUMO

Este trabalho teve como objetivo integrar diferentes abordagens para investigar a ocorrência de acidose subclínica em vacas leiteiras, bem como o comportamento alimentar e estratégias nutricionais e/ou de monitoramento voltadas à sua prevenção, com potencial impacto sobre a produtividade e a saúde animal em sistemas leiteiros. Para isso, foram conduzidos três estudos complementares, combinando experimentação controlada, integração de bancos de dados experimentais e avaliação em condições comerciais. No primeiro estudo, avaliou-se o uso de fosfato de magnésio (MgP) como fonte de magnésio e fósforo para vacas em lactação. A suplementação com MgP, isoladamente ou associada ao óxido de magnésio, não alterou o consumo de matéria seca, a produção de leite ou variáveis de pH ruminal e fermentação. No entanto, maior fornecimento de magnésio aumentou a concentração de gordura do leite, associado a maiores proporções de ácidos graxos pré-formados e maior digestibilidade de extrato etéreo. No segundo estudo, foram avaliadas associações entre consumo de carboidratos, comportamento ingestivo e dinâmica do pH ruminal, utilizando dados integrados de dois experimentos com mensuração contínua de pH via bólus ruminal. A ingestão de fibra, especialmente FDN de forragem e FDN fisicamente efetiva em relação ao peso corporal, esteve positivamente associada ao tempo de ruminação e mastigação e a indicadores de maior estabilidade do pH ruminal. A ruminação modulou a relação entre ingestão de fibra e pH, enquanto efeitos de ordem de parto indicaram maior responsividade de vacas multíparas e maior susceptibilidade de primíparas a condições desfavoráveis de pH. Variáveis de pH também se associaram a desempenho produtivo, perfil de ácidos graxos do leite e marcadores fisiológicos. No terceiro estudo, avaliou-se o potencial do tempo de ruminação obtido por colares de monitoramento como ferramenta para monitoramento nutricional em nível de lote, em condições comerciais. O tempo de ruminação apresentou associação positiva com produção de leite e consumo de matéria seca, além de relação com variáveis de composição da dieta e ingestão de nutrientes, especialmente lignina. Essas associações foram mais evidentes no início da lactação, e os resultados indicam que dados de ruminação derivados de sensores podem ser utilizados como indicadores úteis do manejo nutricional em sistemas comerciais. De forma integrada, os resultados demonstram que a dinâmica do pH ruminal e sua relação com desempenho produtivo dependem da interação entre ingestão de fibra, comportamento ingestivo e características dos animais, e que ferramentas baseadas em sensores apresentam potencial para apoiar o monitoramento nutricional e a tomada de decisão em sistemas leiteiros.

Palavras-chave: acidose subclínica; pH ruminal; atividade mastigatória; ruminação; magnésio.

ABSTRACT

This work aimed to integrate different approaches to investigate the occurrence of subacute ruminal acidosis in dairy cows, as well as feeding behavior and nutritional and/or monitoring strategies for its prevention, with potential implications for productivity and animal health in dairy systems. To achieve this, three complementary studies were conducted, combining controlled experimentation, integration of experimental datasets, and evaluation under commercial conditions. In the first study, magnesium phosphate (MgP) was evaluated as a source of magnesium and phosphorus for lactating dairy cows. Supplementation with MgP, either alone or in combination with magnesium oxide, did not affect dry matter intake, milk yield, or ruminal pH and fermentation variables. However, increased dietary magnesium supply increased milk fat concentration, which was associated with greater proportions of preformed fatty acids and higher ether extract digestibility, as well as improved total-tract nutrient digestibility. In the second study, associations among carbohydrate intake, feeding behavior, and ruminal pH dynamics were evaluated using integrated data from two experiments with continuous ruminal pH measurement via bolus sensors. Fiber intake, particularly forage NDF and physically effective NDF relative to body weight, was positively associated with rumination and chewing time and with indicators of greater ruminal pH stability. Rumination modified the relationship between fiber intake and ruminal pH, whereas parity effects indicated greater responsiveness in multiparous cows and increased susceptibility of primiparous cows to unfavorable pH conditions. Ruminal pH variables were also associated with productive performance, milk fatty acid profile, and physiological markers. In the third study, the potential of collar-derived rumination time as a tool for nutritional monitoring at the pen level under commercial conditions was evaluated. Rumination time was positively associated with milk yield and dry matter intake and showed strong relationships with diet composition and nutrient intake variables, particularly lignin. These associations were more pronounced in early lactation, and the results indicate that sensor-derived rumination data can serve as useful indicators of nutritional management in commercial dairy systems. Overall, the results demonstrate that ruminal pH dynamics and their relationship with productive performance depend on the interaction among fiber intake, feeding behavior, and animal characteristics, and that sensor-based tools have potential to support nutritional monitoring and decision-making in dairy production systems.

Keywords: subacute ruminal acidosis; ruminal pH; chewing behavior; rumination; magnesium.

INDICADORES DE IMPACTO

O presente trabalho teve como objetivo investigar fatores nutricionais e comportamentais relacionados à acidose ruminal subclínica em vacas leiteiras e avaliar estratégias para seu monitoramento e prevenção. Os resultados geraram conhecimentos que contribuem para o aprimoramento do manejo nutricional, demonstrando que a suplementação de magnésio pode favorecer a digestibilidade dos nutrientes e o teor de gordura do leite. Além disso, a pesquisa evidenciou que dados de ruminação obtidos por sensores podem ser utilizados como ferramenta de apoio ao manejo nutricional e à tomada de decisão em fazendas leiteiras, contribuindo para a adoção de tecnologias de pecuária de precisão. Do ponto de vista econômico, os conhecimentos gerados fornecem subsídios para aumentar a eficiência alimentar, reduzir perdas associadas à acidose ruminal subclínica e melhorar a produtividade dos rebanhos. A pesquisa também apresenta impacto social ao disponibilizar informações que podem ser aplicadas por produtores e técnicos na melhoria do manejo dos animais. Parte dos estudos foi conduzida em fazendas comerciais parceiras, fortalecendo a integração entre a UFLA e o setor produtivo e favorecendo a transferência de conhecimento para condições reais de produção. Os impactos diretos incluem a formação de recursos humanos e a geração de conhecimento aplicado para fazendas leiteiras, enquanto os impactos potenciais envolvem a adoção das estratégias propostas por produtores, técnicos e empresas do setor. O trabalho enquadra-se principalmente nas áreas de Tecnologia e Produção e Educação da Política Nacional de Extensão e está alinhado aos Objetivos de Desenvolvimento Sustentável (ODS) da Agenda 2030 da Organização das Nações Unidas, especificamente o ODS 2 (Fome Zero e Agricultura Sustentável) e o ODS 12 (Consumo e Produção Responsáveis).

IMPACT INDICATORS

This work aimed to investigate nutritional and behavioral factors related to subclinical ruminal acidosis in dairy cows and to evaluate strategies for its monitoring and prevention. The results generated knowledge that contributes to improving nutritional management, demonstrating that magnesium supplementation can enhance nutrient digestibility and milk fat content. In addition, the research showed that rumination data obtained through sensors can be used as a tool to support nutritional management and decision-making on dairy farms, contributing to the adoption of precision livestock farming technologies. From an economic standpoint, the knowledge generated provides support for increasing feed efficiency, reducing losses associated with subclinical ruminal acidosis, and improving herd productivity. The research also has social impact by making available information that can be applied by producers and technicians to improve animal management. Part of the studies was conducted on partner commercial farms, strengthening the integration between UFLA and the productive sector and fostering knowledge transfer to real production conditions. Direct impacts include human resource training and the generation of applied knowledge for dairy farms, while potential impacts involve the adoption of the proposed strategies by producers, technicians, and companies in the sector. The work falls primarily within the areas of Technology and Production and Education of the National Extension Policy and is aligned with the Sustainable Development Goals (SDGs) of the United Nations' 2030 Agenda, specifically SDG 2 (Zero Hunger and Sustainable Agriculture) and SDG 12 (Responsible Consumption and Production).

SUMÁRIO

1. INTRODUÇÃO

O presente trabalho teve como objetivo integrar diferentes abordagens para investigar a ocorrência de acidose subclínica em vacas leiteiras, bem como o comportamento alimentar e estratégias nutricionais e/ou de monitoramento voltadas à sua prevenção, com potencial impacto positivo sobre a produtividade e a saúde animal em sistemas leiteiros. A primeira parte consiste em um referencial teórico focado na etiologia da acidose subclínica. A segunda parte reúne três artigos. O primeiro descreve um experimento conduzido para avaliar o fornecimento de fosfato de magnésio para vacas em lactação. O segundo utiliza um banco de dados integrando dois experimentos (incluindo o primeiro desta tese), nos quais foram utilizados bólus ruminais para investigar associações entre consumo de fibra, atividade mastigatória, pH ruminal, desempenho e variáveis fisiológicas. O terceiro artigo avalia o uso de colares de monitoramento de ruminação como ferramenta para monitoramento nutricional em nível de lote, em condições comerciais.

2. REFERENCIAL TEÓRICO

2.1 Acidose subclínica em vacas leiteiras

Vacas leiteiras têm alta exigência nutricional. Assim, para suprir as exigências de energia e proteína, é comum aumentar a oferta de alimentos concentrados e reduzir a quantidade de forragem nas dietas. Tipicamente, em dietas de vacas em lactação em sistemas confinados, o teor de amido varia de 20 a 30% na matéria seca (MS - NASEM, 2021). Valores próximos de 30% são frequentemente encontrados, pois aumentam o aporte de energia para o animal, aumentam a eficiência alimentar e maximizam a síntese de proteína microbiana (Hall e Derek, 2001; NASEM, 2021). No entanto, isso também aumenta o risco de acidose subclínica (ASC). Caso a prevalência de ASC em um rebanho seja alta, os benefícios de se fornecer uma dieta com alto amido não serão observados, além de que provavelmente ocorrerá queda na produção e teor de gordura (Bauman e Griinari, 2001) e proteína (Chang et al., 2018) do leite, além de aumento nos casos de deslocamento de abomaso, problemas de casco e problemas hepáticos (Plaizier et al., 2008). Nesse contexto, um dos maiores desafios na nutrição de vacas leiteiras é o balanceamento de carboidratos (considerando velocidade de degradação do amido e tamanho de partícula da fibra) da dieta para promover adequada produção de ácidos graxos de cadeia curta (AGCC) e crescimento microbiano, já que o animal possui grande demanda por energia e proteína, mas sem comprometer a saúde do trato gastrointestinal.

A ASC é um distúrbio metabólico altamente prevalente em rebanhos leiteiros. Levantamentos realizados nos Estados Unidos, Irã, Holanda e Alemanha mostraram que a prevalência de ASC, determinada quando o pH do líquido ruminal coletado por ruminocentese foi ≤ 5,5, variou de 13,8 a 27,6% (Garret et al., 1997; Tajik et al., 2009; Kleen et al., 2009; Kleen et al., 2012). Embora não haja dados semelhantes disponíveis para o Brasil, é possível especular que a prevalência de ASC em fazendas brasileiras seja ainda maior que nos outros países mencionados. Isso principalmente porque a silagem de milho, frequentemente com pequeno tamanho de partícula, é a principal forragem conservada utilizada no país (Bernardes et al., 2018; Daniel et al., 2019); a maior parte das fazendas parece não incluir uma segunda fonte de forragem na dieta (Bernardes et al., 2018); e os teores de gordura e proteína do leite no país são historicamente baixos (Rezende, 2022), mesmo com o baixo nível de produção médio relatado em levantamentos nacionais (IBGE, 2017).

Mensuração do pH ruminal e detecção de acidose subclínica

Em trabalhos que mediram a prevalência de ASC utilizando a técnica de ruminocentese, considera-se que o distúrbio metabólico ocorre quando pH do líquido ruminal ≤ 5,5 (Garret et al., 1997). No entanto, apesar de útil para levantamentos em fazendas comerciais, a mensuração de pH por ruminocentese possui limitações, como o potencial trauma ao animal e a coleta em um único horário do dia. Sabidamente, o pH ruminal varia ao longo do dia, sendo inversamente proporcional ao acúmulo de AGCC no meio (atingindo o nadir normalmente de 8 a 12 horas após a primeira alimentação – Pereira e Armentano, 2000; Resende, 2022). Resultados de pH ruminal em amostras coletadas por sonda esofágica também apresentam problemas, apesar de serem frequentemente relatado em artigos (Cueva et al., 2021; Stefenoni et al., 2021; Lobo et al., 2023). Nesse caso, as amostras podem sofrer com alta contaminação de saliva (Terré et al., 2013), o que resulta em valores de pH mais elevados. Além disso, assim como na ruminocentese, quase sempre é possível coletar apenas uma amostra por dia, já que o uso da sonda esofágica repetidas vezes aumenta o risco de traumas e, consequentemente, queda no consumo de matéria seca (CMS) e desempenho dos animais.

Assim, em estudos que avaliam estratégias de controle da ASC, metodologias mais sólidas são preferencialmente adotadas. O uso de animais com fístula de rúmen possibilita a coleta de amostras várias vezes por dia. A possibilidade de traçar a curva de pH ruminal ao longo do dia é muito útil, já que o efeito da dieta sobre o pH ruminal pode depender do tempo de amostragem. Outra vantagem evidente do uso de vacas fistuladas é a possibilidade de analisar outros parâmetros do líquido ruminal, como concentração de AGCC, ao longo do dia.

Entretanto, é importante observar que poucos centros de pesquisa possuem acesso a um grande número de vacas fistuladas. Nesse contexto, o uso de bólus eletrônicos que permitem a medição contínua do pH ruminal tem ganhado destaque em pesquisas recentes (Humer et al., 2015; Petri et al., 2019; Stauder et al., 2020; Resende, 2022).

Os bólus ficam localizados no retículo e mensuram o pH em intervalos programados, a cada 5, 10 ou 15 minutos, por exemplo. Essa informação permite traçar uma curva de pH ruminal ao longo do dia bastante robusta, desde que o equipamento em questão seja validado em pesquisa e corretamente calibrado. Assim, além da simples comparação das médias de pH ruminal entre os tratamentos, é possível avaliar valores máximos e mínimos, bem como o tempo diário em que o pH ruminal fica abaixo de 5,6; 5,8 ou qualquer outro limite. Na literatura, existem sugestões de limite de tempo diário de pH ruminal abaixo de um determinado valor específico para caracterização de ASC. Por exemplo, Plaizier et al., (2008) considera ASC quando o pH permanece < 5,6 por mais que 3 horas por dia, enquanto Zebeli et al., (2012) a considera quando o pH permanece < 5,8 por mais de 5 horas por dia.

Adicionalmente, o tempo que o animal permanece em ASC é importante para determinar a severidade desse distúrbio. Dohme et al., (2008) desafiaram vacas em lactação (restrição de consumo para 50% do *ad libitum* no dia 4 do período experimental e fornecimento de 2 kg de cevada + 2 kg de trigo antes da alimentação no dia 5) com acidose por três períodos consecutivos de 14 dias e observaram que os menores valores de pH ruminal foram encontrados no terceiro desafio (médias de pH de 6,13; 6,03 e 5,77; nadir de pH 5,52; 5,34 e 5,14; para os períodos desafio 1, 2 e 3, respectivamente). Essa maior queda em pH ao longo dos desafios provavelmente ocorre porque a repetição do quadro de ASC reduz a capacidade de absorção de AGCC do epitélio ruminal (Aschenbach et al., 2019), tornando o rúmen mais propenso ao acúmulo de ácidos. O uso de bólus de pH ruminal também é interessante nesse sentido, já que permite o monitoramento diário de pH de forma não invasiva, durante um período relativamente longo de tempo. Contudo, o uso de bólus apresenta como principais desvantagens o alto custo, a duração do sensor de pH (normalmente dura apenas um experimento) e a possiblidade do sensor mudar o seu local dentro do rúmen, pela própria motilidade. Caso isso aconteça, não é possível utilizar o dado do animal em questão, já que o sensor estará mensurando o pH em um local diferente dos outros animais, o que confunde o efeito de tratamento.

Demais variáveis e acidose subclínica

Indo além, os sintomas de ASC não se limitam à diminuição do pH ruminal. Portanto, uma avaliação dessa desordem metabólica deve levar em consideração uma combinação de variáveis, incluindo outros parâmetros do rúmen, bem como parâmetros sanguíneos, do leite e das fezes (Plaizier et al., 2018). Ainda no próprio rúmen, a ASC está associada a uma alteração da microbiota e perfil fermentativo, produção de endotoxinas, falha na função de barreira do epitélio e até rumenites em casos mais graves.

O uso de dietas com maior teor de amido e menor teor de FDN favorece a produção de propionato (produzido principalmente por bactérias amilolíticas) e desfavorece a produção de acetato (produzido principalmente por bactérias celulolítcas). Sendo assim, a relação acetato/propionato no rúmen funciona como um bom indicador do risco de ASC (Pereira e Armentano, 2000). Isso ocorre pelo efeito de substrato e pela diferença entre esses grupos de bactérias na tolerância ao abaixamento do pH. Como bactérias celulolíticas são menos resistentes ao baixo pH (Russel, 2002), uma queda na degradação de FDN e, consequentemente, menor produção de acetato são esperados com o aumento no teor de amido da dieta. A relação acetato/propionato varia de 3/1 (dietas com alto teor de FDN) para 2/1 (dietas com alto teor de amido) (Pereira e Armentano, 2000). Geralmente, o que se busca na formulação de dietas para vacas de alta produção é a redução da relação acetato/propionato no rúmen, já que isso aumenta a eficiência energética do animal. O ganho em eficiência energética ocorre porque substituir FDN por amido aumenta a digestibilidade da dieta e a conversão de energia digestível para energia metabolizável (reduz perda de energia pela produção de CH4 no rúmen). Porém, em uma relação acetato/propionato mais baixa, provavelmente a produção de AGCC no rúmen estará mais rápida, aumentando o risco de acidose.

A concentração de butirato no rúmen, que aumenta com o maior fornecimento de carboidratos rapidamente fermentáveis (principalmente sacarose - Gao e Oba, 2016), é importante para a etiologia da ASC. Em bezerros em aleitamento, o aumento da produção/suplementação de butirato aumenta o crescimento de papilas no rúmen, o que geralmente resulta em maior superfície de absorção de nutrientes e, provavelmente, melhor desempenho produtivo (Gorka et al., 2018).

Por outro lado, em vacas adultas consumindo dietas com moderado/alto teor de carboidratos rapidamente fermentáveis, o aumento exacerbado da concentração ruminal de butirato pode promover hiperqueratose e paraqueratose excessiva (Costa et al., 2008; Kauffold et al., 1977 – citado por Aschenbach et al., 2019). Tais efeitos são indesejáveis, já que a hiperqueratose reduz a capacidade metabólica (absorção) do epitélio ruminal (Baldwin e Jesse,

1991), enquanto a paraqueratose excessiva reduz a função de barreira, o que o aumenta o risco de absorção de endotoxinas. Adicionalmente, o aumento da concentração ruminal de butirato e propionato, mas não acetato (Kendall e McLeay, 1996), parece inibir motilidade ruminal pela ativação dos receptores químicos do epitélio, o que pode prejudicar a absorção de AGCC como um todo, além de causar queda no CMS.

Indo além, os efeitos do butirato sobre o epitélio ruminal e intestinal são complexos. Utilizando ratos com fístula ileal, Sakata et al., (1987) compararam os efeitos do butirato, propionato e acetato sobre a proliferação de células da parede do ceco e jejuno *in vivo* e, no mesmo experimento, coletaram tecido desses órgãos para testar os efeitos dos mesmos AGCC *in vitro*. Interessantemente, o butirato teve o maior efeito estimulatório *in vivo*, mas o maior efeito inibitório *in vitro*. Esse trabalho sugere que o butirato atua de forma direta e indireta sobre o epitélio do trato gastrointestinal.

O efeito direto acontece pelo contato desse AGCC com as células do epitélio, enquanto o efeito indireto acontece de forma sistêmica e é provavelmente mediado por hormônios. Sabe-se que o butirato possui um potente efeito positivo sobre a secreção de insulina e IGF-1 (De Jong, 1982 - citado por Fukumori et al., 2021), o que causa crescimento de papilas e aumento da capacidade absorção de AGCC do epitélio ruminal. Mais recentemente, uma relação entre butirato e o hormônio do trato gastrointestinal peptídeo-2 semelhante ao glucagon (GLP-2) também foi demonstrada (ElSabagh et al., 2017; Fukumori et al., 2021). Em vacas secas consumindo dietas de alta forragem, a suplementação de butirato de sódio (0,04% do peso corporal) durante 22 dias triplicou o GLP-2 no sangue (Fukumori et al., 2021). Essa relação entre butirato e GLP-2 pode ser importante para rebanhos com alta prevalência de ASC, já que o aumento de GLP-2 no sangue está associado a melhor função de barreira e menor resposta inflamatória no trato gastrointestinal (Baldassano e Amato, 2014).

Considerando os efeitos diretos e indiretos do butirato, é possível dizer que a influência desse AGCC no epitélio ruminal depende do balanço entre esses efeitos. Para obter uma influência benéfica, é provável que a produção de butirato no rúmen deva ser suficiente para estimular a ação hormonal, sem, no entanto, causar danos ao epitélio ruminal pelo efeito direto. Esse contexto, inclusive, ajuda a explicar por que os efeitos do butirato são geralmente positivos em bezerros, mas geram controvérsias em vacas em lactação, especialmente em dietas com teor de concentrado mais elevado. Dado que a produção de butirato é baixa em bezerros (Gorka et al., 2018), é plausível que o efeito predominante ao suplementar butirato seja o indireto. Por outro lado, em vacas em lactação que consomem dietas ricas em concentrado, a produção de

butirato já está aumentada (Aschenbach et al., 2019; Gao e Oba, 2016) e, nesse contexto, estratégias nutricionais que aumentem ainda mais podem favorecer o efeito direto.

Ainda explorando os possíveis efeitos da ASC no rúmen, uma revisão de literatura recente (Aschenbach et al., 2019) discute a relação entre a ocorrência de ASC e a função de barreira (integridade) do epitélio ruminal. Dentre os componentes da função de barreira do epitélio ruminal, que incluem fatores mecânicos e químicos, as junções de oclusão (*tight junctions*) destacam-se como um fator-chave, com o papel de atuar como uma barreira seletiva que previne a entrada de moléculas nocivas (e até bactérias) na circulação portal (Aschenbach et al., 2019). Existem algumas evidências que comprovam a influência da dieta nas junções de oclusão do epitélio ruminal. Steele et al., (2016), utilizando vacas secas, demonstraram que, ao alterar a dieta dos animais de 0 para 65% de grãos, ocorreu uma dessensibilização de proteínas transmembranas associadas às junções de oclusão do epitélio ruminal, indicando uma função de barreira comprometida.

A menor eficiência das junções de oclusão em condições de ASC é explicada pelos efeitos do baixo pH, alta concentração de AGCC e hiperosmolaridade sobre as mesmas (Aschenbach et al., 2019). Embora o pH ruminal desempenhe um papel crucial na eficiência da função de barreira do epitélio, a concentração de AGCC parece ter um papel igualmente importante. Estudando o epitélio ruminal *ex vivo*, Meissner et al., (2017) notaram que somente a redução do pH do meio para 5,1 não foi suficiente para alterar a permeabilidade do epitélio ruminal, enquanto a redução do pH para 5,1 somada à alta concentração de AGCC no meio promoveu um aumento de permeabilidade significativo. A contribuição da hiperosmolaridade para menor integridade da barreira epitelial também já foi estudada e confirmada *ex vivo* (Penner et al., 2010). Em condições *in vivo*, porém, provavelmente ocorrerão queda no pH, hiperosmolaridade e aumento da concentração de AGCC de forma simultânea, já que a concentração de AGCC é justamente a principal causadora da queda de pH e hiperosmolaridade.

Além de reduzir a integridade e função de barreira, a ASC pode reduzir a capacidade de absorção do epitélio ruminal (Aschenbach et al., 2019). Apesar de quedas moderadas de pH estimularem a absorção de AGCC (Dijkstra et al., 1993; Aschenbach et al., 2009), quedas acentuadas reduzem a absorção (Schwaiger et al., 2013). É válido salientar que a função de barreira e capacidade de absorção do epitélio ruminal representam processos que acontecem de forma paracelular e transcelular, respectivamente, e podem ser afetados de forma opostas pela ASC (Aschenbach et al., 2019). A redução da absorção de AGCC com a ocorrência de ASC

ocorre, provavelmente, para evitar a acidificação interna das células do epitélio ruminal (Aschenbach et al., 2019).

De forma bilateral, a ASC reduz a capacidade de absorção do epitélio ruminal, mas a própria menor capacidade de absorção aumenta o risco de ASC. Interessantemente, Penner et al., (2009) demonstraram que ovelhas com baixa capacidade de absorção de AGCC desenvolveram ASC rapidamente, ao receberem um *drench* oral de glicose na dose de 5 g/kg de peso corporal. Por outro lado, ovelhas com alta capacidade de absorção de AGCC receberam o mesmo *drench* e foram capazes de manter o pH ruminal acima do limite de ASC. No mesmo sentido, Schlau et al., (2012) observaram que vacas com maior tendência genética à ASC (índice de acidose 61,7 *versus* 13,5 min de pH abaixo do limiar/kg CMS) também apresentaram maior concentração ruminal de AGCC e menor capacidade de absorção.

Além dos efeitos sobre o epitélio, a ASC também está associada à maior produção e liberação de endotoxinas e aminas biogênicas (principalmente histamina) no fluído ruminal. Tal fato tem grande importância porque, somado à falha na função de barreira, a absorção desses compostos pode causar um estado de inflamação sistêmica nos animais (Aschenbach et al., 2019). Endotoxinas são uma forma de lipopolissacarídeo (LPS), componente de membrana de bactérias gram-negativas, amplamente produzidas e liberadas no meio ruminal quando o pH cai. É interessante ressaltar que o próprio aumento da concentração de endotoxinas no rúmen, e não só os outros fatores já discutidos, pode reduzir a função de barreira do epitélio ruminal (Aschenbach et al., 2019). De fato, na maioria dos trabalhos que mensuraram essa variável, o aumento de LPS no fluído ruminal se traduz em um aumento de LPS no plasma e, consequentemente, aumento de mercadores de resposta inflamatória nos animais (tabela 1). A tabela 1 traz um compilado da literatura sobre os efeitos da ASC sobre a produção de endotoxinas, aminas biogênicas e resposta inflamatória em vacas leiteiras.

Tabela 1. Efeito da acidose subclínica (ASC) sobre a concentração de endotoxinas (LPS), proteína de ligação a lipopolissacarídeo (LBP), aminas biogênicas (histamina) e resposta inflamatória em vacas leiteiras.

Estudo	Variável - Unidade	Amostragem	Controle	ASC	EPM	P valor
Khafipour et al 2009	LPS – EU/mL	Fluido ruminal	28193	111520	34590	< 0,01
Memon et al 2019	LPS – EU/mL	Fluido ruminal	47470	78430	6180	< 0,01
Gozho et al 2007	LPS – Log 10 EU/mL	Fluido ruminal	4,39	5,11	0,15	< 0,01
Zhao et al 2018	LPS – EU/mL	Fluido ruminal	30768	130589	1675	< 0,01
Khafipour et al 2009	LPS – EU/mL	Plasma	< 0,05	0,52	0,18	< 0,01

Memon et al 2019	LPS – EU/mL	Plasma arterial	0,45	0,85	0,09	< 0,01
Memon et al 2019	LPS – EU/mL	Plasma venoso	0,15	0,25	0,09	< 0,01
Zhao et al 2018	LPS – EU/mL	Plasma	< 0,05	0,21	0,06	< 0,01
Wang et al 2013	Histamina - µmol/L	Fluido ruminal	46,4	161,2	3,40	< 0,01
Wang et al 2013	Histamina - µmol/L	Plasma	2,03	7,92	0,34	< 0,01
Khafipour et al 2009	Amiloide A, µg/mL	Plasma	167,4	438,6	51,0	< 0,01
Gozho et al 2007	Amiloide A, µg/mL	Plasma	286,6	498,8	0,15	< 0,01
Khafipour et al 2009	Haptoglobina, µg/mL	Plasma	ND[1]	476	48,0	< 0,01
Gozho et al 2007	Haptoglobina, mg/mL	Plasma	0,244	0,265	0,03	0,59
Khafipour et al 2009	LBP - µg/mL	Plasma	18,2	53,1	10,8	0,02
Khafipour et al 2009	LBP - µg/mL	Leite	3,02	6,94	1,24	0,02

[1]ND = Não detectado.

Inicialmente, pensava-se que, após a absorção por falha na função de barreira do epitélio do trato gastrointestinal, LPS e/ou histamina atuavam diretamente em órgãos periféricos e causavam distúrbios de locomoção (Irwin et al., 1979). Posteriormente, foi proposto que o principal local de ação de LPS e histamina é o fígado, que possui papel-chave no desenvolvimento de um estado de inflamação sistêmica (Aschenbach et al., 2019). Sendo assim, a mensuração de algumas proteínas de fase aguda, como LBP, amiloide sérico A e haptoglobina, é utilizada para auxiliar no diagnóstico de ASC (tabela 1; Humer et al., 2018). No entanto, é necessário ponderar que alguns animais desenvolvem rápida tolerância à LPS e não apresentam a resposta inflamatória esperada (Elsasser et al., 2005), além de que outras doenças também podem promover aumento de proteínas de fase aguda no plasma (Bradford et al., 2015). Interessantemente, vacas em ASC sofreram maior queda na produção de leite e CMS quando expostas à infusão intramamária de LPS do que vacas que não estavam em ASC (Aditya et al., 2017).

É bem estabelecido na literatura que a ativação de um estado de inflamação sistêmica em vacas leiteiras possui um custo energético significativo (Kvidera et al., 2017). Por exemplo, existe relato que a concentração ruminal de LPS teve correlação negativa com produção de gordura do leite, leite corrigido para gordura e eficiência alimentar em vacas leiteiras, possivelmente pelo custo energético da inflamação sistêmica e, consequentemente, menor disponibilidade de nutrientes para a produção de leite (Dong et al., 2011). Chang et al (2018) testaram o fornecimento de uma dieta mais acidogênica (60% concentrado e 40% de forragem, 31,9% FDN e 32,3% amido *versus* 40% concentrado e 60% forragem, 37,7% FDN e 25,3% amido) por 18 semanas, reduziu expressivamente o pH ruminal, a produção de leite (28,1 *versus*

26,9 kg/d, P < 0,01) e os teores de gordura (3,54 *versus* 2,94%, P < 0,01) e proteína do leite (3,02 *versus* 2,88%). Nesse trabalho, a maior inclusão de concentrado aumentou significativamente a concentração de histidina no fluído ruminal, sangue, fígado e glândula mamária. Na glândula mamária, a maior concentração de histidina foi associada à uma maior expressão de genes relacionados à inflamação e menor expressão de genes associados à síntese de caseína.

Caso as condições dietéticas e/ou do animal façam com que uma grande quantidade de amido chegue ao intestino grosso, a acidose também pode acontecer neste compartimento do trato gastrointestinal (Steele et al., 2016; Pederzolli et al., 2018), simultaneamente ou não à sua ocorrência no rúmen. Nesse caso, a acidose intestinal também poderia causar inflamação sistêmica, seguindo um mecanismo semelhante ao proposto para o rúmen (Aschenbach et al., 2019). De fato, é possível especular que, pelo tamanho do compartimento e pelas diferenças no epitélio, o intestino grosso seja até mais sensível à falha de função de barreira que o rúmen. Como o epitélio ruminal é estratificado e o epitélio intestinal não, teoricamente pode ser mais fácil que a absorção de LPS e histamina aconteça a partir do intestino.

No entanto, recentemente diversos trabalhos com vacas leiteiras (Piantoni et al., 2022; Abeyta et al., 2023a; Abeyta et al., 2023b; Abeyta et al., 2022; Abeyta et al., 2023c; Abeyta el., 2023d), em diferentes condições de animal e dieta, induziram acidose no intestino grosso com a infusão de até 4 kg de amido puro diretamente no abomaso e não detectaram respostas consistentes em marcadores de resposta inflamatória no sangue e desempenho dos animais, o que sugere que a acidose intestinal é menos importante do que o esperado. Contudo, a acidose intestinal ainda precisa ser melhor estudada. Notavelmente, a resposta mais consistente nesses trabalhos foi em pH fecal, que reduziu em todos os tratamentos com infusão de amido no abomaso.

No leite, a ASC pode causar queda no teor e produção de gordura e proteína. A depressão de gordura do leite ocorre quando as condições do meio ruminal levam à rota alternativa de biohidrogenação dos ácidos graxos insaturados da dieta (Bauman e Griinari, 2001). A rota de biohidrogenação alternativa tem maior chance de ocorrer quando o pH ruminal está baixo e/ou a inclusão dietética de ácidos graxos insaturados, principalmente ácido linoleico, é alta (Lock et al., 2006). Essa rota alternativa leva à formação de ácidos linoleicos conjugados (CLA) intermediários altamente bioativos, que escapam do rúmen e inibem as enzimas que são responsáveis pela síntese de gordura na glândula mamária. Desses CLA bioativos, o CLA trans-10, cis-12 é o mais estudado. Baumgard et al., (2000) observaram uma acentuada queda no teor

(3,22 *versus* 2,36%) e produção de gordura do leite (1034 *versus* 742 g/dia) quando infundiram 10 g/dia de CLA trans-10, cis-12 no abomaso de vacas leiteiras. De forma mais específica, a depressão de gordura do leite (DGL) afeta de forma mais intensa a síntese de novo de ácidos graxos pela glândula mamária, reduzindo, portanto, principalmente a produção de ácidos graxos de até 16 carbonos no leite (Woolpert et al., 2017; Barbano, 2018).

Fatores dietéticos que afetam o risco de acidose subclínica

Aprofundar discussão em fisiologia de rúmen.

Para ruminantes, a fibra pode ser definida como a matéria orgânica lentamente digestível ou indigestível que ocupa espaço no rúmen (Mertens, 1997). Nesse contexto nutricional, a metodologia de análise de fibra insolúvel em detergente neutro (FDN; Van Soest, 1964 – citados por Hall e Mertens, 2017) revolucionou a nutrição de ruminantes, já que, até o momento, não existia uma forma eficaz de caracterizar quimicamente os carboidratos dos alimentos.

No entanto, o tamanho de partículas (característica física) da fibra também é importante e afeta a fisiologia do animal. Há décadas este fato é reconhecido. Porter et al., (1953), por exemplo, mostraram que o fornecimento de alfafa moída ou peletizada resultou em menor teor de gordura do leite do que o fornecimento de alfafa picada com maior tamanho de partícula. Outros trabalhos, com diferentes fontes de forragens, encontraram resultados semelhantes no teor e/ou produção de gordura do leite (Powell, 1939; Tyznik e Allen, 1951). Além dos resultados em gordura do leite, descobriu-se que o tamanho de partícula da fibra afeta sua taxa de passagem (Moore, 1964), a atividade mastigatória necessária para reduzir o tamanho de partícula (Balch, 1971; Sudweeks e tal., 1981) e a consistência do conteúdo ruminal (Welch, 1982).

Tipo da amostra (úmida ou seca). Sendo assim, Lammers et al., (1996) propuseram um conjunto de peneiras, atualmente chamado de Separador de Partículas Penn State (SPPS), composto de peneiras com crivos de 19 mm, 8 mm e um fundo, com o objetivo de monitorar o tamanho de partículas em alimentos e na dieta total misturada (TMR). Na literatura, existem propostas da adição de mais uma peneira ao SPPS, com crivos de 1,18 mm (Kononoff et al., 2003) ou 4 mm (Heinrichs, 2013). Contudo, em dietas de vacas leiteiras, a quantidade de FDN > 8 mm (FDN fisicamente efetivo – FDNfe) teve melhor correlação com tempo de ruminação do que os outros crivos mencionados (Zebeli et al., 2012). Zebeli não testou 4 mm. A metodologia do SPPS permitiu uma grande evolução no balanceamento de carboidratos para

vacas leiteiras, por padronizar a forma de mensuração do tamanho de partículas nos alimentos e dietas e, além disso, por ser uma forma prática e facilmente aplicável nas fazendas.

Por afetar a ruminação, produção de saliva, motilidade, formação de um colchão fibroso no rúmen (MAT), velocidade de absorção de AGCC e pH (Mertens, 1997; Zebeli et al., 2012), a FDNfe da dieta tem grande importância para o risco de ASC. De fato, ao realizarem uma metanálise e modelarem os efeitos de parâmetros dietéticos sobre o pH ruminal, o teor de FDNfe da dieta apareceu como um dos efeitos principais, juntamente com o teor de amido (Khorrami et al., 2021). Como esperado, maior teor de FDNfe na dieta gerou menor risco de ASC, enquanto maior teor de amido dietético causou maior risco de ASC (Khorrami et al., 2021).

É necessário fazer uma ressalva sobre a metodologia normalmente empregada para se calcular o FDNfe na TMR. Normalmente, se multiplica a porcentagem do material retida acima de 8 mm pelo teor de FDN da TMR (Khorrami et al., 2021; Lammers et al., 1996), com resultados semelhantes quando a conta é feita na matéria natural ou MS (Havekes et al., 2020). No entanto, essa metodologia assume que o teor de FDN é constante nas caixas do SPPS, o que obviamente não é verdade, já que a maior parte do concentrado se concentra no fundo. Sendo assim, os teores de FDNfe na metanálise de Khorrami et al., (2021) estão provavelmente subestimados. O mesmo conceito é válido para silagem de milho. Khorrami et al., (2021) reconhecem essa falha, mas argumentam que a maior parte dos trabalhos não reporta o teor de FDN em cada caixa do SPPS, o que torna impossível o cálculo do FDNfe real das dietas. Além disso, apesar de metodologicamente correto, mensurar o FDN de cada caixa do SPPS pode ser inviável em condições de fazenda. Essa ressalva na determinação do FDNfe tem importância para ASC, já que esse é um dos principais parâmetros da dieta que influenciarão no risco desse distúrbio metabólico. É importante que os trabalhos analisem separadamente as caixas do SPPS para que, no futuro, possamos ter melhores referências de FDNfe.

Apesar da importância amplamente reconhecida do FDNfe, o NASEM (2021) ainda traz recomendações para o mínimo de FDN de forragem (FDNF) de acordo com o teor de amido da dieta. Isso provavelmente ocorre pela falta de sistematização na mensuração de FDNfe na literatura. Como a FDNfe da dieta normalmente vem de forragens (a exceção é o caroço de algodão), essa abordagem pode fazer sentido para formulação de dietas quando o tamanho de partícula da forragem é adequado. Contudo, quando silagem de milho com pequeno tamanho de partícula é utilizada, condição comumente encontrada no Brasil (Bernardes et al., 2018; Daniel et al., 2019), a formulação por FDNF não será suficiente para minimizar o risco de ASC.

Considerando a importância do tamanho de partícula da FDN, o NASEM (2021) sugere o uso do FDN fisicamente ajustado (FDNfa; White et al., 2017). Contudo, o uso desse parâmetro exige a determinação da matéria seca de cada caixa do SPPS e ainda não foi validado em condições comerciais. O maior gasto de tempo para utilização desse parâmetro na formulação pode ser um obstáculo para seu uso rotineiro por nutricionistas. Incluir solução.

Considerando o uso do SPPS para a formulação de dietas pelo parâmetro FDNfe, uma questão importante é se partículas > 19 mm possuem a mesma efetividade física que partículas entre 8 a 19 mm. Visando responder essa questão, Piran Filho et al., (2023) utilizaram o SPPS para isolar manualmente frações de silagem de milho < 8 mm, entre 8 a 19 mm e > 19 mm. As dietas utilizadas foram 1) controle - 17% FDNF advindo de silagem de milho, com 31,5% amido e 31,9% FDN; 2) partículas < 8 mm – 17% FDNF advindo de silagem de milho + 9% de FDN advindo de partículas de silagem de milho < 8 mm, com 25,9% amido e 37,9% FDN; 3) partículas entre 8 a 19 mm – 17% FDNF advindo de silagem de milho + 9% de FDN advindo de partículas de silagem de milho entre 8 a 19 mm, com 25,5% amido e 38,3% FDN; 4) partículas > 19 mm – 17% FDNF advindo de silagem de milho + 9% de FDN advindo de partículas de silagem de milho > 19 mm, 24,9% amido e 38,8% FDN. Vacas consumindo mais partículas entre 8 a 19 mm apresentaram maior CMS e leite corrigido para energia (22,4 e 26,9 kg/dia, respectivamente) que vacas consumindo a dieta controle (20,8 e 24,7 kg/dia) e vacas consumindo mais partículas acima de 19 mm (21,2 e 24,8 kg/dia). Interessantemente, considerando as outras variáveis mensuradas no trabalho, o que parece explicar o pior desempenho de vacas consumindo mais partículas > 19 mm em relação a vacas consumindo mais partículas entre 8 a 19 mm é a maior seleção contra FDN (95,6 *versus* 97,8%), que resultou em menor CMS e, consequentemente, menor consumo de FDN (7,94 *versus* 8,37 kg/dia) e amido (5,27 *versus* 5,71 kg/dia), além de menor tempo de mastigação (657 *versus* 718 min/dia).

Dietas com alta inclusão de FDNfe são mais saudáveis e eliminam o risco de ASC, mas, por outro lado, podem limitar o consumo por enchimento físico do rúmen (NASEM, 2021) e, consequentemente, limitar a produção dos animais. Nesse sentido, em dietas modernas de ruminantes a inclusão de carboidratos não fibrosos (CNF – principalmente amido) é alta e torna o balanceamento adequado desafiador (Hall e Mertens, 2017; Plaizier et al., 2022).

Do lado de CNF, é necessário separar amido de pectina + sacarose (CNF não amiláceo), já que amido possui maior variação na degradação ruminal que CNF não amiláceo (NASEM, 2021), gera um perfil fermentativo diferente no rúmen (Bradford e Allen, 2004; Gozho e Mutsvangwa, 2008; Oba e Allen, 2003; Gao e Oba, 2016) e maximiza a síntese de Pmic (Hall

e Herejk, 2001). Dietas de vacas leiteiras em sistemas confinados normalmente variam de 20 a 30% de amido (NASEM, 2021). Além de considerar o teor de amido da dieta, é necessário considerar o quanto desse amido será degradável no rúmen e o quanto desse amido será digerido no trato total. Em geral, a meta é maximizar a degradabilidade ruminal do amido, já que isso aumenta o aporte de energia e proteína para a vaca (NASEM, 2021), além de normalmente aumentar a eficiência alimentar. No entanto, quanto maior a degradação ruminal do amido, maior o risco de ASC e maior deveria ser o teor de FDNfe da dieta (Khorrami et al., 2021).

A degradação ruminal do amido depende de diversos fatores, destacando-se tipo de grão, híbrido utilizado, maturidade à colheita do grão e processamento (Ferrareto et al., 2013). Mundialmente, o milho é a principal fonte de amido para vacas leiteiras, seja na forma de silagem de planta inteira ou na forma de milho grão. Sabidamente, a maioria do milho comercializado no Brasil possui endosperma duro e apresenta, portanto, menor degradação ruminal e menor digestão no trato total (Correia et al., 2002). Nesse contexto, o processamento do milho ganha importância como forma de otimizar o desempenho dos animais.

Por exemplo, Salvati et al., (2019) compararam silagens de planta inteira de milho colhidas por máquina autopropelida com processador de grãos com três tamanhos teóricos de corte diferentes (6, 12 e 18 mm) *versus* silagem colhida por máquina tracionada por trator sem processador de grãos (tamanho teórico de corte de 6 mm). Comparando apenas o tratamento máquina autopropelida com tamanho teórico de corte 6 mm *versus* máquina tracionada por trator, com o objetivo de isolar o efeito do processamento de grãos, os autores relataram maior digestibilidade aparente do amido no trato total (95,3 *versus* 93,1%, P = 0,04), maior concentração de glicose no plasma (64,7 *versus* 61,6 mg/dL, P = 0,03) e, consequentemente, maior produção de leite (29,4 *versus* 28,2 kg/dia, P = 0,02), maior produção de proteína do leite (956 *versus* 920 g/dia, P = 0,01), lactose (1357 *versus* 1296 g/dia, P = 0,02), maior eficiência alimentar (leite corrigido para energia/CMS; 1,54 *versus* 1,46, P = 0,04) e menor nitrogênio ureico do leite (10,3 *versus* 11,4 mg/dL, P < 0,01). Por outro lado, o maior processamento de grãos tendeu a aumentar amiloide sérico A, um marcador de resposta inflamatória, no sangue (6,0 *versus* 3,9 µg/mL, P = 0,08), indicando que o maior processamento do amido possivelmente aumentou o risco de ASC. Tamanho de partícula foi menor também, considerar isso.

No que diz respeito à relação entre processamento do milho grão, degradação ruminal do amido e digestibilidade do amido no trato total, existem inúmeros trabalhos publicados na literatura (Moharrery et al., 2014; Rafiee e Darabighane, 2021; Ferrareto et al., 2013; Castro et

al., 2019; Silva et al., 2025). Caso o processamento do grão resulte em maior digestão do amido para vacas leiteiras, a resposta mais consistente parece ocorrer em eficiência alimentar (produção de leite/CMS), seja por redução do CMS e/ou aumento da produção de leite.

Os efeitos do aumento de açúcar (sacarose) na dieta de vacas leiteiras são controversos. Hall e Mertens (2017) discutem que o aumento na produção de gordura do leite encontrado em alguns trabalhos que aumentaram o fornecimento de açúcar (Nombekele e Murphy, 1995; Broderick et al., 2008) pode ser associado à maior proporção molar de butirato no rúmen, já que esse AGCC é, juntamente com acetato, precursor para síntese de gordura do leite. Um outro possível mecanismo que relaciona teor de açúcar da dieta com produção de gordura do leite envolve a biohidrogenação de ácidos graxos insaturados no rúmen. Penner e Oba (2009), ao elevarem o nível de sacarose da dieta para 4,7%, observaram tendência de aumento na produção de gordura do leite e redução da concentração do ácido graxo trans 18:1, um marcador da rota de biohidrogenação alternativa, na gordura do leite. Isso pode ter ocorrido porque algumas bactérias responsáveis pela rota de biohidrogenação normal utilizam glicose e podem ter sido favorecidas pelo aumento de sacarose na dieta. No entanto, o aumento de butirato no rúmen pode ser indesejável do ponto de vista de ASC, como discutido anteriormente. De qualquer forma, os trabalhos que avaliaram o aumento de açúcar na dieta ainda são escassos e não existem recomendações dietéticas sólidas para esse parâmetro.

Nível de consumo, estágio de lactação e ordem de parto

Khorrami et al., (2021) realizaram uma metanálise para entender os principais fatores que afetam o risco de ASC em vacas leiteiras e, ao incluírem efeitos do animal no modelo, o CMS apareceu como efeito mais importante. Quanto maior o CMS, maior o risco de ASC. Em vacas de alta produção, o metabolismo microbiano no rúmen chega a produzir mais que 100 mols/dia de AGCC, o que é equivalente a mais que 7 kg de ácido puro (Aschenbach et al., 2011). Considerando este dado, a correlação positiva entre CMS e risco de ASC faz sentido, uma vez que o maior CMS aumenta a quantidade de substrato para fermentação ruminal e, consequentemente, aumenta o risco do acúmulo de AGCC no meio. Também é necessário ressaltar que, provavelmente, vacas com maior CMS tenham maior nível de produção, tendo, portanto, maior exigência de energia e maior risco de regulação do consumo por enchimento físico (NASEM, 2021).

Considerando a fisiologia da vaca leiteira, que muda radicalmente ao longo do ciclo produtivo (Baumgard et al., 2017), é provável que o risco de ASC também varie

consideravelmente ao longo da lactação. Com todas as mudanças abruptas (dieta, manejo, ambiente, sociais) que acontecem ao redor do parto, é plausível que o risco de ASC seja maior no pós-parto. Contudo, a condução de pesquisa nessa fase da lactação é desafiadora e os dados disponíveis ainda não são totalmente claros.

Vacas no período pós-parto necessitam de precursores de glicose (principalmente propionato, advindo da degradação ruminal de amido) para atender ao rápido aumento da produção de leite enquanto o CMS está reduzido (NASEM, 2021). No entanto, a degradação ruminal do amido pode reduzir ainda mais o CMS, pela teoria da oxidação hepática (Allen et al., 2009). Alguns experimentos testaram o teor de amido da dieta no período pós-parto (Andersen et al., 2003; Rabelo et al., 2005; Dann e Nelson, 2011; McCarthy et al., 2015; Albornoz e Allen, 2018). Andersen et al., (2003) e Rabelo et al., (2005) relataram maior CMS e maior produção de leite ao elevar o teor de amido da dieta pós-parto. É interessante notar que, nesses experimentos, o aumento de amido da dieta foi alcançado substituindo forragem por grãos de cereais. Nesse sentido, pode ser que o aumento de CMS tenha acontecido por menor risco de enchimento físico. Por outro lado, quando o aumento de amido dietético (21,5 *versus* 26,2%) foi alcançado com a substituição de subprodutos fibrosos por milho, Dann e Nelson (2011) relataram menor CMS (- 1,5 kg/dia) para a dieta de amido mais alto.

Albornoz e Allen (2018) alimentaram vacas em período pós-parto em um fatorial 2x2, em que os fatores eram degradabilidade do amido (milho seco moído *versus* milho grão úmido) e teor de amido da dieta (baixo, 22% *versus* alto, 28%). Interessantemente, o milho grão úmido reduziu o CMS (17,6 *versus* 18,6 kg/dia em baixo amido, 16,3 *versus* 20,2 kg/dia em alto amido, P para teor de amido = 0,96, P para fonte de amido = 0,01, P para interação teor x fonte de amido = 0,07). Considerando a produção de leite, o milho grão úmido teve efeito negativo independentemente do teor de amido da dieta (36,8 *versus* 41,1 kg/dia, P para fonte de amido = 0,02). Esses resultados sugerem que, no período pós-parto, vacas leiteiras são mais afetadas pela degradabilidade do amido e não simplesmente pelo teor na dieta. No entanto, os autores não observaram efeito significativo de tratamento sobre a produção de leite cumulativa até 72 dias de lactação (em média 2485 kg), o que dificulta a elaboração de recomendações para o teor de amido adequado para o pós-parto a partir desse experimento. Em outra publicação com dados de resposta inflamatória do mesmo experimento, Albornoz et al., (2020) relatam que o grão úmido só promoveu maior resposta inflamatória quando utilizado na dieta de alto amido. Infelizmente, o pH ruminal não foi mensurado nesse experimento.

É provável que o CMS no pós-parto posso ser controlado tanto pela oxidação hepática quanto pela distensão do rúmen (enchimento físico), sendo que o mecanismo dominante é dependente da disponibilidade de acetil-CoA para oxidação hepática, degradabilidade ruminal do amido e do risco de enchimento físico da dieta (NASEM, 2021). O fornecimento de fontes de amido com degradabilidade moderada (milho moído fino, por exemplo) provavelmente permitirá o fornecimento de dietas com maior teor de amido e aumentará o CMS (Albornoz e Allen, 2018), reduzindo a intensidade do balanço energético negativo dos animais (NASEM, 2021). Até o momento, pensando exclusivamente em ASC, os efeitos da composição da dieta pós-parto ainda não são bem entendidos e precisam ser melhor estudados.

Vacas em final de lactação possuem menor exigência para produção de leite e, consequentemente, estão mais propensas ao ganho de escore de condição corporal que, quando excessivo, pode prejudicar a produção de leite, eficiência reprodutivo e aumentar o risco de distúrbios metabólicos na próxima lactação (NASEM, 2021). Pensando nisso, a dieta para o final de lactação pode ser formulada com menor teor de amido e maior inclusão de subprodutos fibrosos (Firkins, 1997), o que, inclusive, reduziria o risco de ASC. No entanto, como nessa fase da lactação o CMS já está mais baixo (NASEM, 2021), essa decisão é provavelmente muito mais impactada pelo custo e disponibilidade de subprodutos fibrosos na fazenda.

Por fim, existem dados na literatura que sugerem que primíparas são mais sensíveis à ASC do que multíparas (Krause e Oetzel, 2006; Bramley et al., 2008 – citados por Humer et al., 2018). Humer et al., (2015) observaram que, quando alimentadas com a mesma dieta de 20 até 80 dias pós-parto, primíparas apresentaram maior tempo de pH reticular < 6,0 do que multíparas (370 *versus* 97 min/dia, P = 0,02). No mesmo sentido, Khorrami et al., (2021), avaliando dados internos de cinco experimentos diferentes, relataram que vacas de primeira e segunda lactação estão sob maior risco de ASC, já que apresentaram menor pH ruminal médio (- 0,20) e maior tempo de pH ruminal < 5,8/dia (dobro do tempo), mesmo com menor CMS e menor produção de leite que vacas mais velhas.

É possível especular que primíparas possuem um epitélio ruminal menos adaptado, já que consomem dietas altamente energéticas somente quando entram na primeira lactação (Humer et al., 2018). Adicionalmente, podem existir diferenças no comportamento alimentar que dependem da ordem de parto, sendo que primíparas parecem apresentar menor tempo de mastigação do que multíparas (Maekawa et al., 2002). Quando primíparas são mantidas junto com multíparas, a hierarquia social pode influenciar no risco de ASC em primíparas, já que, nesse contexto, as primíparas terão dificuldade de acesso à alimentação e provavelmente

consumirão maiores refeições de forma menos frequente ao longo do dia, o que aumenta o risco de ASC (Macmillan et al., 2017). Por fim, diferenças no peso corporal e, consequentemente, volume do rúmen podem ter um papel importante na susceptibilidade do animal à ASC. Interessantemente, Nasrollahi et al., (2017) relataram que vacas mais sensíveis à ASC eram, em média, 50 kg mais leves que vacas resistentes à ASC.

Manejo alimentar e acidose subclínica

As principais variáveis de manejo alimentar que afetam o risco de ASC são: fornecimento ou não de TMR, número de alimentações por dia, homogeneidade da TMR, consistência na composição da dieta ao longo dos dias, ocorrência de seleção por ingredientes e/ou tamanho de partículas, lotação de curral, espaço de cocho e forma de agrupamento dos animais.

De forma geral, o fornecimento de TMR para vacas leiteiras possui grandes benefícios. Segunda a revisão de Schingoethe (2017), destacam-se como benefícios: a seleção por ingredientes ou tamanho de partículas é dificultada, fazendo com que a composição da dita em cada bocado do animal seja o mais consistente possível ao longo do dia; menor risco de desordens digestivas no início da lactação, quando as vacas passam de dietas com alta forragem (pré-parto) para dietas com alto concentrado (pós-parto); o fornecimento de nitrogênio não proteico (principalmente ureia) de forma mais segura; aumenta a possibilidade do fornecimento de ingredientes/aditivos pouco palatáveis. O fornecimento de TMR também reduz o risco de ASC e depressão de gordura do leite, principalmente quando comparado com sistemas de produção a pasto que fornecem altas quantidades de concentrado durante a ordenha/de forma infrequente no dia (NASEM, 2021)

No entanto, os benefícios do fornecimento de TMR são dependentes do manejo alimentar. Ao serem confinados, os animais ficam totalmente dependentes da alimentação que está disponível no cocho. Sendo assim, para maximizar o CMS de vacas leiteiras confinadas, é fundamental que a dieta esteja disponível no cocho 24 horas por dia e que os animais tenham, no mínimo, 20 horas de acesso ao cocho (DeVries, 2017). Como o alimento precisa estar disponível 24 horas por dia, isso significa que o cocho nunca pode ficar vazio. Metas de sobras comuns em fazendas leiteiras vão de 3 a 10% do total fornecido. Caso falte alimentos no cocho por um período grande de tempo, com o fornecimento da próxima alimentação pode acontecer o que normalmente é chamado de “slug feeding”. Nesse caso, as vacas aumentam o tamanho da primeira refeição, fazendo com que uma grande quantidade de substrato chegue ao rúmen

rapidamente, causando uma produção de AGCC exacerbada e queda no pH (Resende, 2022). Essa condição é o contrário do que se busca no manejo alimentar de vacas leiteiras. Um manejo alimentar que estimule várias refeições pequenas ao longo do dia é desejável para minimizar o risco de ASC e aumentar o teor de sólidos do leite (DeVries, 2017). O CMS e a produção de leite em diversos estudos apresentaram uma relação positiva com número de refeições e tempo de ruminação (Johnson e DeVries, 2018).

Nesse sentido, o número de alimentações por dia já foi avaliado em alguns trabalhos. Os benefícios de aumentar o número de vezes que a dieta é oferecida envolvem aumentar o número de refeições por dia e reduzir o tamanho médio de refeição, com o objetivo de manter a fermentação ruminal mais estável. Contudo, os efeitos do número de alimentações são controversos. Enquanto alguns trabalhos mostraram benefícios na produção de leite e/ou teor de sólidos com o aumento do número de alimentações por dia (Woolpert et al., 2017; Macmillian et al., 2017), outros trabalhos não encontraram diferenças (Hart et al., 2014; Niu et al., 2014). Sendo assim, é plausível dizer que o número ideal de alimentações por dia depende da condição de cada fazenda. Caso existam outros problemas de manejo alimentar (baixa frequência de aproximação da dieta dos animais, baixa estabilidade aeróbia da TMR), é provável que aumentar o número de alimentações seja benéfico. No entanto, caso a fazenda tenha um bom manejo alimentar, pode ser que reduzir o número de alimentações por dia facilite a operação e reduza custos, sem, no entanto, prejudicar o desempenho dos animais.

O número de vezes que a dieta é aproximada dos animais por dia parece bastante importante para maximizar o CMS, a produção de leite e reduzir a ocorrência de comportamento de seleção. Vários levantamentos realizados em fazendas comerciais no Canadá associaram essa variável de manejo alimentar com produção de leite e comportamento animal. King et al., (2016) relataram + 0,1 hora/dia de tempo da vaca deitada para 2 aproximações da dieta extras/dia, sendo que, em média, as fazendas aproximavam a dieta 8 vezes/dia. Matson et al., (2021) relataram + 0,35 kg de leite/dia para cada 5 aproximações da dieta extras/dia, sendo que, em média, as fazendas aproximavam a dieta 12,8 vezes/dia. Por fim, Castro et al., (2022) mostraram que fazendas que possuíam robô de aproximar a dieta dos animais de forma automática tiveram média de produção de leite maior (+ 2,1 kg/dia) do que fazendas que não possuíam esse robô. Nesse trabalho, os autores discutem que o que explica o aumento de leite não é especificamente o robô, mas sim a consistência que esse traz para o manejo.

Idealmente, em uma TMR com adequada qualidade de mistura, cada bocado deveria ter a mesma composição da dieta formulada. Mas, na realidade, vacas selecionam ingredientes e/ou tamanho de partícula, na maioria das vezes contra as partículas mais longas (Miller-Cushon e DeVries, 2017; Piran-Filho et al., 2019). Miller-Cushon e DeVries (2017) verificaram que para cada 10% de seleção contra partículas longas, aconteceu queda de 0,10 unidades percentuais no teor de gordura do leite. Essa relação entre seleção contra partículas longas e teor de gordura do leite faz sentido, uma vez que provavelmente a seleção contra partículas longas leva ao consumo de uma dieta com menor teor de FDNfe e maior teor de amido degradável no rúmen do que o planejado pela dieta formulada. A baixa qualidade de mistura da TMR pode estimular a o comportamento de seleção (Oelberg e Stone, 2014), seja por erros na ordem de carregamento do vagão, tempo de mistura inadequado ou o uso de feno inteiro, com tamanho de partícula grande. Em uma dieta de alta palha de trigo para vacas secas, a redução do tamanho de partícula da palha de trigo resultou em maior CMS e menor comportamento de seleção (Havekes et al., 2020). A ordem de carregamento e tempo de mistura dependem do modelo de vagão (horizontal ou vertical) e de quais ingredientes (densidade, tamanho de partícula, umidade) estão sendo utilizados (Oelberg e Stone, 2014).

O teor de umidade da TMR também afeta o comportamento de seleção. Leonardi et al., (2005) relataram que a adição de água para reduzir a MS da dieta de 81 para 64% resultou em menor comportamento de seleção, com tendência de aumentar o consumo de FDN e o teor de gordura do leite, sem, no entanto, afetar o CMS ou a produção de leite. Por outro lado, a adição de água para reduzir a MS da dieta de 56 para 51 ou 44%, durante os meses quentes de verão, aumentou o comportamento de seleção e reduziu o CMS de vacas em lactação, provavelmente porque o aumento de umidade piorou a estabilidade aeróbia da TMR (Felton e DeVries, 2010). Apesar de ser uma estratégia adotada por muitas fazendas, a adição de água à TMR só parece se justificar caso a dieta tenha teor de MS muito alto, o que dificilmente acontecerá em dietas à base de silagem de milho colhida com teor de MS adequado (30 a 40%). Por sua alta relevância prática, esse é um tópico que merece mais estudos.

O comportamento alimentar dos animais também pode ser afetado pela hierarquia social dentro do lote. Vacas dominantes, normalmente mais pesadas e maiores, passam mais tempo comendo do que vacas de posição hierárquica inferior, especialmente quando o espaço de cocho é limitado (Albright, 1993). A competição no cocho parece aumentar o tamanho de refeição e reduzir a frequência de refeição (Hosseinkhani et al., 2008), o que também pode ser associado a “slug feeding” e à ASC. Também por hierarquia social, o agrupamento de primíparas junto

com multíparas piora o desempenho das primíparas. Existe um relato na literatura de maior produção de leite (+ 725 kg na lactação) quando primíparas estavam separadas, em vez de misturadas com multíparas (Phelps e Drew, 1992 – citados por NASEM, 2021).

A recomendação clássica de espaço de cocho mínimo para vacas leiteiras é de 46 cm/animal (Albright, 1993), mas referências mais modernas sugerem um mínimo de 60 cm/animal para minimizar o risco de ASC (Humer et al., 2018). No entanto, é provável que o espaço de cocho ideal dependa de outros vários aspectos do manejo alimentar, como, por exemplo, agrupamento dos animais e tempo de acesso ao alimento por dia (NASEM, 2021). Adicionalmente, a posição do cocho parece ser importante. Albright (1993) mostra evidências que vacas se alimentando com a cabeça abaixada produzem 17% mais saliva que vacas se alimentando com a cabeça na posição horizontal.

Bicarbonato de sódio

Introduzir melhor.

Uma metanálise (Hu e Murphy, 2005) avaliou os efeitos do bicarbonato de sódio (BicNa) sobre vacas em lactação. O banco de dados incluiu 27 estudos, publicados entre 1980 e 1999, que incluíram dados de 30 experimentos, 73 tratamentos e 369 vacas. Em média, o CMS foi de 18,5 kg/dia e a produção de leite de 29,3 kg/dia, com 3,34% de gordura e 3,21% de proteína. Em dietas baseadas em silagem de milho, o BicNa aumentou o CMS (+ 1,24 kg/dia, P = 0,02), o teor de gordura do leite (+0,27 pontos percentuais, P = 0,02) e a produção de gordura do leite (+ 105 g/dia, P = 0,01), mas não afetou a produção de leite, teor ou produção de proteína. Ainda em dietas à base de silagem de milho, o BicNa aumentou o pH ruminal médio (+0,13, P = 0,01) e a relação acetato/propionato (+0,26, P = 0,04), além de reduzir a concentração de propionato no rúmen (-23,8 mmol/mol, P = 0,04). Quando comparada com a dose média de BicNa (0,7 a 1% da MS), a dose alta (1,05 a 1,5%) não teve efeito sobre nenhum parâmetro avaliado, o que sugere que a dose ótima de BicNa está entre 0,7 a 1,0% da MS. Interessantemente, nas dietas que não eram baseadas em silagem de milho o BicNa não apresentou efeito sobre nenhuma das variáveis analisadas. Apesar de não ter sido relatado nesse trabalho, isso provavelmente sugere que o efeito do BicNa depende do teor de amido da dieta.

Diversas teorias tentam explicar os efeitos do BicNa sobre o desempenho e o risco de ASC em vacas leiteiras. O maior consumo de Na parece resultar em maior concentração de Na na urina (Hu e Murphy, 2004) ou maior excreção diária de urina (Resende, 2022), com possível aumento no consumo de água (Edwards e Poole, 1983). Ao realizarem infusão de BicNa no

rúmen de ovelhas, Harrison et al (1975) relataram aumento na taxa de passagem de líquida, que foi associado a maior fluxo de amido para fora do rúmen e maior relação acetato/propionato. Considerando os resultados desses trabalhos em conjunto, o maior consumo de água pode ser associado a uma maior taxa de passagem no rúmen, o que aumentaria a passagem de amido (provavelmente digerido no intestino delgado) e AGCC (absorvidos no omaso), reduzindo o risco de ASC. A maior taxa de passagem no rúmen, inclusive, explicaria a maior produção de proteína microbiana com o fornecimento de BicNa, observada por Resende (2022). Apesar desse mecanismo ser frequentemente comentado na literatura, poucos trabalhos realmente forneceram BicNa e mensuraram consumo de água e taxa de passagem.

Em vacas de alta produção, o metabolismo microbiano no rúmen chega a produzir mais que 100 mols/dia de ácidos graxos de cadeia curta (AGCC), o que é equivalente a mais que 7 kg de ácido puro (Aschenbach et al., 2011). Normalmente, a inclusão de BicNa na dieta varia de 0,7 a 1,0% da MS (Hu e Murphy, 2005). Em um consumo de 25 kg de MS/dia, uma vaca comeria 250 g/dia de BicNa (dose 1% MS). Como o bicarbonato de sódio possui 84,1 g/mol, isso significa que essa vaca comeria apenas 2,97 mols desse neutralizante, o que sugere que os efeitos do fornecimento de bicarbonato de sódio vão além da neutralização de ácidos no rúmen.

É interessante notar que a absorção de AGCC no rúmen é afetada por Na, seja pelo mecanismo de absorção na forma protonada ou dissociada. Ao ser absorvido na forma protonada, o AGCC rapidamente libera o H^+ no interior da célula do epitélio ruminal, sendo que esse H^+ sai da célula em uma troca dependente de Na^+ (Aschenbach et al., 2011). Como acúmulo de H^+ no meio intracelular tem consequências gravíssimas para a função celular, é provável que, com a falta de Na^+, a absorção de AGCC seja interrompida (Burhans et al., 2022). Adicionalmente, a menor disponibilidade de Na^+ no rúmen pode reduzir a absorção de AGCC pelo mecanismo dependente de $HCO3^-$, já que o transporte do $HCO3^-$ do sangue para a célula do epitélio ruminal é dependente do Na^+ (Aschenbach et al., 2011).

Indo além, os efeitos do bicarbonato de sódio também podem ser explicados de forma sistêmica. Uma metanálise (Hu e Murphy, 2004) avaliou os efeitos da diferença catiônica-aniônica da dieta (DCAD) sobre a gasometria do sangue e desempenho de vacas leiteiras. O DCAD foi calculado considerando os efetios dos cátions Na e K e do ânion Cl. O efeito do S, bem como o efeito de todos os outros íons dietéticos, não foi considerado. O DCAD médio foi de 261,3 mEq/kg MS, com valor mínimo de – 191,0 e máximo de 635,7 mEq/kg MS. No sangue, o aumento de DCAD afetou o pH (aumento quadrático, $P < 0,01$, $R^2 = 0,83$), a concentração de bicarbonato (HCO3 - aumento quadrático, $P < 0,01$, $R^2 = 0,88$) e a quantidade

de CO2 dissolvida (pCO2 – aumento linear, $P < 0,01$, $R^2 = 0,35$). Os autores discutem que a resposta em pH sanguíneo provavelmente reflete a resposta de HCO3, considerando a equação de Hendersson-Hasselbach [pH sanguíneo = 6,1 + log/10 (HCO3/(0,03 x pCO2)]. Portanto, o aumento de HCO3 aumenta o pH, enquanto o aumento de pCO2 reduz. Nesse sentido, a resposta em pCO2 indica que houve uma compensação respiratória ao aumento de HCO3 no sangue, já que a regulação do pH sanguíneo é fundamental para a sobrevivência do animal. O efeito da diferença catiônica-aniônica do sangue (BCAD) sobre os parâmetros de gasometria também foi avaliado. Como esperado, o aumento de BCAD aumentou o pH (efeito linear, $P < 0,01$, $R^2 = 0,72$) e HCO3 (efeito linear, $P < 0,01$, $R^2 = 0,85$) no sangue.

Interessantemente, o DCAD também afetou o desempenho dos animais (Hu e Murphy, 2004). Em média, o CMS dos animais foi de 18,41 kg/dia e a produção de leite de 22,98 kg/dia, com 3,68% de gordura e 3,18% de proteína. O aumento do DCAD foi associado ao aumento quadrático do CMS, produção de leite, leite corrigido para 4% de gordura (LCG4%) e produção de gordura do leite. O maior CMS, produção de leite, LCG4% e produção de gordura do leite ocorreram com DCAD de 396, 336, 489 e 550 mEq/kg MS, respectivamente. A resposta máxima em produção de leite foi de + 1,6 kg/dia.

Apesar de não isolar o efeito do Na, a metanálise sobre DCAD para vacas em lactação de Hu e Murphy (2004) traz informações interessantes de como íons dietéticos (Na, K e Cl) podem afetar a gasometria do sangue, que ajudam a explicar os efeitos sistêmicos do fornecimento de BicNa (Goff, 2018). Além disso, os resultados de desempenho com o aumento do DCAD da dieta (Hu e Murphy, 2004) são consistentes com os efeitos relatados com o fornecimento de BicNa (Hu e Murphy, 2005), principalmente em CMS e produção de gordura do leite.

Óxido de magnésio

O óxido de magnésio (MgO) é, depois do BicNa, o principal agente neutralizante utilizado na dieta de vacas leiteiras (NASEM, 2021). É importante ressaltar que, diferentemente do que acontece com BicNa, o processamento do MgO afeta seus efeitos no ambiente ruminal e a sua absorção (Khiaosa-arda et al., 2023). Enquanto para Na o coeficiente de absorção é de 100% para todas as fontes, o coeficiente de absorção do Mg em MgO é de apenas 23% (NASEM, 2021), sendo esse um valor médio, já que a solubilidade de um MgO pode variar de acordo com o tamanho de partícula e temperatura de calcinação (Khiaosa-arda et al., 2023).

Alguns trabalhos recentes avaliaram o fornecimento de MgO para vacas leiteiras (Resende, 2022; Bach et al., 2023; Razzaghi et al., 2022). Resende (2022) comparou o fornecimento de um MgO com processamento especial com BicNa ou uma dieta controle, sem inclusão de agentes neutralizantes. A dose de MgO foi de 0,5% da MS, enquanto a dose de BicNa foi de 1% da MS. O experimento foi conduzido em um quadrado latino 3x3, com períodos de 21 dias. No dia 17 de cada período, a dieta foi removida do cocho às 21:00 horas e, no dia 18, o teor de amido dietético foi abruptamente aumentado de 22,6 para 31,6% da MS, com o objetivo de induzir acidose ruminal. Contrastes foram utilizados para comparar controle *versus* MgO e BicNa *versus* MgO. Não foram encontradas diferenças na produção de leite (27,5 kg/dia) ou CMS (22,6 kg/dia), mas o MgO tendeu a aumentar o teor de gordura do leite (3,81%) quando comparado com o controle (3,63%) e com BicNa (3,65%). A digestibilidade do extrato etéreo (EE) no trato total tendeu a ser maior para MgO do que para o controle, o que sugere que o aumento do Mg da dieta pode ter levado a formação de sabões de Mg no rúmen e, consequentemente, passagem de maior quantidade de AG insaturados para o intestino. Ainda no trabalho de Resende (2022), o pH reticular mínimo tendeu a ser maior para MgO (5,90) do que para o controle (5,82). Interessantemente, esse resultado em pH aconteceu mesmo que as vacas consumindo MgO tenham tendido a passar menos tempo ruminando que o controle (460 *versus* 489 min/dia). A concentração de Mg no sangue foi maior para MgO do que para os outros dois tratamentos. De fato, o maior consumo de Mg (por maior teor na dieta) normalmente resulta em maior absorção e, consequentemente, maior concentração desse no sangue (Khiaosa-arda et al., 2023).

Bach et al., (2023) testaram o mesmo produto de MgO que Resende (2022). Nesse experimento (Bach et al., 2023), o MgO (dose de 0,25% da MS) foi comparado com uma dieta controle, sem adição de neutralizantes, ou com uma dieta com 0,82% de BicNa. Quinze vacas por tratamento foram avaliadas de forma contínua, por 63 dias. Após o fornecimento de uma dieta basal por 7 dias, a cada 14 dias a quantidade de forragem da dieta foi diminuída (retirando feno de festuca) e a quantidade de concentrado (incluindo cevada) foi aumentada. A relação forragem/concentrado da dieta passou pelos níveis de 48/52; 44/56; 40/60 e 36/64. A interação entre agente neutralizante (MgO ou BicNa) e relação forragem/concentrado da dieta foi significativa para CMS, eficiência alimentar, parâmetros de comportamento ingestivo e tempo diário de pH ruminal < 5,8. O CMS não diferiu entre tratamentos quando a relação forragem/concentrado foi de 48/52 ou 44/56, mas foi maior para o MgO quando a relação forragem/concentrado foi de 40/60 e 36/64. Por outro lado, a produção de leite e leite corrigido

para energia reduziu com a diminuição da relação forragem/concentrado da dieta, independente do tratamento. O tempo de pH ruminal diário < 5,8 não foi afetado por tratamento nas relações forragem/concentrado 48/52, 44/56 e 40/60, mas foi menor para MgO do que para BicNa quando a relação forragem/concentrado foi de 36/64. De forma bastante interessante, quando consideraram os últimos 7 dias de cada período e fizeram uma média de pH ruminal por hora, os autores notaram que pH ruminal das vacas consumindo BicNa foi maior que o pH ruminal das vacas consumindo MgO de 6 a 9 horas após a primeira refeição do dia. Contudo, o pH ruminal das vacas consumindo MgO foi maior que o das vacas consumindo BicNa de 11 a 23 horas após a primeira refeição do dia. Como nessas horas o pH ruminal estava mais baixo, é provável que a solubilidade do MgO tenha aumentado, explicando assim a diferença de efeito no pH do meio ao longo do tempo.

Por fim, Razzaghi et al., (2022) induziram DGL (dieta com 35,2% de amido e 4,8% de ácidos graxos totais na MS) e, posteriormente, avaliaram o fornecimento de MgO (0,4% da MS) ou de calcário dolomítico (0,8% da MS) na recuperação da DGL. Nesse trabalho, o fornecimento das duas fontes de Mg aumentou rapidamente (a partir de 4 dias do fornecimento) o teor e produção de gordura do leite, que foram associados a uma menor relação entre os ácidos graxos trans-10/trans-11 no leite, indicando que o mecanismo envolveu a rota de biohidrogenação de ácidos graxos insaturados no rúmen. A retirada dos suplementos de Mg dieta rapidamente causou DGL novamente (a partir de 7 dias), reduzindo o teor e produção de gordura do leite. Infelizmente, esse trabalho não mensurou pH ruminal.

Avaliando esses dados em conjunto, o fornecimento de MgO parece ser uma estratégia interessante para reduzir o risco de ASC e/ou aumentar a produção de gordura do leite. No entanto, a fonte de MgO e a dieta basal devem ser cuidadosamente consideradas.

REFERÊNCIAS

ABEYTA, M. A. et al. Effects of hindgut acidosis on inflammation, metabolism, and productivity in lactating dairy cows fed a high-fiber diet. Journal of Dairy Science, v. 106, n. 4, p. 2879-2889, 2023.

ABEYTA, M. A. et al. Effects of hindgut acidosis on metabolism, inflammation, and production in dairy cows consuming a standard lactation diet. Journal of Dairy Science, v. 106, n. 2, p. 1429-1440, 2023.

ABEYTA, M. A. et al. Effects of hindgut acidosis on production, metabolism, and inflammatory biomarkers in previously immune-activated lactating dairy cows. Journal of Dairy Science, v. 106, n. 6, p. 4324-4335, 2023.

ABEYTA, M. A. et al. Effects of hindgut acidosis on production, metabolism, and inflammatory biomarkers in feed-restricted lactating dairy cows. Journal of Dairy Science, v. 106, n. 4, p. 2890-2903, 2023.

ABOU-RJEILEH, Ursula et al. Oleic acid abomasal infusion limits lipolysis and improves insulin sensitivity in adipose tissue from periparturient dairy cows. Journal of Dairy Science, v. 106, n. 6, p. 4306-4323, 2023.

ADITYA, S. et al. Intramammary infusion of Escherichia coli lipopolysaccharide negatively affects feed intake, chewing, and clinical variables, but some effects are stronger in cows experiencing subacute rumen acidosis. Journal of Dairy Science, v. 100, n. 2, p. 1363-1377, 2017.

AHVANOOEI, MR Rezaei et al. Effects of monensin supplementation on rumen fermentation, methane emissions, nitrogen balance, and metabolic responses of dairy cows: a systematic review and dose–response meta-analysis. Journal of Dairy Science, 2023.

ALBORNOZ, R. I. et al. Diet starch concentration and starch fermentability affect markers of inflammatory response and oxidant status in dairy cows during the early postpartum period. Journal of Dairy Science, v. 103, n. 1, p. 352-367, 2020.

ALBORNOZ, Rodrigo I.; ALLEN, Michael S. Highly fermentable starch at different diet starch concentrations decreased feed intake and milk yield of cows in the early postpartum period. Journal of dairy science, v. 101, n. 10, p. 8902-8915, 2018.

ALBRIGHT, J. L. Feeding behavior of dairy cattle. Journal of dairy science, v. 76, n. 2, p. 485-498, 1993.

ALLEN, M. S.; BRADFORD, B. J.; OBA, M. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of animal science, v. 87, n. 10, p. 3317-3334, 2009.

ANDERSEN, J. B. et al. The effects of low vs. high concentrate level in the diet on performance in cows milked two or three times daily in early lactation. Livest. Prod. Sci. 81:119–128. 2003.

APPER-BOSSARD, E. et al. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regulation in dairy cows receiving 2 contrasting levels of concentrate in diets. Journal of dairy science, v. 93, n. 9, p. 4196-4210, 2010.

APPUHAMY, JAD Ranga Niroshan et al. Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. Journal of Dairy Science, v. 96, n. 8, p. 5161-5173, 2013.

ASCHENBACH, J. R. et al. 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. J. Anim. Sci. 89:1092–1107.

ASCHENBACH, J. R. et al. Ruminant nutrition symposium: Role of fermentation acid absorption in the regulation of ruminal pH. Journal of animal science, v. 89, n. 4, p. 1092-1107, 2011.

ASCHENBACH, Jörg R. et al. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. Journal of Dairy Science, v. 102, n. 2, p. 1866-1882, 2019.

ATTEBERY, John T.; JOHNSON, Harold D. Effects of environmental temperature, controlled feeding and fasting on rumen motility. Journal of animal science, v. 29, n. 5, p. 734-737, 1969.

BACH, A. et al. Associations between nondietary factors and dairy herd performance. Journal of dairy science, v. 91, n. 8, p. 3259-3267, 2008.

BACH, Alex et al. Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacity. Journal of Dairy Science, 2023.

BACH, Alex et al. Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacity. Journal of Dairy Science, 2023.

BACH, C. C. 1971. Proposal to use time spent chewing as an index of the extent of which diets for ruminants possess the physical property of fibrousness characteristic of roughages. Br. J. Nutr. 26:383–392.

BALDASSANO, Sara; AMATO, Antonella. GLP-2: what do we know? What are we going to discover? Regulatory peptides, v. 194, p. 6-10, 2014.

BALDWIN, RL t; JESSE, B. W. Isolation and characterization of sheep ruminal epithelial cells. Journal of animal science, v. 69, n. 9, p. 3603-3609, 1991.

BALES, A. M. et al. Effect of increasing dietary inclusion of whole cottonseed on milk production of high-producing dairy cows. Abstract. American Dairy Science Association Meeting, 2023.

BARBANO, D. et al. New Milk Analysis Metrics for Dairy Herd Management. 2018.

BAUMAN, D. E; GRIINARI, J. M. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. **Livestock Production Science**, v. 70, n. 1-2, p. 15-29, 2001.

BAUMGARD, L. H.; COLLIER, Robert J.; BAUMAN, D. E. A 100-Year Review: Regulation of nutrient partitioning to support lactation. Journal of Dairy Science, v. 100, n. 12, p. 10353-10366, 2017.

BAUMGARD, Lance H. et al. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, v. 278, n. 1, p. R179-R184, 2000.

BAUMGARD, Lance H.; RHOADS JR, Robert P. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci., v. 1, n. 1, p. 311-337, 2013.

BECKER, C. A.; COLLIER, R. J.; STONE, A. E. Invited review: Physiological and behavioral effects of heat stress in dairy cows. Journal of dairy science, v. 103, n. 8, p. 6751-6770, 2020.

BEEDE, D. K.; COLLIER, R. J. Potential nutritional strategies for intensively managed cattle during thermal stress. Journal of animal science, v. 62, n. 2, p. 543-554, 1986.

BERNARDES, T. F. et al. Silage review: Unique challenges of silages made in hot and cold regions. Journal of dairy science, v. 101, n. 5, p. 4001-4019, 2018.

BLOCK, Elliot. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. Journal of dairy science, v. 67, n. 12, p. 2939-2948, 1984.

BRADFORD, B. J. et al. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. Journal of dairy science, v. 98, n. 10, p. 6631-6650, 2015.

BRADFORD, B. J; ALLEN, M. S. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. Journal of Dairy Science, v. 87, n. 11, p. 3800-3807, 2004

BRAMLEY, E. et al. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. Journal of dairy science, v. 91, n. 1, p. 308-321, 2008.

BRODERICK, G. A. et al. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. Journal of dairy science, v. 91, n. 12, p. 4801-4810, 2008.

BURHANS, W. S.; BURHANS, CA Rossiter; BAUMGARD, L. H. Invited review: Lethal heat stress: The putative pathophysiology of a deadly disorder in dairy cattle. Journal of Dairy Science, v. 105, n. 5, p. 3716-3735, 2022.

CARNEIRO, Jorge Henrique; SANTOS, Junio Fabiano dos; ALMEIDA, Rodrigo de. Accuracy and physical characteristics of total mixed rations and feeding sorting behavior in dairy herds of Castro, Paraná. Revista Brasileira de Zootecnia, v. 50, p. e20200174, 2021.

CASTRO, Lucas P. et al. Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and proportion in the diet. Journal of dairy science, v. 102, n. 11, p. 9857-9869, 2019.

CASTRO, M. M. D. et al. Association of housing and management practices with milk yield, milk composition, and fatty acid profile, predicted using Fourier transform mid-infrared spectroscopy, in farms with automated milking systems. Journal of Dairy Science, v. 105, n. 6, p. 5097-5108, 2022.

CHANG, Guangjun et al. Histamine activates inflammatory response and depresses casein synthesis in mammary gland of dairy cows during SARA. BMC veterinary research, v. 14, n. 1, p. 1-9, 2018.

CHARBONNEAU, E.; PELLERIN, D.; OETZEL, G. R. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. Journal of dairy science, v. 89, n. 2, p. 537-548, 2006.

CHEN, Lianmin et al. Megasphaera elsdenii lactate degradation pattern shifts in rumen acidosis models. Frontiers in microbiology, v. 10, p. 162, 2019.

CLAYTON, E. H. et al. Effects of feeding virginiamycin and sodium bicarbonate to grazing lactating dairy cows. Journal of dairy science, v. 82, n. 7, p. 1545-1554, 1999.

COCITO, C. Antibiotics of the virginiamycin family, inhibitors which contain synergistic components. Microbiological Reviews, v. 43, n. 2, p. 145-192, 1979.

CONTE, Giuseppe et al. Feeding and nutrition management of heat-stressed dairy ruminants. Italian Journal of Animal Science, v. 17, n. 3, p. 604-620, 2018.

CONTRERAS-JODAR, Alexandra et al. Heat stress modifies the lactational performances and the urinary metabolomic profile related to gastrointestinal microbiota of dairy goats. PLoS One, v. 14, n. 2, p. e0202457, 2019.

CORREA, C. E. S. et al. Relationship between corn vitreousness and ruminal in situ starch degradability. Journal of dairy science, v. 85, n. 11, p. 3008-3012, 2002.

COSTA, S. F. et al. Alterações morfológicas induzidas por butirato, propionato e lactato sobre a mucosa ruminal e a epiderme de bezerros: I Aspectos histológicos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 60, p. 1-9, 2008.

CRUYWAGEN, C. W. et al. The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. Journal of dairy Science, v. 98, n. 8, p. 5506-5514, 2015.

CUEVA, S. F. et al. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. Journal of dairy science, v. 104, n. 9, p. 9827-9841, 2021.

DANIEL, João Luiz Pratti et al. Production and utilization of silages in tropical areas with focus on Brazil. Grass and forage Science, v. 74, n. 2, p. 188-200, 2019.

DANN, H. M.; NELSON, B. H. Early lactation diets for dairy cattle–focus on starch. Department of Animal Science at the New York State College of Agriculture and Life Sciences (A Statutory College of the State University of New York) Cornell University, v. 46, 2011.

DE JESUS, E. Ferreira et al. Influence of a blend of functional oils or monensin on nutrient intake and digestibility, ruminal fermentation and milk production of dairy cows. Animal Feed Science and Technology, v. 219, p. 59-67, 2016.

DE JONG, A. Patterns of plasma concentrations of insulin and glucagon after intravascular and intraruminal administration of volatile fatty acids in the goat. journal of Endocrinology, v. 92, n. 3, p. 357-370, 1982.

DE SOUZA, J. Balanceamento de ácidos graxos em dietas de vacas leiteiras. Anais do IX SIMLEITE. Simpósio Internacional de Bovinocultura Leiteira. Universidade Federal de Viçosa. 2023.

DE SOUZA, J. et al. Predicting the concentration and yield of milk fatty acids from diet nutrient composition in dairy cows. Abrasct. American Dairy Science Association Meeting, 2018.

DE SOUZA, J.; LOCK, A. L. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. Journal of dairy science, v. 101, n. 4, p. 3044-3056, 2018.

DE SOUZA, J.; LOCK, A. L. Long-term palmitic acid supplementation interacts with parity in lactating dairy cows: Production responses, nutrient digestibility, and energy partitioning. Journal of dairy science, v. 101, n. 4, p. 3044-3056, 2018.

DE SOUZA, J.; PRESEAULT, C. L.; LOCK, A. L. Altering the ratio of dietary palmitic, stearic, and oleic acids in diets with or without whole cottonseed affects nutrient digestibility, energy partitioning, and production responses of dairy cows. Journal of dairy science, v. 101, n. 1, p. 172-185, 2018.

DE SOUZA, J.; PROM, C. M.; LOCK, A. L. Altering the ratio of dietary palmitic and oleic acids affects production responses during the immediate postpartum and carryover periods in dairy cows. Journal of Dairy Science, v. 104, n. 3, p. 2896-2909, 2021.

DESNOYERS, Marion et al. Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science, v. 92, n. 4, p. 1620-1632, 2009.

DEVRIES, T. J. Ensuring access to feed to optimize health and production of dairy cows. Large Dairy Herd Management. 3rd edition. 2017.

DIJKSTRA, J. et al. Ruminal pH regulation and nutritional consequences of low pH. Animal Feed Science and Technology, v. 172, n. 1-2, p. 22-33, 2012.

DIJKSTRA, Jan et al. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. British Journal of Nutrition, v. 69, n. 2, p. 385-396, 1993.

DOHME, F et al. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Ruminal pH. Journal of dairy science, v. 91, n. 9, p. 3554-3567, 2008.

DONG, Guozhong et al. Diet-induced bacterial immunogens in the gastrointestinal tract of dairy cows: impacts on immunity and metabolism. Acta Veterinaria Scandinavica, v. 53, p. 1-7, 2011.

DUFFIELD, T. F.; RABIEE, A. R.; LEAN, I. J. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. Journal of dairy science, v. 91, n. 4, p. 1334-1346, 2008.

DUFFIELD, T. F.; RABIEE, A. R.; LEAN, I. J. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. Journal of Dairy Science, v. 91, n. 4, p. 1347-1360, 2008.

EDWARDS, Sandra A.; POOLE, D. A. The effects of including sodium bicarbonate in the diet of dairy cows in early lactation. Animal Science, v. 37, n. 2, p. 183-188, 1983.

Elsasser, T. H., J. W. Blum, and S. Kahl. 2005. Characterization of calves exhibiting a novel inheritable TNF-alpha hyperresponsiveness to endotoxin: Associations with increased pathophysiological complications. J. Appl. Physiol. 98:2045–2055.

FELTON, C. A.; DEVRIES, T. J. Effect of water addition to a total mixed ration on feed temperature, feed intake, sorting behavior, and milk production of dairy cows. Journal of Dairy Science, v. 93, n. 6, p. 2651-2660, 2010.

FERRARETTO, L. F.; CRUMP, P. M.; SHAVER, R. D. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. Journal of dairy science, v. 96, n. 1, p. 533-550, 2013.

FIEVEZ, V. et al. Milk odd-and branched-chain fatty acids as biomarkers of rumen function—An update. Animal Feed Science and Technology, v. 172, n. 1-2, p. 51-65, 2012.

FIRKINS, Jeffrey L. Effects of feeding nonforage fiber sources on site of fiber digestion. Journal of Dairy Science, v. 80, n. 7, p. 1426-14

FUKUMORI, Rika et al. The effect of sodium butyrate supplementation on ruminal and fecal pH and serum lipopolysaccharide-binding protein after ruminal acidosis challenge in nonlactating cows. Animal Science Journal, v. 92, n. 1, p. e13673, 2021.

GAO, X.; OBA, M. Effect of increasing dietary nonfiber carbohydrate with starch, sucrose, or lactose on rumen fermentation and productivity of lactating dairy cows. Journal of Dairy Science, v. 99, n. 1, p. 291-300, 2016.

Garrett, E. F., K. V. Nordlund, W. J. Goodger, and G. R. Oetzel. A cross-sectional field study investigating the effect of periparturient dietary management on ruminal pH in early lactation dairy cows. J. Dairy Sci. 80(Suppl. 1):169.(Abstr.), 1997.

GOFF, J. P.; RUIZ, R.; HORST, R. L. Relative acidifying activity of anionic salts commonly used to prevent milk fever. Journal of dairy science, v. 87, n. 5, p. 1245-1255, 2004.

GOFF, Jesse P. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of dairy science, v. 101, n. 4, p. 2763-2813, 2018.

GÓRKA, Paweł et al. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. Journal of dairy science, v. 101, n. 6, p. 4785-4800, 2018.

GOZHO, G. N. et al. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. Journal of dairy science, v. 90, n. 2, p. 856-866, 2007.

GOZHO, G. N; MUTSVANGWA, T. Influence of carbohydrate source on ruminal fermentation characteristics, performance, and microbial protein synthesis in dairy cows. Journal of Dairy Science, v. 91, n. 7, p. 2726-2735, 2008.

HALL, M. B.; HEREJK, C. Differences in yields of microbial crude protein from in vitro fermentation of carbohydrates. Journal of Dairy Science, v. 84, n. 11, p. 2486-2493, 2001.

HALL, Mary Beth; MERTENS, David R. A 100-year review: Carbohydrates—Characterization, digestion, and utilization. Journal of dairy science, v. 100, n. 12, p. 10078-10093, 2017.

HARRISON, D. G. et al. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. The Journal of Agricultural Science, v. 85, n. 1, p. 93-101, 1975.

HART, K. D. et al. Effect of frequency of feed delivery on the behavior and productivity of lactating dairy cows. Journal of Dairy Science, v. 97, n. 3, p. 1713-1724, 2014.

HAVEKES, C. D. et al. Effects of wheat straw chop length in high-straw dry cow diets on intake, health, and performance of dairy cows across the transition period. Journal of dairy science, v. 103, n. 1, p. 254-271, 2020.

Havekes, C. D., T. F. Duffield, A. J. Carpenter, and T. J. DeVries. 2020. Effects of wheat straw chop length in high-straw dry cow diets on intake, health, and performance of dairy cows across the transition period. J. Dairy Sci. 103:254–271.

HEINRICHS, Jud; KONONOFF, P. The Penn state particle separator. Penn State Extension, University Park, PA. DSE, v. 186, p. 1-8, 2013.

HOSSEINKHANI, A. et al. The effects of feed bunk competition on the feed sorting behavior of close-up dry cows. Journal of dairy science, v. 91, n. 3, p. 1115-1121, 2008.

HU, W.; MURPHY, M. R. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. Journal of dairy science, v. 87, n. 7, p. 2222-2229, 2004.

HU, W.; MURPHY, M. R. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. Journal of dairy science, v. 87, n. 7, p. 2222-2229, 2004.

HU, Wenping; MURPHY, Michael R. Statistical evaluation of early-and mid-lactation dairy cow responses to dietary sodium bicarbonate addition. Animal feed science and technology, v. 119, n. 1-2, p. 43-54, 2005.

HUMER, E. et al. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of dairy science, v. 101, n. 2, p. 872-888, 2018.

HUMER, E. et al. Peripartal changes in reticuloruminal pH and temperature in dairy cows differing in the susceptibility to subacute rumen acidosis. Journal of dairy science, v. 98, n. 12, p. 8788-8799, 2015.

HUMER, E. et al. Signals for identifying cows at risk of subacute ruminal acidosis in dairy veterinary practice. Journal of animal physiology and animal nutrition, v. 102, n. 2, p. 380-392, 2018.

IBGE. Censo Agropecuário 2017. 2019. Disponível em: <https://biblioteca.ibge.gov.br/visualizacao/periodicos/3096/agro_2017_resultados_definitivos.pdf>. Acesso em: 25/11/12023.

IRWIN, Lyndon N. et al. Histamine, tyramine, tryptamine and electrolytes during glucose induced lactic acidosis. Journal of Animal Science, v. 48, n. 2, p. 367-374, 1979.

IWANIUK, M. E.; ERDMAN, R. A. Intake, milk production, ruminal, and feed efficiency responses to dietary cation-anion difference by lactating dairy cows. Journal of dairy science, v. 98, n. 12, p. 8973-8985, 2015.

JENKINS, T. C. Lipid metabolism in the rumen. Journal of dairy science, v. 76, n. 12, p. 3851-3863, 1993.

JENKINS, T. Fatty acids in diets of dairy cattle. Webinar. 2016. Disponível em: https://www.youtube.com/watch?v=CJx_Hkc2vx8. Acesso em: 27/11/2023.

JITTAKHOT, Surasak et al. Effect of high magnesium intake on apparent magnesium absorption in lactating cows. Animal feed science and technology, v. 113, n. 1-4, p. 53-60, 2004.

JOHNSTON, C.; DEVRIES, T. J. Associations of feeding behavior and milk production in dairy cows. Journal of dairy science, v. 101, n. 4, p. 3367-3373, 2018.

KADZERE, Charles T. et al. Heat stress in lactating dairy cows: a review. Livestock production science, v. 77, n. 1, p. 59-91, 2002.

KAUFFOLD, P.; VOIGT, J.; HERRENDÖRFER, G. The effect of nutritional factors on the ruminal mucosa. 3. Condition of the mucosa after infusion of propionic acid, acetic acid and butyric acid. Archiv fur Tierernahrung, v. 27, n. 3, p. 201-211, 1977.

KHAFIPOUR, E.; KRAUSE, D. O.; PLAIZIER, J. C. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. Journal of dairy science, v. 92, n. 3, p. 1060-1070, 2009.

KHIAOSA-ARDA, Ratchaneewan et al. Magnesium in dairy cattle nutrition: a meta-analysis on magnesium absorption in dairy cattle and assessment of simple solubility tests to predict magnesium availability from supplemental sources. Journal of Dairy Science, 2023.

KHORRAMI, Behzad; KHIAOSA-ARD, Ratchaneewan; ZEBELI, Qendrim. Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: A meta-analysis. Journal of Dairy Science, v. 104, n. 7, p. 7761-7780, 2021.

KING, M. T. M. et al. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. Journal of dairy science, v. 99, n. 11, p. 9069-9079, 2016.

KLEEN, J. L.; CANNIZZO, C. Incidence, prevalence and impact of SARA in dairy herds. Animal Feed Science and Technology, v. 172, n. 1-2, p. 4-8, 2012.

KLEEN, Joachim Lübbo et al. Subacute ruminal acidosis in Dutch dairy herds. Veterinary Record, v. 164, n. 22, p. 681-684, 2009.

KNAPP, D. M.; GRUMMER, Ric R. Response of lactating dairy cows to fat supplementation during heat stress. Journal of dairy science, v. 74, n. 8, p. 2573-2579, 1991.

KNOWLTON, K. F.; HERBEIN, J. H. Phosphorus partitioning during early lactation in dairy cows fed diets varying in phosphorus content. Journal of Dairy Science, v. 85, n. 5, p. 1227-1236, 2002.

KOCH, Franziska et al. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. Proceedings of the National Academy of Sciences, v. 116, n. 21, p. 10333-10338, 2019.

KONONOFF, P. J.; HEINRICHS, A. J.; BUCKMASTER, D. R. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. Journal of dairy science, v. 86, n. 5, p. 1858-1863, 2003.

KRAUSE, K. Marie; OETZEL, Garrett R. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Animal feed science and technology, v. 126, n. 3-4, p. 215-236, 2006.

KUNG JR, L. et al. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. Journal of Dairy science, v. 91, n. 12, p. 4793-4800, 2008.

KVIDERA, S. K. et al. Glucose requirements of an activated immune system in lactating Holstein cows. Journal of dairy science, v. 100, n. 3, p. 2360-2374, 2017.

LAMBERT, G. P. et al. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. Journal of applied physiology, v. 92, n. 4, p. 1750-1761, 2002.

LAMMERS, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922–928.

LASCANO, Gustavo J. et al. Changes in fermentation and biohydrogenation intermediates in continuous cultures fed low and high levels of fat with increasing rates of starch degradability. Journal of Dairy Science, v. 99, n. 8, p. 6334-6341, 2016.

LEONARDI, C.; ARMENTANO, L. E. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. Journal of Dairy Science, v. 86, n. 2, p. 557-564, 2003.

LEONARDI, C.; GIANNICO, F.; ARMENTANO, L. E. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. Journal of Dairy Science, v. 88, n. 3, p. 1043-1049, 2005.

LOBO, Richard Roberto et al. Production, physiological response, and calcium and magnesium balance of lactating Holstein cows fed different sources of supplemental magnesium with or without ruminal buffer. Journal of Dairy Science, v. 106, n. 2, p. 990-1001, 2023.

LOCK, Adam L. et al. A conjugated linoleic acid supplement containing trans-10, cis-12 reduces milk fat synthesis in lactating sheep. Journal of Dairy Science, v. 89, n. 5, p. 1525-1532, 2006.

LOPEZ, H. et al. Effect of dietary phosphorus on performance of lactating dairy cows: milk production and cow health. Journal of Dairy Science, v. 87, n. 1, p. 139-145, 2004.

LUCAS, H. L.; LOOSLI, J. K. The effect of fat upon the digestion of nutrients by dairy cows. Journal of Animal Science, v. 3, n. 1, p. 3-11, 1944.

MA, N. et al. A high-concentrate diet induces an inflammatory response and oxidative stress and depresses milk fat synthesis in the mammary gland of dairy cows. Journal of Dairy Science, v. 105, n. 6, p. 5493-5505, 2022.

MACMILLAN, K.; GAO, X.; OBA, M. Increased feeding frequency increased milk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. Journal of Dairy Science, v. 100, n. 2, p. 1045-1054, 2017.

MACMILLAN, K.; GAO, X.; OBA, M. Increased feeding frequency increased milk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. Journal of Dairy Science, v. 100, n. 2, p. 1045-1054, 2017.

MAEKAWA, M.; BEAUCHEMIN, K. A.; CHRISTENSEN, D. A. Chewing activity, saliva production, and ruminal pH of primiparous and multiparous lactating dairy cows. Journal of dairy science, v. 85, n. 5, p. 1176-1182, 2002.

MATSON, R. D. et al. Benchmarking of farms with automated milking systems in Canada and associations with milk production and quality. Journal of Dairy Science, v. 104, n. 7, p. 7971-7983, 2021.

MCCARTHY, M. M. et al. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. Journal of Dairy Science, v. 98, n. 5, p. 3335-3350, 2015.

MCGUFFEY, R. K.; RICHARDSON, L. F.; WILKINSON, J. I. D. Ionophores for dairy cattle: current status and future outlook. Journal of Dairy Science, v. 84, p. E194-E203, 2001.

MCGUIRK, S. M.; BUTLER, D. G. Metabolic alkalosis with paradoxic aciduria in cattle. Journal of the American Veterinary Medical Association, v. 177, n. 6, p. 551-554, 1980.

MCLEAY, Lance M.; PASS, M. A. Inhibitory effects of short intravascular infusions of propionate on reticulo-rumen motility in the sheep. Comparative Biochemistry and Physiology Part A: Physiology, v. 115, n. 1, p. 63-71, 1996.

MCNEIL, C. J. et al. Whole-body and splanchnic amino acid metabolism in sheep during an acute endotoxin challenge. British Journal of Nutrition, v. 116, n. 2, p. 211-222, 2016.

MEISSNER, Svenja et al. Key role of short-chain fatty acids in epithelial barrier failure during ruminal acidosis. Journal of dairy science, v. 100, n. 8, p. 6662-6675, 2017.

MEMON, Meena Arif et al. Lipopolysaccharide induces oxidative stress by triggering MAPK and Nrf2 signalling pathways in mammary glands of dairy cows fed a high-concentrate diet. Microbial Pathogenesis, v. 128, p. 268-275, 2019.

MERTENS, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463–1481.

MILLEN, D. D. et al., Acidose Ruminal. Publicado de forma online. Edição 2022.

MILLER-CUSHON, E. K.; DEVRIES, T. J. Feed sorting in dairy cattle: Causes, consequences, and management. Journal of dairy science, v. 100, n. 5, p. 4172-4183, 2017.

MILLS, Katelyn E.; WEARY, Daniel M.; VON KEYSERLINGK, Marina AG. Identifying barriers to successful dairy cow transition management. Journal of dairy science, v. 103, n. 2, p. 1749-1758, 2020.

MOALLEM, U. Invited review: Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. Journal of dairy science, v. 101, n. 10, p. 8641-8661, 2018.

MOHARRERY, A.; LARSEN, MOGENS; WEISBJERG, MARTIN RIIS. Starch digestion in the rumen, small intestine, and hind gut of dairy cows–A meta-analysis. Animal Feed Science and Technology, v. 192, p. 1-14, 2014.

Mongin, P. 1981. Recent advances in dietary anion-cation balance: Applications in poultry. Proc. Nutr. Soc. 40:285–294

MOORE, L. A. 1964. Symposium on forage utilization: Nutritive value of forage as affected by physical form. Part I. General principles involved with ruminants and effect of feeding pelleted or wafered forage to dairy cattle. J. Anim. Sci. 23:230–238.

MORETTI, Riccardo et al. Heat stress effects on Holstein dairy cows' rumination. Animal, v. 11, n. 12, p. 2320-2325, 2017.

NAGARAJA, T. G.; TAYLOR, M. B. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. Applied and Environmental Microbiology, v. 53, n. 7, p. 1620-1625, 1987.

NAGARAJA, T. G.; TITGEMEYER, E. C. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. Journal of dairy science, v. 90, p. E17-E38, 2007.

NASEM. **Nutrient Requirements of Dairy Cattle: Eighth Revised Edition**. 2021. Washington, DC: The National Academies Press.

NASROLLAHI, S. M. et al. Variability in susceptibility to acidosis among high producing mid-lactation dairy cows is associated with rumen pH, fermentation, feed intake, sorting activity, and milk fat percentage. Animal Feed Science and Technology, v. 228, p. 72-82, 2017.

NEVILLE, E. W. et al. Effects of calcareous marine algae on milk production, feed intake, energy balance, mineral status, and inflammatory markers in transition dairy cows. Journal of Dairy Science, v. 105, n. 8, p. 6616-6627, 2022.

NIU, M. et al. The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows. Journal of dairy science, v. 97, n. 12, p. 7764-7776, 2014.

NOMBEKELA, S. W.; MURPHY, M. R. Sucrose supplementation and feed of dairy cows in early lactation. Journal of dairy science, v. 78, n. 4, p. 880-885, 1995.

NRC. **Nutrient requirements of dairy cattle: 2001**. Washington, DC: National Academies Press, 2001.

OBA, M; ALLEN, M. S. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. Journal of Dairy Science, v. 86, n. 1, p. 174-183, 2003.

OELBERG, Thomas J.; STONE, William. Monitoring total mixed rations and feed delivery systems. Veterinary Clinics: Food Animal Practice, v. 30, n. 3, p. 721-744, 2014.

OLIVEIRA, C. A.; MILLEN, D. D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. Animal Feed Science and Technology, v. 197, p. 64-75, 2014.

PALMQUIST, D. L.; JENKINS, T. C. A 100-Year Review: Fat feeding of dairy cows. Journal of dairy science, v. 100, n. 12, p. 10061-10077, 2017

PEDERZOLLI, Rae-Leigh A. et al. Effect of ruminal acidosis and short-term low feed intake on indicators of gastrointestinal barrier function in Holstein steers. Journal of Animal Science, v. 96, n. 1, p. 108-125, 2018.

PENNER, G. B. et al. A single mild episode of subacute ruminal acidosis does not affect ruminal barrier function in the short term. Journal of dairy science, v. 93, n. 10, p. 4838-4845, 2010.

PENNER, G. B. et al. Ruminant Nutrition Symposium: Molecular adaptation of ruminal epithelia to highly fermentable diets. Journal of Animal Science, v. 89, n. 4, p. 1108-1119, 2011.

PENNER, G. B.; OBA, M. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. Journal of Dairy Science, v. 92, n. 7, p. 3341-3353, 2009.

PENNER, Gregory B. et al. Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to subacute ruminal acidosis in sheep. The Journal of nutrition, v. 139, n. 9, p. 1714-1720, 2009.

PEREIRA, M. N. et al. Partial replacement of forage with nonforage fiber sources in lactating cow diets. I. Performance and health. Journal of dairy science, v. 82, n. 12, p. 2716-2730, 1999.

PEREIRA, M. N.; ARMENTANO, L. E. Partial replacement of forage with nonforage fiber sources in lactating cow diets. II. Digestion and rumen function. Journal of Dairy Science, v. 83, n. 12, p. 2876-2887, 2000.

PETRI, R. M. et al. Adaptive responses in short-chain fatty acid absorption, gene expression, and bacterial community of the bovine rumen epithelium recovered from a continuous or transient high-grain feeding. Journal of dairy science, v. 102, n. 6, p. 5361-5378, 2019.

PIANTONI, P. et al. Evaluation of feed restriction and abomasal infusion of resistant starch as models to induce intestinal barrier dysfunction in healthy lactating cows. Journal of Dairy Science, v. 106, n. 2, p. 1453-1463, 2023.

PIRAN FILHO, F. A. et al. Physical effectiveness of corn silage fractions stratified with the Penn State Particle Separator for lactating dairy cows. Journal of Dairy Science, v. 106, n. 9, p. 6041-6059, 2023.

PLAIZIER, J. C. et al. Effects of duration of moderate increases in grain feeding on endotoxins in the digestive tract and acute phase proteins in peripheral blood of yearling calves. Journal of Dairy Science, v. 97, n. 11, p. 7076-7084, 2014.

PLAIZIER, J. C. et al. Enhancing gastrointestinal health in dairy cows. Animal, v. 12, n. s2, p. s399-s418, 2018.

PLAIZIER, J. C. et al. Invited review: Effect of subacute ruminal acidosis on gut health of dairy cows. Journal of Dairy Science, 2022.

PLAIZIER, J. C. et al. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Veterinary Journal, v. 176, n. 1, p. 21-31, 2008.

PORTER, G. H. et al. Relative value for milk production of field-cured and field-baled, artificially dried-chopped, artificially dried-ground and artificially dried-pelleted alfalfa when fed as the sole source of roughage to dairy cattle. Journal of Dairy Science, v. 36, n. 10, p. 1140-1149, 1953.

POWELL, E. B. 1939. Some relations of the roughage intake to the composition of milk. J. Dairy Sci. 22:453. (Abstr.)

RABELO, E. et al. Effects of pre-and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. Journal of Dairy Science, v. 88, n. 12, p. 4375-4383, 2005.

RAFIEE, Hassan; DARABIGHANE, Babak. A meta-analysis and meta-regression of the effect of substitution of steam flaked corn instead of finely ground corn on dry matter intake, nutrients digestibility, lactation performance, and rumen fermentation in dairy cows. Animal Feed Science and Technology, v. 280, p. 115043, 2021.

RAMIN, M.; HUHTANEN, P. Development of equations for predicting methane emissions from ruminants. Journal of dairy science, v. 96, n. 4, p. 2476-2493, 2013.

RAZZAGHI, Ali et al. Effect of dietary supplementation or cessation of magnesium-based alkalizers on milk fat output in dairy cows under milk fat depression conditions. Journal of Dairy Science, v. 105, n. 3, p. 2275-2287, 2022.

RESENDE, L. C. Effect of rumen neutralizers on performance, digestibility, chewing behavior, and rumen fermentation of dairy cows. Tese de Doutorado. Universidade Federal de Lavras. 2022.

REZENDE, J. P. A. Composição e ritmo circanual do leite em amostras de fazendas brasileiras. Dissertação de Mestrado. Universidade Federal de Lavras. 2022.

RHOADS, M. L. et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. Journal of dairy science, v. 92, n. 5, p. 1986-1997, 2009.

RODNEY, R. M. et al. Effects of dietary fat on fertility of dairy cattle: A meta-analysis and meta-regression. Journal of Dairy Science, v. 98, n. 8, p. 5601-5620, 2015.

ROGERS, J. A. et al. Response of dairy cows to sodium bicarbonate and limestone in early lactation. Journal of Dairy Science, v. 68, n. 3, p. 646-660, 1985.

ROSSOW, H. A.; ALY, S. S. Variation in nutrients formulated and nutrients supplied on 5 California dairies. Journal of dairy Science, v. 96, n. 11, p. 7371-7381, 2013.

RUSSELL, J. B.; HINO, T. Regulation of lactate production in Streptococcus bovis: A spiraling effect that contributes to rumen acidosis. J. Dairy Sci. v.68, p.1712–1721, 1985.

RUSSELL, James B. Rumen microbiology and its role in ruminant nutrition. Department of Microbiology, Cornell University, 2002.

SAKATA, Takashi. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. British Journal of Nutrition, v. 58, n. 1, p. 95-103, 1987.

SALVATI, G. G. S. et al. Effect of kernel processing and particle size of whole-plant corn silage with vitreous endosperm on dairy cow performance. Journal of Dairy Science, v. 104, n. 2, p. 1794-1810, 2021.

SALVATI, G. G. S. et al. Response of lactating cows to live yeast supplementation during summer. Journal of Dairy Science, v. 98, n. 6, p. 4062-4073, 2015.

SALVATI, G. G. S. et al. Response of lactating cows to live yeast supplementation during summer. Journal of Dairy Science, v. 98, n. 6, p. 4062-4073, 2015.

SANCHEZ, W. K.; BEEDE, D. K.; CORNELL, J. A. Interactions of sodium, potassium, and chloride on lactation, acid-base status, and mineral concentrations. Journal of dairy science, v. 77, n. 6, p. 1661-1675, 1994.

SANCHEZ, W. K.; BEEDE, D. K.; DELORENZO, M. A. Macromineral element interrelationships and lactational performance: Empirical models from a large data set. Journal of dairy science, v. 77, n. 10, p. 3096-3110, 1994.

SANTOS, J. E. P. et al. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. Journal of dairy science, v. 102, n. 3, p. 2134-2154, 2019.

SCHINGOETHE, David J. A 100-Year Review: Total mixed ration feeding of dairy cows. Journal of dairy science, v. 100, n. 12, p. 10143-10150, 2017.

SCHLAU, N.; GUAN, L. L.; OBA, M. The relationship between rumen acidosis resistance and expression of genes involved in regulation of intracellular pH and butyrate metabolism of ruminal epithelial cells in steers. Journal of Dairy Science, v. 95, n. 10, p. 5866-5875, 2012.

SCHNEIDER, P. L.; BEEDE, D. K.; WILCOX, C. J. Effects of supplemental potassium and sodium chloride salts on ruminal turnover rates, acid-base and mineral status of lactating dairy cows during heat stress. Journal of animal science, v. 66, n. 1, p. 126-135, 1988.

SCHWAIGER, T.; BEAUCHEMIN, K. A.; PENNER, G. B. Duration of time that beef cattle are fed a high-grain diet affects the recovery from a bout of ruminal acidosis: Short-chain fatty acid and lactate absorption, saliva production, and blood metabolites. Journal of Animal Science, v. 91, n. 12, p. 5743-5753, 2013.

SEO, Ja Kyeom et al. Direct-fed microbials for ruminant animals. Asian-Australasian Journal of Animal Sciences, v. 23, n. 12, p. 1657-1667, 2010.

SINEDINO, Letícia DP et al. Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. Reproduction, v. 153, n. 5, p. 707-723, 2017.

SORIANI, N.; PANELLA, Gand; CALAMARI, LUIGI. Rumination time during the summer season and its relationships with metabolic conditions and milk production. Journal of dairy science, v. 96, n. 8, p. 5082-5094, 2013.

STEELE, Michael A. et al. Development and physiology of the rumen and the lower gut: Targets for improving gut health. Journal of dairy science, v. 99, n. 6, p. 4955-4966, 2016.

STEFENONI, H. A. et al. Effects of the macroalga Asparagopsis taxiformis and oregano leaves on methane emission, rumen fermentation, and lactational performance of dairy cows. Journal of dairy science, v. 104, n. 4, p. 4157-4173, 2021.

ST-PIERRE, Normand R.; COBANOV, B.; SCHNITKEY, Gary. Economic losses from heat stress by US livestock industries. Journal of dairy science, v. 86, p. E52-E77, 2003.

Sudweeks, E. M., L. O. Ely, D. R. Mertens, and L. R. Sisk. 1981. Assessing minimum amounts and form of roughages in ruminant diets: Roughage value index systems. J. Anim. Sci. 53:1406–1411.

SWIFT, R. W. et al. Digestibility of rations for ruminants as affected by proportions of nutrients. Journal of Animal Science, v. 6, n. 4, p. 432-444, 1947.

SWIFT, R. W. et al. The effect of dietary fat on utilization of the energy and protein of rations by sheep. Journal of Animal Science, v. 7, n. 4, p. 475-485, 1948.

TAJIK, J. et al. Prevalence of subacute ruminal acidosis in some dairy herds of Khorasan Razavi province, northeast of Iran. Iranian Journal of Veterinary Research, v. 10, n. 1, p. 28-32, 2009.

TAYLOR, Jeremy G.; BUSHINSKY, David A. Calcium and phosphorus homeostasis. Blood purification, v. 27, n. 4, p. 387-394, 2009.

TERRÉ, M. et al. Comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. Journal of dairy science, v. 96, n. 8, p. 5290-5294, 2013.

TUCKER, W. B.; HARRISON, G. A.; HEMKEN, R. W. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. Journal of Dairy Science, v. 71, n. 2, p. 346-354, 1988.

Tyznik, W., and N. N. Allen. 1951. The relation of roughage intake to the fat content of the milk and the level of fatty acids in the rumen. J. Dairy Sci. 34:493. (Abstr.)

Van Soest, P. J. 1964. Symposium on nutrition and forages and pastures: New chemical procedures for evaluating forages. J. Anim.Sci. 23:838–845

WAMPLER, Jennifer L.; MARTIN, Scott A.; HILL, Gary M. Effects of laidlomycin propionate and monensin on glucose utilization and nutrient transport by Streptococcus bovis and Selenomonas ruminantium. Journal of animal science, v. 76, n. 10, p. 2730-2736, 1998.

WANG, D. S. et al. Effects of subacute ruminal acidosis challenges on fermentation and biogenic amines in the rumen of dairy cows. Livestock Science, v. 155, n. 2-3, p. 262-272, 2013.

WANG, L. et al. Effect of heat stress on endotoxin flux across mesenteric-drained and portal-drained viscera of dairy goat. Journal of animal physiology and animal nutrition, v. 95, n. 4, p. 468-477, 2011.

WASSERMAN, Robert H.; FULLMER, Curtis S. On the molecular mechanism of intestinal calcium transport. Mineral absorption in the monogastric GI tract, p. 45-65, 1989.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54:885–894.

WEST, J. W. et al. Potassium carbonate as a potassium source and dietary buffer for lactating Holstein cows during hot weather. Journal of Dairy Science, v. 70, n. 2, p. 309-320, 1987.

WHITE, Robin R. et al. Physically adjusted neutral detergent fiber system for lactating dairy cow rations. I: Deriving equations that identify factors that influence effectiveness of fiber. Journal of dairy science, v. 100, n. 12, p. 9551-9568, 2017.

WILDMAN, C. D.; WEST, J. W.; BERNARD, J. K. Effect of dietary cation-anion difference and dietary crude protein on performance of lactating dairy cows during hot weather. Journal of dairy science, v. 90, n. 4, p. 1842-1850, 2007.

WOOLPERT, M. E. et al. Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. Journal of Dairy Science, v. 99, n. 10, p. 8486-8497, 2016.

ZEBELI, Q. et al. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. Journal of dairy science, v. 95, n. 3, p. 1041-1056, 2012.

ZHAO, Chenxu et al. Inflammatory mechanism of Rumenitis in dairy cows with subacute ruminal acidosis. BMC Veterinary Research, v. 14, p. 1-8, 2018.

ZIMBELMAN, R. B. et al. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. In: Proceedings of the Southwest Nutrition Conference. p. 158-169. 2009.

ARTIGO 1 – Effect of magnesium phosphate supplementation on lactation performance, ruminal function, nutrient digestibility, and metabolic responses of dairy cows

Artigo formatado de acordo com as normas para submissão ao Journal of Dairy Science.

ABSTRACT

Magnesium phosphate (MgP; 26% Mg, 14% P; MAG26, Phosphea, France) is a potential source of Mg and P for lactating dairy cows, but requires evaluation. This study investigated the effects of MgP on lactation performance, DMI, ruminal pH and fermentation, milk FA profile, total-tract apparent digestibility, mineral utilization, feeding behavior, jugular blood acid–base balance, SARA related blood markers, and fecal pH. Twenty-one individually housed Holstein cows (144 ± 77 DIM) were assigned to a 3 × 3 Latin square design with 21-d periods (14-d of adaptation, 7-d of collection) and received 1 of 3 treatments: dicalcium phosphate (DCP) plus magnesium oxide (MgO) supplying 0.54% P and 0.39% Mg on a DM basis (Control); MgP supplying the same levels of P and Mg as Control (Iso-Mg); or MgP plus MgO supplying the same P level but a greater Mg concentration (0.53% of DM; High-Mg). Diets contained 17.3% forage NDF, 20.1% physically effective NDF, 24.2% starch, 18.2% nonstarch NFC, and 5.0% ether extract (EE). Data were analyzed using a mixed model including treatment and period as fixed effects and square and cow(square) as random effects. Statistical significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. There was no treatment effect on DMI (22.3 kg/d), milk yield (34.5 kg/d), and ECM (33.6 kg/d). Milk fat concentration was greater for High-Mg (3.60%) than for Control (3.45%) and Iso-Mg (3.44%), and milk total solids concentration followed the same pattern. Ruminal pH variables, including time below pH 5.8 (284 min/d), as well as the ruminal fermentation profile, were not affected by treatment. Preformed FA concentration was greater for High-Mg (1.41% of milk) than for Control and Iso-Mg (both 1.33%). Mixed FA concentration was greater for High-Mg (1.28% of milk) than for Iso-Mg (1.21%), whereas de novo FA concentration was unaffected (0.90%).

Total-tract apparent digestibility of DM, OM, EE, and NDF was greater or tended to be greater for High-Mg (72.9, 73.0, 73.2, and 49.4%, respectively) compared with Control (71.2, 71.2, 69.3, and 46.2%) and Iso-Mg (70.1, 70.1, 69.2, and 43.4%). Starch digestibility was greater for High-Mg (99.3%) than for Control (98.9%), with Iso-Mg not differing from either treatment (99.0%), and CP digestibility tended to be greater for High-Mg (75.2%) than for Iso-Mg (73.0%), with Control not differing from either treatment (73.6%). Digestibility of Mg tended to be greater for High-Mg (27%) than for Iso-Mg (21%). Digestibility of P tended to be greater for High-Mg (38%) than for Control (32%). Cows fed High-Mg had a longer first meal than Control and Iso-Mg. The proportion of daily intake consumed in the morning was greater for High-Mg than for Iso-Mg, whereas nighttime intake was lower for High-Mg than for Iso-Mg and tended to be lower than Control. Serum Mg concentration was greater for Iso-Mg (1.94 mg/dL) than for Control (1.85 mg/dL) and High-Mg (1.83 mg/dL). Jugular blood HCO_3^- and total CO_2 concentrations tended to be greater for High-Mg than for Control. Plasma haptoglobin and LBP concentrations were unaffected, whereas plasma D-lactate tended to be greater for Iso-Mg than for High-Mg. Fecal pH was greater for High-Mg than for Control. Overall, MgP effectively replaced MgO and DCP at equivalent dietary Mg and P concentrations. Increasing dietary Mg supply through combined MgP and MgO supplementation increased milk fat concentration, a response associated with greater proportions of preformed and monounsaturated FA in milk and higher EE digestibility.

INTRODUCTION

Lactating dairy cows have high nutritional requirements, which leads to the formulation of diets with increased concentrate inclusion to enhance energy and protein supply. These diets are characterized by high concentrations of rapidly fermentable carbohydrates (starch and nonstarch NFC), at the expense of forage NDF and physically effective NDF (peNDF). Although this strategy increases energy availability and supports microbial protein synthesis in

the rumen, it also increases the risk of SARA (Khorrami et al., 2021). When SARA prevalence within a herd is high, the expected benefits of highly fermentable diets may be compromised, resulting in reduced milk yield and milk fat concentration (Aschenbach et al., 2019; Bach et al., 2018; Bach et al., 2023), as well as a greater incidence of metabolic and digestive disorders (Plaizier et al., 2022).

In this context, buffering and alkalinizing agents are frequently included in dairy cow diets. Sodium bicarbonate is a widely used buffer, whereas magnesium oxide (MgO) acts as an alkalinizing agent (Goff, 2018), and both may be supplemented concurrently to enhance dietary safety (Erdman et al., 1982; Teh et al., 1985). Under this practice, dietary Mg concentrations often exceed requirements estimated using a factorial approach (approximately 0.20% of DM; NASEM, 2021), frequently reaching 0.40% or higher (Erdman et al., 1982; Bach et al., 2018; Razzaghi et al., 2022). This strategy is based on the premise that supplemental Mg may contribute to stabilization of ruminal pH, support rumen fermentation, mitigate the risk of SARA, and preserve milk fat synthesis.

Consistent with this premise, under progressive SARA challenges based on forage reduction and increased barley supply, MgO supplementation attenuated declines in DMI, milk yield, ruminal pH, and milk fat concentration, whereas control cows exhibited pronounced reductions in these responses (Bach et al., 2018; Bach et al., 2023). In addition, Mg supplementation increased milk fat concentration and yield and reduced the milk trans-10:trans-11 C18:1 ratio under diets formulated to induce milk fat depression, indirectly suggesting improved rumen conditions (Razzaghi et al., 2022). In contrast, Resende (2022) reported increased milk fat concentration with MgO supplementation without effects on ruminal pH, along with a tendency for greater ether extract (EE) digestibility, suggesting that, in some contexts, milk fat responses to Mg may occur independently of changes in ruminal pH.

The source of Mg can influence its availability and metabolic responses (Razzaghi et al., 2022; Lobo et al., 2023). Magnesium phosphate (MgP), which combines the supply of both Mg and P, could potentially replace two mineral sources in the diet and reduce purchase, storage and mixing operations. However, the effects of replacing dicalcium phosphate (DCP) and MgO with MgP are poorly characterized, and, to the best of our knowledge, evaluation of MgP in diets for lactating dairy cows has not been reported in the literature.

Therefore, the objective of this study was to evaluate the effects of replacing MgO and DCP with MgP in diets for lactating dairy cows formulated to challenge rumen fermentation without inducing overt acidosis, either while maintaining equivalent dietary Mg and P concentrations or while increasing dietary Mg concentration under constant dietary P supply through combined Mg supplementation (MgP plus MgO). This approach allowed the evaluation of MgP as an alternative Mg and P source and assessment of cow responses to increased dietary Mg.

We hypothesized that replacing MgO and DCP with MgP, while maintaining equivalent dietary Mg and P concentrations, would sustain lactation performance, ruminal conditions, and mineral status comparable to those observed in cows fed the control diet. Additionally, we hypothesized that increasing dietary Mg through the combined inclusion of MgP and MgO would enhance ruminal pH stability and increase milk fat concentration and yield.

MATERIAL AND METHODS

Experimental design, cows, and treatments

The experiment was conducted from January 15th to March 18th, 2024, during the hot-rainy season of southeast Brazil. Twenty-one Holstein cows (9 primiparous and 12 multiparous; 34.6 ± 4.5 kg/d of milk yield; 144 ± 77 DIM; 592 ± 87 kg of BW) were used. Cows were blocked into 7 squares of 3 animals, primarily by parity (3 squares of primiparous and 4 squares

of multiparous cows) and subsequently by milk yield and DIM. Within square, cows were randomly assigned to treatment sequences in a 3 × 3 Latin square design with 21 d periods (14 d of adaptation and 7 d of data collection), balanced for carryover effects.

All cows received the same basal diet, with treatments differing only in the mineral sources. Control treatment included DCP (26% Ca and 18.5% P on a DM basis) as the supplemental source of Ca and P and MgO (56% Mg) as the supplemental source of Mg (0.54% P and 0.39% Mg in the diet; Control). High-Mg treatment replaced DCP with MgP (26% Mg and 14% P; MAG26, Phosphea, France) based on P concentration (0.54% P and 0.53% Mg; High-Mg), while Iso-Mg treatment replaced DCP with MgP based on P concentration combined with removal of MgO to match the Mg concentration of the Control diet (0.54% P and 0.39% Mg; Iso-Mg). In diets containing MgP, limestone was included to maintain dietary Ca concentration equal to the Control diet (0.80% of DM). Once per experimental period, a premix containing limestone, sodium bicarbonate, salt, and a commercial mineral and vitamin source was prepared and mixed with the treatment-specific ingredients before inclusion in the TMR. Diets were based on whole-plant corn silage, corn ear fibrous coproduct (Cargill Agricola, Uberlândia, Brazil; Silva et al., 2022), rehydrated ensiled corn, citrus pulp, whole cottonseed, and soybean meal. Ingredient inclusion rates and the chemical composition of consumed diets are presented in Table 1.

Cows were individually fed in an open-walled, sand-bedded tie stall barn with fans and high-pressure sprinklers and milked 3 times per day starting at 0500, 1200, and 1900 h in an adjacent herringbone parlor. The TMR was mixed in a stationary feed mixer (Unimix 1200, Casale, São Carlos, Brazil) and offered once per day at 0700 h in amounts to allow for 10 to 15% of offered as daily orts per cow (average observed 13.2% of the offered TMR). Feed was pushed up at least 12 x/d. The DM concentrations of whole-plant corn silage, corn ear fibrous

coproduct and rehydrated corn were monitored weekly with a microwave oven and the TMR was adjusted accordingly.

Lactation performance, intake, and ruminal pH

Milk yield and DMI were recorded daily throughout the experiment, and the averages from d 15 to 21 of each experimental period were used for treatment comparisons. Milk samples were collected proportionally to milk yield at each milking on d 15, 16, 19, and 20 of each experimental period and preserved with 2-bromo-2-nitropropane-1,3-diol under refrigeration until shipment to the milk analysis laboratory (Clínica do Leite, Piracicaba, Brazil). Samples from d 15 and 16 and from d 19 and 20 were shipped separately, and the maximum time between collection and analysis was 4 d. Milk fat, protein, lactose, SCC, and milk urea nitrogen were determined by mid-infrared analysis (Milkoscan FT, Foss Inc.). For milk composition variables, the mean of the 4 sampling days was used for treatment comparisons. On d 20 of each period, an additional 500 mL of milk from the morning milking was collected for fatty acid (FA) analysis.

For FA profile determination, milk samples were frozen without chemical preservatives and stored at −20°C until analysis. Milk fat was extracted according to Hara and Radin (1978), and fatty acids were methylated following Christie (1982). Fatty acid methyl esters were analyzed by gas chromatography (Finnigan Focus GC, Thermo Fisher Scientific) using a capillary column (0.25 mm i.d.; 0.20 µm film thickness). De novo FA were calculated as the sum of FA containing 4 to 15 C; mixed FA as the sum of FA containing 16 or 17 C; and preformed FA as the sum of FA containing ≥18 C.

Milk energy secretion (Mcal/d) was calculated according to NASEM (2021) as [(0.0929 × % fat) + (0.055 × % crude protein) + (0.0395 × % lactose)] × kg of milk, and ECM was calculated as milk energy secretion divided by 0.70, assuming milk containing 3.7% fat, 3.2%

protein, and 4.6% lactose. Body weight was measured after the morning milking on d 15 and 16 of each period using an individual walk-over scale (Eziweigh2, Tru-Test). Body condition score was assessed on d 21 of each period as the mean of 3 independent evaluators (Wildman et al., 1982).

Ruminal pH wireless boluses (smaXtec animal cara GmbH, Graz, Austria) were inserted into the reticulum of each cow on the first day of the experiment. Ruminal pH was recorded at 10-min intervals and downloaded at the end of the experiment. Data from d 15 to 21 of each period were used to calculate mean, minimum, and maximum pH; pH range (maximum − minimum); time below pH 6.0, 5.8, and 5.5 (min/d); and the SARA index, calculated as time below each pH threshold divided by DMI. Daily averages from d 15 to 21 were used for treatment comparisons.

Feeds

Daily samples of feed ingredients and orts per cow were collected and frozen during the last week of each experimental period. Feed ingredients samples were composited by period, and orts sample were composited by cow within period. Samples were dried in a forced-air oven at 55°C for 72 h and ground to pass a 1-mm diameter mesh screen in a grinder (Star FT 80, Fortinox Indústria). The DM concentration was determined in a forced air-oven at 105°C for 24 h, and ash was evaluated by incineration at 600°C for 4 h. The NDF concentration was evaluated using heat-stable α-amylase and sodium sulfite, according to Detmann et al. (2012), based on AOAC 2002.04 (AOAC International, 2006) and adapted for autoclave using filter bags. Starch plus free glucose was measured with α-amylase and amyloglucosidase and colorimetry for glucose, as described in Silva et al. (2019). The EE concentration was measured using Soxhlet extraction method with diethyl ether (method 920.39; AOAC, 2005) in a commercial laboratory (Esalqlab, Piracicaba, Brazil). The CP concentration was determined by combustion and detection of N_2 (Leco Analyzer, Leco Corp.).

The concentration of Ca, P, Mg, K, Na, Cl, and S was measured in a commercial laboratory (3R Ribersolo, Ribeirão Preto, Brazil) using inductively coupled plasma optical emission spectroscopy (ICP-OES; method 985.01; AOAC International, 2016). Based on mineral composition, DCAD was calculated using two approaches. According to Ender et al. (1971), DCAD1 (mEq/kg) was calculated as: (Na/0.0023 + K/0.00391) – (Cl/0.00355 + S/0.00321 x 2). Alternatively, following Goff (2000), DCAD2 (mEq/kg) was calculated as: (Na/0.0023 + K/0.00391 + 0.15 x Ca/0.004 x 2 + 0.15 x Mg/0.0024 x 2) – (Cl/0.00355 + 0.25 x S/0.00321 x 2 + 0.5 x P/0.0031 x 3).

Particle size distribution of forages was evaluated using the Penn State Particle Separator (PSPS) with 19-mm and 8-mm screens and a pan, on d 15, 16, and 17 of each experimental period, with daily sieving. The DM and NDF concentration by particle size were determined in composite samples formed by sieve fraction within each period, as previously described. The peNDF was calculated as the sum of NDF contributions from particles retained on sieves >8 mm, obtained by multiplying the proportion of DM retained on the 19- and 8-mm sieves by the NDF concentration of the respective fractions and summing these values. For concentrates, only whole cottonseed NDF was considered as peNDF and assumed to be fully retained above 8 mm.

Total-tract apparent digestibility and ruminal microbial yield

During days 19 to 21 of each experimental period, fecal and urine collection was performed continuously in 3 separates 8 h collection periods, with 8 h intervals, and starting 8 h later on each consecutive day, to represent a 24 h collection. Feces and urine were collected in buckets during excretion, without contact with the ground, by at least 1 person for each 3 cows from 2100 to 0500 h (first collection period), 1300 to 2100 h (second collection period), and 0500 to 1300 h (third collection period). During collection, cows were moved in groups of 3 to the milking routine and immediately returned to their individual stalls after milking to

reduce the daily time without sampling. Fecal samples were collected, immediately weighted, sampled (1% of the fresh weight), and frozen throughout the collection period. A composite sample was formed per cow per period. Samples were dried at 55°C for 72 h. The concentrations of DM, ash, NDF, starch, EE, CP, Ca, P, Mg, and K were measured as previously described, with the exception that fecal EE samples were acidified prior to analysis, and their total-tract apparent digestibility was calculated. Digestible OM intake (DOMI) was estimated, and the ECM/DOMI ratio was calculated as a measure of energy usage efficiency.

A sample from the first defecation of each cow was collected on d 21 for viscosity determination. Viscosity was evaluated using an adaptation of Cannon et al. (2010). Samples of fresh feces (100 g) were diluted in 120 mL of water. The solution was homogenized with a spatula for 30 s and filtered through 2 layers of cheesecloth. The viscosity of the solution was measured with a rotational viscometer (model DV-E, Brookfield Engineering Laboratories) at 100 rpm. Additionally, subsamples (10 g) from each composite sample obtained per period were diluted in 100 mL of distiller water for pH determination.

Immediately after collection, urine volume was measured with a graduated beaker, and a sample (3%) was stored under refrigeration. Urine samples were mixed to a 4% sulfuric acid solution (1:5) and frozen for allantoin determination according to Chen and Gomes (1992). The daily excretion allantoin in urine was used as an indirect estimate of ruminal microbial yield (Valadares et al., 1999).

Ruminal fermentation

Ruminal fluid samples were collected on d 21 of each experimental period using an orogastric tube coupled to a vacuum pump at 11 h after the morning feeding. The first 100 mL of rumen fluid were discarded to minimize saliva contamination. Cows were randomly sampled within squares, and ruminal pH was measured immediately after collection. Aliquots of rumen

fluid were allocated for subsequent analyses: one sample was snap-frozen in liquid nitrogen and stored at −20°C for VFA determination; a 10-mL subsample was diluted (50% vol/vol) in a 36% (vol/vol) formaldehyde solution for protozoa counting; and an additional 20-mL aliquot of undiluted rumen fluid was frozen at −20°C for mineral analysis (Mg, P, and Ca).

Analysis of VFA was conducted using a gas-liquid chromatograph (GC HP 7890 A, Injector HP 7683B, Agilent Technologies) equipped with a capillary column (225 m, 0.32 mm, 0.5 µm; J&W HP-FFAP 1909F-112, Agilent Technologies). For protozoa counting, preserved samples were stained according to Dehority (1984), and protozoa were enumerated using an optical microscope in a Neubauer chamber. Mineral concentrations (Ca, Mg, and P) were determined by ICP-OES following acid digestion of rumen fluid samples in a commercial laboratory (3R Ribersolo, Ribeirão Preto, Brazil). Results were initially expressed on a DM basis, using the DM content of rumen fluid determined by oven drying at 105°C for 24 h. Mineral concentrations were subsequently converted to a volumetric basis (g/L) using the DM content of the rumen fluid, assuming a density of 1.0 kg/L. Therefore, reported values represent total mineral concentrations in rumen fluid samples as collected, including both soluble and fine particulate-associated fractions.

Blood analysis

Blood samples from the coccygeal vessels were collected on d 18 of each experimental period at 0700 h, immediately before feeding, for determination of serum Mg, P, and Ca concentrations at a commercial laboratory (Hemocell, Lavras, Brazil). Jugular blood acid–base balance was evaluated on d 18 at 1400 h (7 h after feeding) using a blood gas analyzer at the same laboratory. Jugular blood samples were collected in heparinized syringes, randomly within square, kept on ice, and analyzed within 1 h of collection. Additional coccygeal blood samples were collected on d 18 at 1800 h for determination of LPS-binding protein (LBP), D-lactate, and haptoglobin. In all samplings, blood from the 21 cows was collected within 20 min.

Samples for D-lactate and haptoglobin analyses were stored at −20°C, whereas samples for LBP analysis were stored at −80°C until analysis. Serum LBP was determined using a commercial ELISA kit (Bovine LBP ELISA Kit, FineTest; intra-assay CV = 8.02%), and plasma D-lactate was analyzed using a commercial enzymatic kit (EnzyChrom D-Lactate Assay Kit, BioAssay Systems; intra-assay CV = 5.68%). Serum haptoglobin concentration was determined by polyacrylamide gel electrophoresis as described by Weber and Osborn (1969).

Chewing behavior

From d 15 to 17 of each experimental period, chewing behavior was recorded by continuous visual observation at 5-min intervals over 24-h periods. Activities were classified as rumination, ingestion, drinking, or idleness. Feeding observations were grouped into meals using an individual meal criterion (the minimum interval between meals) calculated for each cow according to DeVries et al. (2003), with adaptations to account for the 5-min sampling interval (Silva et al., 2025). Meal frequency was defined as the number of meals per day (meals/d). Total meal time (min/d) was calculated as total eating time (number of eating observations × 5 min) plus nonfeeding intervals shorter than the individual meal criterion. Meal duration (min/meal) was calculated as total daily meal time divided by meal frequency, and meal size (kg/meal) as DMI (kg/d) on collection days divided by meal frequency. The duration of the first daily meal was measured using a stopwatch by a single observer from feed delivery at 0700 h until completion of the first meal by all cows.

The proportion of daily intake during the morning (0700 to 1200 h), afternoon (1200 to 1900 h), and night (1900 to 0700 h) was determined from d 15 to 17 of each experimental period. Sorting behavior by time of day was evaluated from d 15 to 17 of each experimental period using a PSPS. Particle distribution of the TMR available to each cow was measured at 0700 h (offered TMR), 1200 h, 1900 h, and 0500 h of the following day. Predicted particle intake (as-fed basis) was calculated as the proportion of TMR retained on each screen multiplied

by TMR intake, whereas observed particle intake was calculated as the proportion of TMR retained on each screen multiplied by TMR offered minus the proportion of orts retained on each screen multiplied by orts. The selection index was calculated as 100 × (observed intake/predicted intake) according to Leonardi and Armentano (2003), with values <100% indicating selective refusal, >100% preferential intake, and 100% no sorting.

Sorting of NDF, starch, Mg, and P was also evaluated based on calculated TMR composition from ingredient analyses and orts collected from each cow from d 15 to 21 of each experimental period. Predicted nutrient intake (DM basis) was calculated as nutrient concentration in the TMR multiplied by TMR intake, whereas observed nutrient intake was calculated as nutrient concentration in the TMR multiplied by TMR offered minus nutrient concentration in orts multiplied by orts. The nutrient selection index was calculated as described previously.

Statistical analysis

Data from one cow assigned to the Iso-Mg treatment during period 3 were removed from the analysis due to a leg injury. Data were analyzed using the MIXED procedure of SAS (University Edition; SAS Institute Inc., Cary, NC). The Latin square model included treatment (1–3) and period (1–3) as fixed effects, and square (1–7) and cow nested within square (1–21) as random effects. Degrees of freedom were estimated using the Kenward–Roger method.

Ruminal pH measured by bolus was analyzed as repeated measures. The fixed effect of hour of the day and the interaction between hour and treatment were included in the model. Cow × period × treatment was specified as a random effect. The covariance structure was selected based on the lowest Akaike information criterion among first-order autoregressive, compound symmetry, and unstructured covariance structures.

Model assumptions were evaluated by inspection of residual diagnostic plots. Observations with studentized residuals greater than |3| were considered outliers and removed from the analysis. Statistical significance was declared at $P \leq 0.05$, and tendencies were declared at $0.05 < P \leq 0.10$. Least squares means were compared using Tukey's test.

RESULTS

Diets

Experimental diets were formulated to be nutritionally similar, differing only in Mg and P source and dietary Mg concentration (Table 1). Considering the consumed diets (ingredients – orts), dietary Mg was 0.39%, 0.40%, and 0.53% of DM for Control, Iso-Mg, and High-Mg, respectively. On % of DM, diets had, on average, 17.4% CP, 29.4% NDF, 17.3% forage NDF, 20.1% peNDF, 24.2% starch, 42.4% NFC, 18.2% nonstarch NFC, 5.0% EE, 5.7% ashes, and 0.54% P. Among ingredients (Table 2), whole-plant corn silage and the corn ear fibrous coproduct contained, on average, 34.8% and 43.8% NDF and 39.1% and 12.0% starch, respectively.

Lactation performance

Dietary Mg source and level did not affect ($P \geq 0.20$) DMI, milk yield, milk components yield, and ECM (Table 3). Milk yield averaged 34.5 kg/d, DMI averaged 22.3 kg/d, and ECM averaged 33.6 kg/d across treatments. However, a treatment effect was observed for milk fat concentration ($P = 0.02$), with greater values for High-Mg (3.60%) than for Control (3.45%) and Iso-Mg (3.44%). Accordingly, milk total solids concentration was also affected by treatment ($P = 0.03$), being greater for High-Mg (12.39%) compared with Control (12.26%) and Iso-Mg (12.24%). Milk protein and lactose concentrations were similar across treatments, averaging 3.22% ($P = 0.72$) and 4.64% ($P = 0.84$), respectively. Milk component yields, feed efficiency, linear SCC, MUN, BW, and BCS were not influenced by treatment ($P \geq 0.12$).

Ruminal pH, ruminal fermentation, and minerals in rumen fluid

Ruminal pH variables were not affected ($P \geq 0.43$) by treatment (Table 4). Mean pH measured by bolus sensors averaged 6.02, whereas mean pH measured by stomach tubing averaged 6.49 across treatments. Time below pH 6.0 averaged 642 min/d, time below pH 5.8 averaged 281 min/d, and time below pH 5.5 averaged 40 min/d. As expected, ruminal pH varied markedly throughout the day (Figure 1; hour effect; $P < 0.01$), but no hour × treatment interaction was detected ($P = 0.31$).

Consistent with ruminal pH variables, ruminal fermentation profile did not respond to treatment (Table 5). No differences ($P \geq 0.12$) were detected in total VFA concentration, individual VFA molar proportions or in the acetate/propionate ratio. Whereas fermentation end products were unaffected, Mg concentration in ruminal fluid responded ($P < 0.01$) to treatment, being greatest for High-Mg (0.26 g/L), intermediate for Control (0.22 g/L), and lowest for Iso-Mg (0.17 g/L). The concentrations of P, Ca, and K in ruminal fluid were not affected by treatment ($P \geq 0.17$).

Milk FA profile

Milk FA composition (% of milk) was affected by treatment, particularly for medium- and long-chain FA (Table 6). Concentration of mixed FA differed among treatments ($P = 0.03$) and was greater for High-Mg (1.28%) than for Iso-Mg (1.21%), whereas Control (1.23%) did not differ from either treatment based on Tukey-adjusted comparisons. Similarly, preformed FA concentration differed among treatments ($P = 0.02$) and was greater for High-Mg (1.41%) compared with Control and Iso-Mg (both 1.33%). In contrast, de novo FA concentration was not affected by treatment ($P = 0.27$), averaging 0.90%.

Among individual FA, C16:0 concentration differed among treatments ($P = 0.03$) and was greater for High-Mg (1.18%) than for Iso-Mg (1.12%), whereas Control presented an

intermediate value (1.14%) that did not differ from either treatment. The cis-9 C18:1 concentration also differed among treatments ($P < 0.01$) and was higher for High-Mg (0.74%) than for Control and Iso-Mg (both 0.69%). Consistent with this response, total monounsaturated FA concentration was affected by treatment ($P < 0.01$) and was greater for High-Mg (0.99%) than for Control (0.93%) and Iso-Mg (0.94%).

In addition, concentrations of C20:0 and C20:5 n-3 differed among treatments ($P \leq 0.05$). The High-Mg presented greater concentrations of C20:0 (0.0039%) and C20:5 n-3 (0.00040%) compared with Control (0.0035 and 0.00028%, respectively), whereas Iso-Mg showed intermediate values (0.0036 and 0.00030%, respectively). A tendency toward greater concentration of C20:4 n-6 was also observed for High-Mg (0.00471%) relative to Control (0.00433%), with Iso-Mg presenting an intermediate value (0.00429%).

Total-tract apparent digestibility of nutrients

Intake of DM, OM, CP, starch, EE, ash, NFC, and nonstarch NFC was not affected by treatment (Table 7; $P \geq 0.12$). In contrast, NDF intake showed a tendency for a treatment effect ($P = 0.08$). The NDF intake was greater for Control (6.69 kg/d) than for High-Mg (6.46 kg/d), whereas Iso-Mg presented an intermediate value (6.56 kg/d).

Fecal excretion of DM and OM varied among treatments ($P < 0.01$). Cows fed High-Mg presented lower fecal excretion (5.96 and 5.60 kg/d, respectively) than Control (6.48 and 6.12 kg/d) and Iso-Mg (6.62 and 6.24 kg/d). A similar treatment effect was observed for fecal NDF and EE ($P < 0.01$), with lower values in High-Mg (3.25 and 0.293 kg/d, respectively) relative to Control (3.57 and 0.346 kg/d) and Iso-Mg (3.67 and 0.340 kg/d). Fecal CP excretion showed a tendency for a treatment effect ($P = 0.06$), with lower values for High-Mg (0.96 kg/d) than for Control and Iso-Mg (both 1.05 kg/d), which did not differ from each other.

Digestibility of DM showed a clear treatment effect ($P < 0.01$). The High-Mg showed greater DM digestibility (72.9%) than Iso-Mg (70.1%), whereas Control (71.2%) presented an intermediate value and tended to differ from High-Mg. Organic matter digestibility also responded to treatment ($P < 0.01$), with greater values for High-Mg (73.0%) than for Control (71.2%) and Iso-Mg (70.1%). Digestibility of NDF was likewise affected by treatment ($P < 0.01$). High-Mg exhibited greater NDF digestibility (49.4%) than Iso-Mg (43.4%), whereas Control (46.2%) was intermediate and tended to differ from High-Mg. Starch digestibility responded to treatment ($P = 0.04$), with higher values for High-Mg (99.3%) than for Control (98.9%), and Iso-Mg (99.0%) presenting an intermediate value. Digestibility of EE was also affected by treatment ($P = 0.02$), being greater for High-Mg (73.2%) than for Control (69.3%) and Iso-Mg (69.2%). Digestibility of CP showed a tendency for a treatment effect ($P = 0.07$), with greater values for High-Mg (75.2%) than for Iso-Mg (73.0%), whereas Control (73.6%) did not differ from either treatment.

As expected by design, Mg intake was affected by treatment (Table 8; $P < 0.01$), being greater for High-Mg (117 g/d) than for Control (89 g/d) and Iso-Mg (88 g/d). P intake showed a tendency for a treatment effect ($P = 0.08$), with a tendency toward greater intake in Control (123 g/d) compared with High-Mg (118 g/d), and Iso-Mg presenting an intermediate value (119 g/d).

A treatment effect was observed for fecal Ca excretion ($P < 0.01$), with values decreasing from Control (130 g/d) to Iso-Mg (122 g/d) and High-Mg (113 g/d). Fecal P excretion was also affected by treatment ($P = 0.03$), being greater for Control (84 g/d) than for High-Mg (74 g/d), whereas Iso-Mg (79 g/d) did not differ from either treatment. Consistent with intake, fecal Mg excretion responded to treatment ($P < 0.01$), with greater excretion in High-Mg (86 g/d) than in Control (67 g/d) and Iso-Mg (69 g/d). Fecal K was also affected by

treatment ($P < 0.01$), being greatest for Control (51 g/d), intermediate for Iso-Mg (46 g/d), and lowest for High-Mg (36 g/d).

Digestibility of Ca was affected by treatment ($P < 0.01$), with greater values for High-Mg (36%) than for Control (28%), whereas Iso-Mg (31%) did not differ from either treatment. Digestibility of P showed a tendency for a treatment effect ($P = 0.07$), increasing from Control (32%) to Iso-Mg (34%) and High-Mg (38%). Mg digestibility also exhibited a tendency ($P = 0.07$), being greater for High-Mg (27%) than for Iso-Mg (21%), with Control (24%) presenting an intermediate value. Digestibility of K responded to treatment ($P < 0.01$) and was greater for High-Mg (88%) than for Control (84%) and Iso-Mg (85%).

Feeding behavior

Meal behavior, daily activities, proportion of intake across periods of the day, and feed particle and nutrient sorting are presented in Table 9. Meal frequency, meal size, meal time, meal duration, and daily time spent eating, ruminating, chewing, and idle were not affected by treatment ($P \geq 0.19$), but first meal duration was ($P = 0.01$). Cows fed High-Mg exhibited a longer first meal (37.2 min/meal) than Control (33.6 min/meal) and Iso-Mg (32.9 min/meal).

The proportion of daily intake varied by treatment during the morning period (0700–1200 h; $P = 0.05$), with greater intake in High-Mg (36.8%) than in Iso-Mg (33.8%), whereas Control (34.3%) did not differ from either treatment. During the nighttime period (1900–0700 h), intake distribution also responded to treatment ($P = 0.04$), being lower in High-Mg (23.9%) than in Iso-Mg (27.3%), with Control (26.7%) presenting intermediate values and tending to be greater than High-Mg.

During the morning period, sorting of feed particles >19 mm responded to treatment ($P = 0.04$), being greater in Iso-Mg (122%) than in Control (92%), with High-Mg (118%) presenting intermediate values and tending to be greater than Control. In the same period,

sorting of particles <8 mm showed a tendency for a treatment effect (P = 0.07), with greater sorting in Control (90%) than in Iso-Mg (85%), whereas High-Mg (89%) did not differ from either treatment.

From 1200 to 1900 h, sorting of particles <8 mm tended to respond to treatment (P = 0.07), being greater in High-Mg (101%) than in Iso-Mg (98%), with Control (100%) not differing from either treatment. In addition, Mg sorting responded to treatment (P = 0.03), being greater in Iso-Mg (100.9%) than in Control (99.7%), whereas High-Mg (100.5%) presented intermediate values and tended to exceed those of Control.

Blood variables

Serum Mg concentration responded to treatment (P < 0.01), being greater for Iso-Mg (1.94 mg/dL) than for Control (1.85 mg/dL) and High-Mg (1.83 mg/dL), which did not differ from each other. Serum P (7.33, 7.30, and 7.28 mg/dL for Control, Iso-Mg, and High-Mg, respectively) and serum Ca (9.70, 10.05, and 9.84 mg/dL) were similar among treatments (P ≥ 0.19).

Jugular blood pH, pCO_2, pO_2, base excess, and O_2 saturation were also similar among treatments (P ≥ 0.22). In contrast, HCO_3^- and total CO_2 showed tendencies for treatment effects (P = 0.10 and P = 0.09, respectively), with greater values in High-Mg (26.09 and 27.33 mM) than in Control (25.09 and 26.24 mM), whereas Iso-Mg (25.67 and 26.81 mM) presented intermediate values and did not differ from either treatment.

Plasma concentrations of haptoglobin and LBP were similar among treatments (P ≥ 0.15). Plasma D-lactate showed a tendency for a treatment effect (P = 0.06), being greater for Iso-Mg (1.23 mM) than for High-Mg (1.08 mM), with Control (1.22 mM) presenting an intermediate value.

Fecal and urinary variables

Fecal pH, fecal viscosity, urine excretion, and urinary allantoin excretion are presented in Table 11. Fecal pH differed among treatments ($P = 0.03$) and was greater for High-Mg (7.53) than for Control (7.44), whereas Iso-Mg (7.47) did not differ from either treatment. In contrast, fecal viscosity was not influenced by treatment ($P = 0.73$). Urine volume and urinary allantoin excretion were also unaffected by treatment ($P \geq 0.27$).

DISCUSSION

Diets and ruminal pH

Using the threshold of ruminal pH < 5.8 for more than 314 min/d (Zebeli et al., 2008), cows in the present study were exposed to a moderate risk of SARA (on average, ruminal pH < 5.8 for 284 min/d), consistent with the objective of formulating diets to challenge rumen fermentation without inducing overt acidosis. Approximately 34% of cow–period observations met this pH-based criterion (data not shown). This condition reflects the high inclusion of rapidly fermentable carbohydrates (24.2% starch and 18.2% nostarch NFC) and the relatively low forage NDF concentration (17.3%) of the basal diet. In addition, dietary starch was supplied primarily through ensiled sources, which are known to exhibit greater ruminal degradability, thereby intensifying ruminal fermentation (Castro et al., 2019; Fernandes et al., 2022).

The absence of differences in ruminal pH between the Control and Iso-Mg treatments was consistent with the hypothesis that MgP can effectively replace MgO without impairing ruminal acid-base conditions. In contrast, the lack of a ruminal pH response in the High-Mg treatment did not support the hypothesis that increasing dietary Mg, through the combined supplementation of MgP and MgO, would enhance ruminal stability. This outcome should be interpreted in the context of the experimental design, because the Control diet already contained MgO and sodium bicarbonate was included in all diets to reflect common feeding practices on

dairy farms, resulting in a basal buffering capacity that may have limited detection of additional alkalinizing effects of increased Mg supply.

To the best of our knowledge, this is the first study to evaluate MgP supplementation in diets for lactating dairy cows. Therefore, direct comparisons with studies using the same Mg source are not possible. Nevertheless, indirect comparisons can be made with studies that evaluated ruminal pH responses to increased dietary Mg supplied as MgO.

Differences in dietary design further distinguish the present study from previous reports in which Mg supplementation increased ruminal pH. In the studies by Bach et al. (2018) and Bach et al. (2023), MgO was evaluated under a SARA challenge, imposed through stepwise increases in dietary barley inclusion and reductions in forage proportion. This approach resulted in significant treatment × diet interactions for ruminal pH, with MgO supplementation attenuating pH declines as diets became increasingly fermentable. In contrast, the present experiment used a stable diet, without progressive or abrupt changes in fermentability, to better represent a challenging but adequate diet, which may have limited the detection of ruminal pH responses to increased dietary Mg concentration.

Beyond that, evidence from the literature indicates that increasing dietary Mg does not consistently result in changes in ruminal pH. Using the same MgO source evaluated by Bach et al. (2018) and Bach et al. (2023), Resende (2022) reported no effects on the time that ruminal pH remained below 5.8 or 6.2, either before or during a dietary challenge designed to increase the risk of SARA. Taken together, these findings suggest that the effectiveness of increased dietary Mg to modify ruminal pH is context dependent and likely influenced by baseline buffering capacity, dietary composition, and the intensity and temporal dynamics of the fermentative challenge.

Lactation performance and milk FA profile

The absence of treatment effects on DMI, milk yield, component yields, and feed efficiency indicates that MgP can effectively replace conventional sources of Mg and P without compromising lactation performance. Cows fed High-Mg exhibited greater milk fat and total solids concentrations. Initially, it was hypothesized that this response would be mediated by improvements in ruminal pH. However, the lack of treatment effects on ruminal pH, ruminal fermentation profile, and de novo FA in milk indicates that the increase in milk fat concentration was not driven by changes in ruminal acid–base status.

Instead, changes in milk FA composition provide important insight into the underlying mechanisms. The increase in milk fat concentration with High-Mg was associated with greater concentrations of mixed and preformed FA, particularly C16:0 and cis-9 C18:1. This FA profile is indicative of an increased supply of long-chain FA to the mammary gland, rather than stimulation of de novo FA synthesis (Lock et al., 2025).

Total-tract apparent digestibility of nutrients

The increase in digestibility of EE observed with High-Mg provides a plausible explanation for the changes in milk fat concentration and FA profile. Although the role of UFA in affecting Mg digestibility is recognized (NASEM, 2021), the potential influence of Mg on FA utilization remains underexplored.

Mg has been suggested to form soaps with UFA in the rumen (Jenkins, 1993), which can reduce the extent of biohydrogenation and increase the flow of UFA to the small intestine. From a chemical perspective, this interaction involves the formation of Mg–FA carboxylate salts through deprotonation of the carboxyl group and ionic association with Mg^{2+}. These salts are less soluble and less reactive than free UFA, thereby reducing their availability for microbial attachment and enzymatic hydrogenation. Consequently, this mechanism would be expected to slow the kinetics of ruminal biohydrogenation, allowing a greater proportion of UFA to escape

the rumen and reach the small intestine. Because UFA are more digestible than saturated FA (Daley et al., 2020), this mechanism would be expected to enhance EE digestibility and increase the availability of preformed FA for milk fat synthesis.

Support for this interpretation is provided by previous work reporting responses similar to those observed in the present study. Resende (2022) reported tendencies toward greater EE digestibility, accompanied by tendencies toward increased milk fat concentration and a greater proportion of UFA in milk fat under increased dietary Mg supply. The similarity between those findings and the present results is particularly noteworthy, as both studies included whole cottonseed at similar dietary inclusion rates (12.5% in the present study and 11.5% in Resende, 2022), an ingredient rich in UFA that probably increases the likelihood of Mg–FA interactions in the rumen.

Notably, despite the greater dietary supply of 18:2, the increased concentration of UFA in milk was primarily driven by greater cis-9 18:1. Although cis-9 18:1 is typically considered to be of dietary origin, there is also evidence that it may arise as a transient intermediate of ruminal biohydrogenation (Toral et al., 2024). Accordingly, one possible explanation is that Mg interacted preferentially with cis-9 18:1, either supplied directly by the diet or formed as a biohydrogenation intermediate, resulting in the formation of relatively stable Mg–FA complexes that limited its microbial utilization in the rumen without markedly altering the metabolism of 18:2. Under this scenario, the effect would be a greater intestinal availability of cis-9 18:1, which is consistent with the observed milk FA profile. Moreover, increased intestinal supply of cis-9 18:1 has been associated with enhanced digestibility of 16:0 (Burch et al., 2021; Prom and Lock, 2021), providing a plausible explanation for the greater milk 16:0 concentration observed with High-Mg.

Beyond that, even if Mg–FA soap formation also occurred with C18:2 and increased its intestinal absorption, this would not necessarily be expected to result in proportional increases

of C18:2 in milk. Dos Santos Neto et al. (2024) demonstrated that abomasal infusion of C18:2 n-6 primarily promotes its incorporation into plasma phospholipids and cholesteryl esters (lipid fractions associated with FA conservation) rather than into triacylglycerols or nonesterified FA, which more directly supply the mammary gland (Lock et al., 2025). In that study, infusion of C18:2 n-6 increased both plasma C18:2 n-6 and its long-chain derivative C20:4 n-6 within these slow-turnover lipid pools, indicating enhanced systemic availability and metabolic conversion without immediate transfer to milk fat. A similar pattern was observed for C18:3 n-3, which increased plasma C20:5 n-3 in phospholipids and cholesteryl esters, followed by a delayed increase in plasma triacylglycerols later during infusion. This temporal pattern supports a delayed redistribution of long-chain PUFA into lipid fractions capable of supplying FA to the mammary gland.

Interestingly, in the present study cows fed High-Mg exhibited greater milk 20:5 n-3 than Control, along with a tendency toward greater milk 20:4 n-6 compared with Iso-Mg. These responses are consistent with a partial redistribution of long-chain PUFA into plasma triacylglycerols following increased systemic availability, as recently demonstrated by dos Santos Neto et al. (2024). Because plasma triacylglycerols constitute the primary lipid pool supplying FA to the mammary gland, even modest increases in the abundance of long-chain PUFA within this fraction would be expected to result in detectable, albeit biologically small, increases in their concentrations in milk.

Taken together, these observations support the mechanistic interpretation that increased dietary Mg may promote the formation of Mg–UFA complexes in the rumen, thereby modulating ruminal biohydrogenation, increasing intestinal FA availability, and influencing milk FA composition. Because this mechanism was not part of the experimental hypothesis, FA digestibility and plasma FA concentrations were not evaluated in the present study. Future

research explicitly addressing the interaction between dietary Mg supply and FA metabolism should include these measurements to further validate the underlying mechanisms.

In addition to effects on EE digestibility, High-Mg increased NDF digestibility relative to Iso-Mg and numerically compared with the Control. This response occurred in the absence of changes in ruminal pH or VFA molar proportions, suggesting that factors other than ruminal fermentation intensity were involved. Fibrolytic bacteria are particularly sensitive to UFA (Maia et al., 2010), and the formation of Mg soaps may have attenuated the inhibitory effects of these FA.

Increases in NDF digestibility associated with higher dietary Mg supply have been reported previously, although the proposed mechanisms differ among studies. Bach et al. (2023) observed greater NDF digestibility when supplementing MgO in a diet with a forage:concentrate ratio of 36:64, an effect that was accompanied by differences in ruminal pH. Tebbe et al. (2018) reported a tendency for an interaction between monensin supplementation and Mg source (MgO vs. $MgSO_4$), in which $MgSO_4$ resulted in lower NDF digestibility than MgO when monensin was included in the diet. These findings indicate that the mechanisms by which dietary Mg influences NDF digestibility are not uniform across studies and are likely contingent upon basal diet characteristics, including forage NDF or peNDF level, FA profile, and the use of rumen modifiers such as ionophores.

Changes in starch and CP digestibility were statistically detectable but small in magnitude and likely of limited biological relevance, particularly given the already high starch digestibility of the diets. Nevertheless, these responses further support the notion that dietary Mg concentration may subtly influence microbial activity and nutrient utilization beyond its traditional role.

Feeding behavior

High-Mg increased first-meal duration and morning intake proportion relative to Iso-Mg, with Control intermediate, while reducing nighttime intake proportion compared with Iso-Mg. A similar pattern has been reported by Resende (2022), who observed a tendency toward a reduced proportion of daily intake during the night in cows supplemented with MgO compared with a control diet (19.2 vs. 21.7%) prior to a SARA challenge. During the challenge period, this response became more pronounced, with MgO-supplemented cows tending to consume a greater proportion of intake during the morning (44.3 vs. 41.7%) and a lower proportion during the night (15.7 vs. 19.6%) relative to controls.

Feeding behavior in dairy cows is regulated by multiple interacting factors, including ruminal fill dynamics, metabolic feedback, and acid accumulation (NASEM, 2021). In the present experiment, these behavioral responses occurred without concurrent changes in ruminal pH, ruminal fermentation profile, or rumination activity, indicating that classical indicators of ruminal acid load were not markedly altered. Nevertheless, subtle changes in systemic acid–base status may have contributed to the observed shifts in intake distribution.

Both jugular blood HCO_3^- and total CO_2 showed tendencies for treatment effects, with values tending to be higher in High-Mg than in Control, whereas Iso-Mg presented intermediate values. Absorbed cations such as Mg^{2+} can influence systemic acid–base balance by increasing the systemic strong cation load, which must be counterbalanced to maintain electroneutrality (Goff, 2018). This adjustment favors retention or generation of HCO_3^- in extracellular fluid, which can be reflected as higher total CO_2 concentrations, given that HCO_3^- constitutes the major component of total CO_2. Because jugular blood for acid–base evaluation was collected at 1400 h, the tendency toward greater HCO_3^- and total CO_2 observed in cows fed High-Mg may be associated with greater Mg absorption coinciding with the higher proportion of feed intake consumed during the morning period. However, despite helping to contextualize the observed feeding behavior, these responses were not accompanied by differences in blood pH,

pCO_2, or total DMI, indicating that cows were able to effectively regulate acid–base homeostasis and accommodate the increased cation load without physiologically meaningful disturbances.

With respect to particle-size and nutrient sorting, treatment effects and tendencies were detected during specific periods of the day, but responses were inconsistent across fractions and time. Accordingly, these differences likely reflect minor variation in short-term feed selection rather than a primary driver of the observed intake distribution or production responses.

Minerals in rumen fluid, blood, and total-tract apparent digestibility

As expected by the experimental design, cows fed High-Mg had greater Mg intake, which resulted in increased fecal Mg excretion and a tendency toward greater Mg digestibility compared with Iso-Mg. This tendency is consistent with the physiology of Mg absorption in ruminants, in which passive transport across the ruminal epithelium increases as luminal Mg concentration rises (Goff et al., 2018). Overall Mg digestibility values (approximately 21 to 27%) were close to those assumed by current feeding systems (absorption coefficient of 23% for MgO; NASEM, 2021), indicating that the observed responses fall within physiologically realistic limits.

Serum Mg concentrations across treatments (1.83 to 1.94 mg/dL) remained within the established physiological range for lactating dairy cows (1.80 to 2.40 mg/dL; NASEM, 2021). Unexpectedly, cows fed Iso-Mg exhibited greater serum Mg concentrations than cows fed Control or High-Mg, despite Control and Iso-Mg providing similar dietary Mg concentrations and exhibiting comparable Mg digestibility. Because Mg absorption becomes saturable only at very high dietary concentrations (≥1.1% of DM; Jittakhot et al., 2004), the lower serum Mg concentration observed in High-Mg should not be interpreted as evidence of absorptive saturation.

Instead, differences in serum Mg concentrations should be interpreted in the context of feeding patterns and sampling time. Blood samples were collected immediately before the first feeding of the day, representing a basal state potentially influenced by the temporal distribution of intake on the previous day. In this regard, differences in intake distribution between Iso-Mg and High-Mg cows, particularly the lower proportion of intake during the nighttime in High-Mg, may have contributed to differences in basal serum Mg concentration. In contrast, differences between Control and High-Mg may also be related to differences in Mg source solubility and, consequently, the timing of Mg absorption throughout the day. However, this interpretation remains speculative, as Control and Iso-Mg exhibited similar Mg digestibility and source solubility was not directly evaluated in the present study.

Based on current feeding guidelines, dietary P requirements for lactating dairy cows are approximately 0.35% of DM, depending on the assumed absorption coefficient of the sources used (NASEM, 2021). In commercial dairy systems, however, dietary P concentrations frequently exceed requirements (Harrison et al., 2021). In the present study, dietary P concentration exceeded (0.54% of DM) requirements because the objective was to evaluate MgP, and formulating diets to meet P requirements would not have allowed the delivery of a nutritionally meaningful MgP dose. Accordingly, all diets were formulated to contain the same dietary P concentration, ensuring that comparisons among treatments were not confounded by differences in P supply.

P digestibility tended to be greater for High-Mg than for Control, whereas serum P concentration was not affected by treatment (averaging 7.30 mg/dL) and remained within the established physiological range for lactating dairy cows (4.4 to 9.2 mg/dL; Forar et al., 1982). Interpretation of apparent P digestibility should be made with caution, as fecal P includes a substantial endogenous component derived from salivary secretion, which can mask changes in true intestinal P absorption (NASEM, 2021). This limitation complicates the attribution of

differences in apparent digestibility to alterations in systemic P utilization, particularly when no concurrent changes are observed in serum P concentrations. Current feeding systems assume a high absorption coefficient for inorganic P sources (e.g., 0.80 for DCP; NASEM, 2021). However, a study formulating diets closer to P requirements (approximately 0.35% of DM) reported apparent P digestibility values closer to 40% (Tebbe et al., 2018).

In addition, because dietary P supply in the present study exceeded requirements, relatively low apparent P digestibility values were expected, as excess P is predominantly excreted in the feces (Mogodiniyai Kasmaei and Holtenius, 2013). Under these conditions, the tendency toward greater apparent P digestibility observed with High-Mg may be attributable to its slightly lower P intake. Collectively, these results indicate that replacing DCP with MgP did not meaningfully alter P utilization and that, at the level of supplementation evaluated, P supplied by MgP was nutritionally equivalent to P supplied by DCP.

SARA related markers and fecal pH

D-lactate is produced exclusively by microbial fermentation, originating from both ruminal and hindgut microbial activity, and its accumulation in blood has been associated with ruminal acidosis and SARA (Quanz et al., 2022). However, direct comparison of absolute plasma or serum D-lactate concentrations across studies remains challenging due to the limited number of available datasets and methodological heterogeneity. Previous studies differ in analytical techniques (enzymatic assays versus chromatographic separation), biological matrices (serum versus plasma), and sampling protocols, including the timing of collection relative to feeding and fermentative challenges. These factors likely contribute to the wide range of reported D-lactate concentrations and hinder the establishment of robust reference values.

In beef cattle, Schwaiger et al. (2013) reported marked diurnal variation in serum D-lactate concentration during an acidosis challenge, with numerically greater values

(approximately 0.70 mM) occurring 8 to 12 h after feeding. In dairy cattle, Resende (2022) reported lower plasma D-lactate concentrations and no effect of increasing dietary Mg, averaging 0.39 mM before and 0.32 mM during a SARA challenge; notably, the acidotic challenge in that study was less severe, as indicated by a shorter daily duration of ruminal pH below 5.8 (40 min/d before and 80 min/d during the challenge). In calves, Ewaschuk et al. (2004) reported serum D-lactate concentrations ranging from 0.8 to 3.8 mM in healthy animals and from 1.2 to 22.7 mM in diarrheic calves, reinforcing the association between elevated D-lactate and altered gastrointestinal fermentation.

In the present study, cows fed High-Mg exhibited lower plasma D-lactate concentrations compared with Iso-Mg, with values also numerically lower than those observed for Control ($P = 0.12$), despite the absence of treatment effects on mean ruminal pH or on markers of systemic inflammation and ruminal epithelial challenge, including haptoglobin and LBP (Aschenbach et al., 2019). This response suggests subtle shifts in microbial metabolism that are not captured by bulk measures of ruminal fermentation.

One possible explanation is that greater dietary Mg supply increased the flow of unabsorbed Mg to the hindgut, as supported by increased fecal Mg excretion, thereby modulating hindgut fermentation pathways and microbial lactate production. Consistent with this interpretation, fecal pH was greater in cows fed High-Mg compared with Control, whereas the difference between High-Mg and Iso-Mg was numerical only ($P = 0.16$). Increases in fecal pH with greater dietary Mg supply have been consistently reported in the literature (Resende, 2022; Bach et al., 2023; Erdman et al., 1982), suggesting potential effects of Mg on hindgut fermentation and, possibly, intestinal and overall health (Neubauer et al., 2020). Nonetheless, the relationship between fecal pH and indicators of intestinal health or systemic inflammation in dairy cows remains inconsistent and incompletely understood (Abeyta et al., 2022; Abeyta et al., 2023).

CONCLUSION

Replacing MgO and DCP with MgP at equivalent dietary Mg and P concentrations maintained ruminal pH, lactation performance, digestibility, and mineral status comparable to the Control diet. Increasing dietary Mg through combined MgP and MgO supplementation did not enhance ruminal pH stability but increased milk fat concentration, a response associated with greater proportions of preformed FA in milk and higher EE digestibility. Collectively, these findings indicate that MgP is a suitable Mg and P source and that, under the conditions of this study, the primary response to increased dietary Mg was altered lipid utilization rather than improved ruminal buffering.

NOTES

Animal care and experimental procedures were approved by the Federal University of Lavras Bioethics Committee in Utilization of Animals (protocol number 3443040923). The authors are grateful to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Grupo do Leite, and INPPAR of Federal University of Lavras. We would like to thank Phosphea for partially funding this experiment. The authors have not stated any conflicts of interest.

REFERENCES

Abeyta, M.A., E.A. Horst, E.J. Mayorga, B.M. Goetz, M. Al-Qaisi, C.S. Mccarthy, M.R. O'Neil, B.C. Dooley, P. Piantoni, G.F. Schroeder, and L.H. Baumgard. 2022. Effects of hindgut acidosis on metabolism, inflammation, and production in dairy cows consuming a standard lactation diet. J. Dairy Sci. 106:1429–1440. doi:10.3168/jds.2022-22303.

Abeyta, M.A., E.A. Horst, B.M. Goetz, S. Rodriguez-Jimenez, E.J. Mayorga, M. Al-Qaisi, and L.H. Baumgard. 2023. Effects of hindgut acidosis on inflammation, metabolism, and productivity in lactating dairy cows fed a high-fiber diet. J. Dairy Sci. 106:2879–2889. doi:10.3168/jds.2022-22680.

Aschenbach, J.R., Q. Zebeli, A.K. Patra, G. Greco, S. Amasheh, and G.B. Penner. 2018. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J. Dairy Sci. 102:1866–1882. doi:10.3168/jds.2018-15243.

Bach, A., I. Guasch, G. Elcoso, J. Duclos, and H. Khelil-Arfa. 2018. Modulation of rumen pH by sodium bicarbonate and a blend of different sources of magnesium oxide in lactating dairy cows submitted to a concentrate challenge. J. Dairy Sci. 101:9777–9788. doi:10.3168/jds.2017-14353.

Bach, A., I. Guasch, G. Elcoso, J. Duclos, and H. Khelil-Arfa. 2018. Modulation of rumen pH by sodium bicarbonate and a blend of different sources of magnesium oxide in lactating dairy cows submitted to a concentrate challenge. J. Dairy Sci. 101:9777–9788. doi:10.3168/jds.2017-14353.

Burch, A.M., A. Pineda, and A.L. Lock. 2021. Effect of palmitic acid-enriched supplements containing stearic or oleic acid on nutrient digestibility and milk production of low- and high-producing dairy cows. J. Dairy Sci. 104:8673–8684. doi:10.3168/jds.2020-19913.

Cannon, S.J., G.C. Fahey, M.R. Murphy, C.L. Dikeman, B.L. Miller, and J.K. Drackley. 2010. Inclusion of psyllium in milk replacer for neonatal calves. 1. Effects on growth, digesta viscosity, rate of passage, nutrient digestibilities, and metabolites in blood. J. Dairy Sci. 93:3652–3660. doi:10.3168/jds.2009-2731.

Castro, L.P., M.N. Pereira, J.D.L. Dias, D.V.D. Lage, E.F. Barbosa, R.P. Melo, K. Ferreira, J.T.R. Carvalho, F.F. Cardoso, and R.A.N. Pereira. 2019. Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and proportion in the diet. J. Dairy Sci. 102:9857–9869. doi:10.3168/jds.2019-16559.

Chen, X. B., and M. J. Gomes. 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details. International Feed Resources Unit, Rowett Research Institute.

Christie, W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipid Res. 23:1072–1075. doi:10.1016/s0022-2275(20)38081-0.

Daley, V.L., L.E. Armentano, P.J. Kononoff, and M.D. Hanigan. 2020. Modeling fatty acids for dairy cattle: Models to predict total fatty acid concentration and fatty acid digestion of feedstuffs. J. Dairy Sci. 103:6982–6999. doi:10.3168/jds.2019-17407.

Dehority, B.A. 1984. Evaluation of subsampling and fixation procedures used for counting rumen protozoa. Appl. Environ. Microbiol. 48:182–185. doi:10.1128/aem.48.1.182-185.1984.

Detmann, E. 2012. Methods for feed analysis. INCT–Ciência Animal, Visconde do Rio Branco, Brazil.

Devries, T.J., M.A.G. Von Keyserlingk, D.M. Weary, and K.A. Beauchemin. 2003. Measuring the Feeding Behavior of Lactating Dairy Cows in Early to Peak Lactation. J. Dairy Sci. 86:3354–3361. doi:10.3168/jds.s0022-0302(03)73938-1.

dos Santos Neto, J.M., L.C. Worden, J.P. Boerman, C.M. Bradley, and A.L. Lock. 2024. Long-term effects of abomasal infusion of linoleic and linolenic acids on the enrichment of n-6 and n-3 fatty acids into plasma lipid fractions of lactating cows. J. Dairy Sci. 107:7996–8008. doi:10.3168/jds.2024-24907.

Ender, F., I.W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. Z. Tierphysiol. Tierernaehr. Futtermittelk. 28:233–256. doi:10.1111/j.1439-0396.1971.tb01573.x.

Erdman, R.A., R.W. Hemken, and L.S. Bull. 1982. Dietary Sodium Bicarbonate and Magnesium Oxide for Early Postpartum Lactating Dairy Cows: Effects of Production, Acid-Based Metabolism, and Digestion. J. Dairy Sci. 65:712–731. doi:10.3168/jds.s0022-0302(82)82259-5.

Ewaschuk, J.B., J.M. Naylor, R. Palmer, S.J. Whiting, and G.A. Zello. 2004. D-Lactate Production and Excretion in Diarrheic Calves. J. Vet. Intern. Med. 18:744–747. doi:10.1111/j.1939-1676.2004.tb02615.x.

Fernandes, T., K.T. Da Silva, B.F. Carvalho, R.F. Schwan, R.A.N. Pereira, M.N. Pereira, and C.L. Da Silva Ávila. 2022. Effect of amylases and storage length on losses, nutritional value, fermentation, and microbiology of silages of corn and sorghum kernels. Anim. Feed Sci. Technol. 285:115227. doi:10.1016/j.anifeedsci.2022.115227.

Forar, F.L., R.L. Kincaid, R.L. Preston, and J.K. Hillers. 1982. Variation of Inorganic Phosphorus in Blood Plasma and Milk of Lactating Cows. J. Dairy Sci. 65:760–763. doi:10.3168/jds.s0022-0302(82)82264-9.

Goff, J.P. 2000. Pathophysiology of calcium and phosphorus disorders.Vet. Clin. North Am. Food Anim. Pract. 16:319–337. doi:10.1016/s0749-0720(15)30108-0.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. J. Dairy Sci. 101:2763–2813. doi:10.3168/jds.2017-13112.

Hara, A., and N.S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90:420–426. doi:10.1016/0003-2697(78)90046-5.

Harrison, B.P., M. Dorigo, C.K. Reynolds, L.A. Sinclair, and P.P. Ray. 2021. Phosphorus feeding practices, barriers to and motivators for minimising phosphorus feeding to dairy cows in diverse dairy farming systems. Animal 15:100248. doi:10.1016/j.animal.2021.100248.

Jenkins, T.C. 1993. Lipid Metabolism in the Rumen. J. Dairy Sci. 76:3851–3863. doi:10.3168/jds.s0022-0302(93)77727-9.

Jittakhot, S., J.T. Schonewille, H. Wouterse, C. Yuangklang, and A.C. Beynen. 2004. Apparent magnesium absorption in dry cows fed at 3 levels of potassium and 2 levels of magnesium intake. J. Dairy Sci. 87:379–385. doi:10.3168/jds.s0022-0302(04)73177-x.

Khorrami, B., R. Khiaosa-Ard, and Q. Zebeli. 2021. Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: A meta-analysis. J. Dairy Sci. 104:7761–7780. doi:10.3168/jds.2020-19890.

Leonardi, C., and L.E. Armentano. 2003. Effect of Quantity, Quality, and Length of Alfalfa Hay on Selective Consumption by Dairy Cows. J. Dairy Sci. 86:557–564. doi:10.3168/jds.s0022-0302(03)73634-0.

Lock, A.L., J.M. Dos Santos Neto, and J. De Souza. 2025. Invited review: Moving from dietary fat to fatty acids-New insights into how fatty acids affect digestibility, metabolism, and performance in dairy cows.. J. Dairy Sci. 108:11733–11756. doi:10.3168/jds.2025-27040.

Lobo, R.R., J.A. Arce-Cordero, S. So, M. Soltis, M. Nehme Marinho, B.C. Agustinho, A.D. Ravelo, J.R. Vinyard, M.L. Johnson, H.F. Monteiro, E. Sarmikasoglou, and A.P. Faciola. 2022. Production, physiological response, and calcium and magnesium balance of lactating Holstein cows fed different sources of supplemental magnesium with or without ruminal buffer. J. Dairy Sci. 106:990–1001. doi:10.3168/jds.2022-22583.

Maia, M.R., L.C. Chaudhary, C.S. Bestwick, A.J. Richardson, N. Mckain, T.R. Larson, I.A. Graham, and R.J. Wallace. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butyrivibrio fibrisolvens. BMC Microbiol. 10. doi:10.1186/1471-2180-10-52.

Mogodiniyai Kasmaei, K., and K. Holtenius. 2012. Phosphorus net absorption in dairy cows subjected to abomasal infusion of inorganic phosphorus – a pilot study. J. Anim. Physiol. Anim. Nutr. 97:599–603. doi:10.1111/j.1439-0396.2012.01282.x.

NASEM. 2021. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. National Academies Press, Washington, DC.

Neubauer, V., R.M. Petri, E. Humer, I. Kröger, N. Reisinger, W. Baumgartner, M. Wagner, and Q. Zebeli. 2020. Starch-Rich Diet Induced Rumen Acidosis and Hindgut Dysbiosis in Dairy Cows of Different Lactations.. Animals 10:1727. doi:10.3390/ani10101727.

Plaizier, J.C., F.J. Mulligan, E.W. Neville, L.L. Guan, M.A. Steele, and G.B. Penner. 2022. Invited review: Effect of subacute ruminal acidosis on gut health of dairy cows. J. Dairy Sci. 105:7141–7160. doi:10.3168/jds.2022-21960.

Prom, C.M., and A.L. Lock. 2021. Replacing stearic acid with oleic acid in supplemental fat blends improves fatty acid digestibility of lactating dairy cows. J. Dairy Sci. 104:9956–9966. doi:10.3168/jds.2020-19985.

Quanz, S.T., K.E. Griswold, L.K. Mamedova, S.K. Kvidera, M.J. Brouk, R.S. Fry, and B.J. Bradford. 2022. Case Study: Combined risk factors and digestive disorders in mid-lactation Holstein cows. Appl. Anim. Sci. 38:505–517. doi:10.15232/aas.2022-02313.

Razzaghi, A., A.R. Vakili, B. Khorrami, M.H. Ghaffari, and D.E. Rico. 2022. Effect of dietary supplementation or cessation of magnesium-based alkalizers on milk fat output in dairy cows under milk fat depression conditions. J. Dairy Sci. 105:2275–2287. doi:10.3168/jds.2021-20457.

Resende, L. C. 2022. Effect of rumen neutralizers on performance, digestibility, chewing behavior, and rumen fermentation of dairy cows. PhD Dissertation. Universidade Federal de Lavras, Lavras, MG, Brazil.

Schwaiger, T., K.A. Beauchemin, and G.B. Penner. 2013. The duration of time that beef cattle are fed a high-grain diet affects the recovery from a bout of ruminal acidosis: Dry matter intake and ruminal fermentation1. J. Anim. Sci. 91:5729–5742. doi:10.2527/jas.2013-6471.

Silva, B.D.C., L.A. Godoi, S.D.C. Valadares Filho, D. Zanetti, P.D.B. Benedeti, and E. Detmann. 2019. A suitable enzymatic method for starch quantification in different organic matrices. MethodsX 6:2322–2328. doi:10.1016/j.mex.2019.09.040.

Silva, W.R., F.R. Carvalho, R.B. Silva, R.A.N. Pereira, C.L.S. Ávila, T.J. Devries, and M.N. Pereira. 2022. Fibrous coproducts of corn and citrus as forage and concentrate sources for dairy cows.. J. Dairy Sci. 105:8099–8114. doi:10.3168/jds.2022-21918.

Silva, W.R., A.J.C. Silva, M.A. Tiengo, J.P.A. Rezende, S.P. Souza, R.A.N. Pereira, T.J. Devries, and M.N. Pereira. 2025. Effect of ensiled or finely ground α-amylase-enabled corn grain on lactation performance, chewing, ruminal fermentation, digestibility, and nitrogen partition of dairy cows.. J. Dairy Sci. 109. doi:10.3168/jds.2025-26966.

Teh, T.H., R.W. Hemken, and R.J. Harmon. 1985. Dietary Magnesium Oxide Interactions with Sodium Bicarbonate on Cows in Early Lactation. J. Dairy Sci. 68:881–890. doi:10.3168/jds.s0022-0302(85)80905-x.

Tebbe, A.W., D.J. Wyatt, and W.P. Weiss. 2017. Effects of magnesium source and monensin on nutrient digestibility and mineral balance in lactating dairy cows. J. Dairy Sci. 101:1152–1163. doi:10.3168/jds.2017-13782.

Toral, P.G., G. Hervás, and P. Frutos. 2024. Invited review: Research on ruminal biohydrogenation—Achievements, gaps in knowledge, and future approaches from the perspective of dairy science. J. Dairy Sci. 107:10115–10140. doi:10.3168/jds.2023-24591.

Valadares, R.F.D., G.A. Broderick, S.C.V. Filho, and M.K. Clayton. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of Total Purine Derivatives. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.s0022-0302(99)75525-6.

Weber, K., and M. Osborn. 1969. The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. J. Biol. Chem. 244:4406–4412. doi:10.1016/s0021-9258(18)94333-4.

Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt, and T.N. Lesch. 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy Sci. 65:495–501. doi:10.3168/jds.s0022-0302(82)82223-6.

Zebeli, Q., J. Dijkstra, M. Tafaj, H. Steingass, B.N. Ametaj, and W. Drochner. 2008. Modeling the Adequacy of Dietary Fiber in Dairy Cows Based on the Responses of Ruminal pH and Milk Fat Production to Composition of the diet. J. Dairy Sci. 91:2046–2066. doi:10.3168/jds.2007-0572.

TABLES AND FIGURES

Table 1. Ingredient composition of offered diets and nutrient composition of the consumed diets (offered – orts).

Item, % of DM	Control	Iso-Mg	High-Mg
Ingredient			
Whole-plant corn silage	36.1	36.1	36.1
Corn ear fibrous coproduct	12.5	12.5	12.5
Rehydrated ensiled corn grain	11.5	11.5	11.5
Citrus pulp pellets	12.0	11.9	11.7
Whole cottonseed	12.5	12.5	12.5
Soybean meal	13.2	13.2	13.2
Dicalcium phosphate	0.41	0.00	0.00
Magnesium phosphate	0.00	0.50	0.50
Magnesium oxide	0.23	0.00	0.23
Limestone	0.23	0.45	0.45
Sodium bicarbonate	0.68	0.68	0.68
Salt (NaCl)	0.23	0.23	0.23
Minerals and vitamins[1]	0.45	0.45	0.45
Nutrient			
CP	17.4	17.6	17.5
NDF	29.5	29.3	29.4
Forage NDF	17.3	17.3	17.3
peNDF[2]	20.1	20.1	20.1
Starch	24.0	24.2	24.3
NFC	42.3	42.4	42.4
Nonstarch NFC	18.3	18.2	18.1
EE	5.0	5.0	5.0
Total FA[3]	4.0	4.0	4.0
18:1[3]	0.71	0.71	0.71

18:2[3]	2.11	2.11	2.11
18:3[3]	0.13	0.13	0.13
Ashes	5.8	5.7	5.7
Ca	0.80	0.80	0.80
P	0.54	0.54	0.54
Mg	0.39	0.40	0.53
K	1.33	1.33	1.33
Na	0.34	0.34	0.34
Cl	0.33	0.33	0.33
S	0.30	0.30	0.30
DCAD1[4], mEq/kg	213	213	213
DCAD2[5], mEq/kg	199	200	217

[1]Mineral and vitamins: 23.8% Ca, 16.9% P, 1.85% Mg, 2.27% S, 100 mg/kg Co, 1231 mg/kg Cu, 3080 mg/kg Mn, 7323 mg/kg Zn, 50 mg/kg Se, 124 mg/kg I, 615385 IU/kg vitamin A, 153600 IU/kg vitamin D, 3825 IU/kg vitamin E.

[2]Physically effective NDF. Particle size was evaluated using the Penn State Particle Separator with 19- and 8-mm sieves and a bottom pan. NDF concentration was determined for each sieve fraction, and only forage NDF retained on particles >8 mm was considered physically effective. Among concentrates, only whole cottonseed was included, with all of its NDF assumed to be physically effective.

[3]Estimated using NASEM (2021) feed library values.

[4]DCAD 1 = (Na/0.0023 + K/0.00391) – (Cl/0.00355 + S/0.00321 x 2)

[5]DCAD 2 = (Na/0.0023 + K/0.00391 + 0.15 x Ca/0.004 x 2 + 0.15 x Mg/0.0024 x 2) – (Cl/0.00355 + 0.25 x S/0.00321 x 2 + 0.5 x P/0.0031 x 3)

Table 2. Mean ± SD for composition of feed ingredients (1 composite sample/period; 3 total samples) and particle size of forages (3 samples/period; 9 total samples).

Item, % of DM unless otherwise stated	Whole-plant corn silage	Corn ear fibrous coproduct	Rehydrated ensiled corn grain[1]	Whole cottonseed[1]	Citrus pulp pellets[1]	Soybean meal[1]
DM, % of as-fed	36.0 ± 1.3	50.8 ± 4.1	58.1 ± 0.2	91.5 ± 0.4	89.1 ± 0.4	88.8 ± 0.3
CP	9.3 ± 0.3	17.1 ± 0.2	8.3 ± 1.5	23.2 ± 1.8	8.9 ± 0.7	50.3 ± 0.5
NDF	34.8 ± 2.1	43.8 ± 4.3	8.3 ± 3.6	51.8 ± 3.8	27.5 ± 1.2	12.9 ± 0.0
Starch	39.1 ± 2.1	12.0 ± 2.7	68.0 ± 3.7	-	-	-
EE	3.3 ± 0.1	2.7 ± 0.2	5.2 ± 0.8	16.4 ± 0.7	-	1.7 ± 0.1
NFC	49.3 ± 2.1	33.4 ± 4.3	8.3 ± 3.6	5.5 ± 4.0	55.8 ± 1.8	30.9 ± 1.0
Nonstarch NFC[2]	10.3 ± 0.2	21.4 ± 1.6	6.5 ± 2.0	5.1 ± 4.0	54.5 ± 1.8	28.5 ± 1.0
Ash	3.2 ± 0.4	3.0 ± 0.4	3.8 ± 0.2	3.0 ± 0.1	4.9 ± 0.3	4.2 ± 1.3
Ca	0.27 ± 0.03	0.15 ± 0.01	0.08 ± 0.03	0.15 ± 0.01	2.28 ± 0.17	0.39 ± 0.02
P	0.21 ± 0.01	0.64 ± 0.03	0.21 ± 0.05	0.65 ± 0.09	0.17 ± 0.02	0.74 ± 0.01
Mg	0.16 ± 0.01	0.39 ± 0.02	0.10 ± 0.02	0.44 ± 0.04	0.16 ± 0.01	0.40 ± 0.01
K	1.08 ± 0.08	1.78 ± 0.07	0.41 ± 0.09	1.50 ± 0.06	1.25 ± 0.03	2.72 ± 0.01
Na	0.03 ± 0.01	0.22 ± 0.08	0.02 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.01
Cl	0.24 ± 0.04	0.38 ± 0.01	0.03 ± 0.02	0.08 ± 0.01	0.13 ± 0.02	0.31 ± 0.01
S	0.19 ± 0.01	0.45 ± 0.02	0.12 ± 0.04	0.48 ± 0.05	0.20 ± 0.01	0.52 ± 0.01
Particle size[3]						
>19 mm	7.5 ± 1.0	24.0 ± 2.0	-	-	-	-
8-19 mm	63.3 ± 5.6	35.9 ± 3.1	-	-	-	-
< 8 mm	29.2 ± 6.1	40.2 ± 1.9	-	-	-	-
NDF by sieve[4]						
>19 mm	54.3 ± 3.9	58.4 ± 1.6	-	-	-	-
8-19 mm	38.7 ± 1.5	49.9 ± 3.4	-	-	-	-
< 8 mm	28.9 ± 6.8	38.6 ± 2.6	-	-	-	-
peNDF	27.2 ± 2.9	31.0 ± 2.3	-	-	-	-

[1]For nutrients indicated by "- ", chemical analyses were not performed and values were obtained from the NASEM (2021) feed library. Particle size distribution was evaluated only for forages.

[2]Nonstarch NFC = NFC – starch

[3]Particle size distribution was determined using the Penn State Particle Separator.

[4]The NDF concentration by particle size was analyzed in composite samples by sieve fraction within period (n = 3). Physically effective NDF (peNDF) was calculated as the sum of NDF retained on sieves >8 mm, obtained by multiplying the proportion of DM retained on each sieve by the NDF concentration of the respective fraction (19- and 8-mm sieves).

Table 3. Effect of treatments[1] on DMI, lactation performance, linear SCC, feed efficiency, BW, and BCS of dairy cows.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
DMI	22.7	22.2	22.1	1.00	0.20
DOMI	15.2	14.8	15.2	0.78	0.20
Milk yield	34.9	34.5	34.1	1.59	0.24
ECM	33.8	33.4	33.6	1.24	0.74
		g/d			
Fat	1195	1180	1215	42.6	0.28
Protein	1120	1108	1087	40.7	0.21
Lactose	1621	1605	1588	77.4	0.40
Total solids	4269	4216	4206	167.6	0.55
		%			
Fat	3.45[b]	3.44[b]	3.60[a]	0.105	0.02
Protein	3.22	3.23	3.21	0.063	0.72
Lactose	4.64	4.64	4.64	0.042	0.84
Total solids	12.26[b]	12.24[b]	12.39[a]	0.154	0.03
Linear SCC, 0 – 9[2]	1.62	1.58	1.58	0.44	0.98
MUN, mg/dL	13.7	13.4	13.2	0.40	0.12
Milk/DMI	1.48	1.51	1.48	0.04	0.48
ECM/DMI	1.44	1.46	1.47	0.03	0.39
ECM/DOMI	2.24	2.28	2.23	0.05	0.20
BW, kg	611	618	611	13.1	0.60
BCS, 1 - 5	3.17	3.19	3.21	0.06	0.85

[a,b] Means in a row with different superscripts differs ($P \leq 0.05$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

[2]Equivalency of the linear SCC: 1.62 = 38,400 cells/mL; 1.58 = 37,400 cells/mL.

Table 4. Effect of treatments[1] on ruminal pH variables.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Mean pH, stomach tubing	6.45	6.54	6.49	0.067	0.48
Mean pH, bolus	6.02	6.02	6.02	0.035	0.85
Minimum pH	5.56	5.59	5.58	0.045	0.60
Maximum pH	6.41	6.41	6.40	0.046	0.91
Amplitude[2]	0.85	0.82	0.83	0.059	0.75
pH ≤ 6.0, min/d	660	627	640	89.6	0.47
pH ≤ 5.8, min/d	276	295	282	58.0	0.81
pH ≤ 5.5, min/d	39	48	34	12.8	0.49
SARA index[3] pH 6.0	28	27	29	4.25	0.52
SARA index[3] pH 5.8	12	13	13	2.65	0.77
SARA index[3] pH 5.5	1.6	2.1	1.5	0.55	0.43

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

[2]Amplitude = maximum – minimum pH.

[3]SARA index = min/d of pH ≤ value of reference / DMI.

Table 5. Effect of treatments[1] on VFA molar proportions, mineral concentrations, and protozoa in rumen fluid.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Total VFA, mM	63.54	58.67	65.26	3.56	0.38
Molar % of total VFA					
Acetate	60.54	60.96	61.16	0.81	0.46
Propionate	23.54	23.70	23.62	1.00	0.97
Acetate/Propionate	2.64	2.65	2.68	0.15	0.92
Butyrate	10.12	10.27	10.29	0.31	0.71
Valerate	1.89	1.89	1.84	0.08	0.37
Isobutyrate	1.02	1.06	1.03	0.03	0.25
Isovalerate	2.01	2.12	2.11	0.07	0.12
Mineral concentration, g/L					
Ca	0.47	0.45	0.49	0.02	0.17
P	0.62	0.59	0.62	0.02	0.17
Mg	0.22[b]	0.17[c]	0.26[a]	0.01	<0.01
K	1.36	1.30	1.35	0.04	0.18
Protozoa, 10^5 cells/mL	2.30	2.34	2.27	0.45	0.90

[a,b] Means in a row with different superscripts differs ($P \leq 0.05$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

Table 6. Effect of treatments[1] on milk FA by gas chromatography (% of milk).

Item	Control	Iso-Mg	High-Mg	SEM	P-value
4:0	0.096	0.096	0.100	0.004	0.29
6:0	0.059	0.057	0.061	0.003	0.33
8:0	0.036	0.038	0.039	0.002	0.22
10:0	0.093	0.094	0.095	0.003	0.69
10:1	0.007	0.007	0.008	0.0004	0.30
11:0	0.002	0.002	0.002	0.0002	0.88
12:0	0.103	0.103	0.105	0.004	0.69
12:1	0.00156	0.00166	0.00141	0.0001	0.21
13:0	0.0046	0.0047	0.0046	0.0003	0.78
iso 13:0	0.0034	0.0035	0.0036	0.0002	0.64
anteiso 13:0	0.0021	0.0021	0.0023	0.0001	0.42
14:0	0.38	0.39	0.39	0.01	0.41
iso 14:0	0.0029	0.0026	0.0029	0.0003	0.54
cis-9 14:1	0.026	0.026	0.027	0.002	0.56
15:0	0.041	0.040	0.041	0.002	0.95
iso 15:0	0.0066	0.0066	0.0068	0.0003	0.50
anteiso 15:0	0.0160	0.0167	0.0165	0.0006	0.41
16:0	1.14^{ab}	1.12^{b}	1.18^{a}	0.05	0.03
iso 16:0	0.0065	0.0066	0.0067	0.0007	0.91
cis-9 16:1	0.051	0.051	0.054	0.003	0.24
17:0	0.0193	0.0191	0.0197	0.0007	0.25
17:0 iso	0.0114	0.0111	0.0117	0.0005	0.52
17:1	0.0051	0.0051	0.0054	0.0002	0.36
18:0	0.38	0.38	0.41	0.02	0.14
trans 18:1	0.093	0.100	0.092	0.006	0.29
trans-16 18:1	0.010	0.010	0.011	0.004	0.17
cis-9 C18:1	0.69^{b}	0.69^{b}	0.74^{a}	0.03	< 0.01
cis-11 18:1 cis-11	0.024	0.024	0.025	0.001	0.24

cis-12 18:1	0.017	0.018	0.017	0.001	0.52
cis-13 18:1	0.0013	0.0013	0.0013	0.0002	0.99
cis-15 18:1	0.0023	0.0020	0.0023	0.0002	0.50
cis-9, cis-12 18:2	0.077	0.075	0.0780	0.03	0.43
cis-9, trans-11 18:2	0.012	0.012	0.012	0.001	0.78
trans-10, cis-12 18:2[3]	ND	ND	ND	-	-
18:3 n-3	0.0047	0.0047	0.0050	0.0003	0.34
18:3 n-6	0.0008	0.0008	0.0008	0.00001	0.58
20:0	0.0035^{b}	0.0036^{ab}	0.0039^{a}	0.00001	0.03
20:1	0.0021	0.0021	0.0022	0.00001	0.18
20:3 n-6	0.0008	0.0009	0.0008	0.000006	0.20
20:4 n-6	0.00433^{AB}	0.00429^{B}	0.00471^{A}	0.0003	0.07
20:5 n-3	0.00028^{b}	0.00030^{ab}	0.00040^{a}	0.00005	0.05
22:0	0.0030	0.0029	0.0032	0.0002	0.30
24:0	0.0007	0.0007	0.0007	0.0001	0.82
22:5	0.00079	0.00086	0.00089	0.00008	0.17
Monounsaturated	0.93^{b}	0.94^{b}	0.99^{a}	0.03	< 0.01
Polyunsaturated	0.094	0.092	0.096	0.003	0.38
De novo[2]	0.89	0.89	0.91	0.02	0.27
Mixed[2]	1.23^{ab}	1.21^{b}	1.28^{a}	0.05	0.03
Preformed[2]	1.33^{b}	1.33^{b}	1.41^{a}	0.04	0.02

[a,b] Means in a row with different lowercase superscripts differs ($P < 0.05$; Tukey test).

[A,B] Means in a row with different uppercase superscripts differs ($0.05 \leq P < 0.10$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

[2]De novo = FA with 4 to 15 C. Mixed = FA with 16 or 17 C. Preformed = FA ≥ 18 C.

[3]Trans-10, cis-12 18:2 was not detected in milk fat (ND).

Table 7. Effect of treatments[1] on intake, excretion and total-tract apparent digestibility of nutrients.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Intake, kg/d					
DM	22.7	22.2	22.1	1.00	0.20
OM	21.4	20.9	20.8	0.95	0.21
CP	3.96	3.88	3.86	0.17	0.24
NDF	6.69^{A}	6.56^{AB}	6.46^{B}	0.31	0.08
Starch	5.44	5.34	5.34	0.25	0.54
EE	1.13	1.10	1.09	0.05	0.12
Ashes	1.31	1.26	1.26	0.06	0.17
NFC	9.59	9.74	9.38	0.55	0.46
Nonstarch NFC	4.15	4.07	4.04	0.19	0.29
Fecal excretion, kg/d					
DM	6.48^{a}	6.62^{a}	5.96^{b}	0.25	< 0.01
OM	6.12^{a}	6.24^{a}	5.60^{b}	0.24	< 0.01
CP	1.05^{A}	1.05^{A}	0.96^{B}	0.05	0.06
NDF	3.57^{a}	3.67^{a}	3.25^{b}	0.12	< 0.01
Starch	0.064	0.053	0.038	0.007	0.13
EE	0.346^{a}	0.340^{a}	0.293^{b}	0.019	< 0.01
Ashes	0.360	0.379	0.354	0.021	0.40
NFC	1.42	1.36	1.32	0.08	0.37
Nonstarch NFC	1.36	1.31	1.28	0.08	0.53
Total-tract apparent digestibility, %					
DM	71.2^{ab}	70.1^{b}	72.9^{a}	0.69	< 0.01
OM	71.2^{b}	70.1^{b}	73.0^{a}	0.69	< 0.01
CP	73.6^{AB}	73.0^{B}	75.2^{A}	0.83	0.07
NDF	46.2^{ab}	43.4^{b}	49.4^{a}	1.59	< 0.01
Starch	98.9^{b}	99.0^{ab}	99.3^{a}	0.13	0.04
EE	69.3^{b}	69.2^{b}	73.2^{a}	0.02	0.02

Ashes	72.2	69.9	72.0	1.28	0.28
NFC	85.3	85.6	86.0	0.62	0.60
Nonstarch NFC	67.5	67.9	68.5	1.49	0.84

[a,b] Means in a row with different lowercase superscripts differs ($P < 0.05$; Tukey test).

[A,B] Means in a row with different uppercase superscripts differs ($0.05 \leq P < 0.10$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

Table 8. Effect of treatments[1] on intake, excretion and total-tract apparent digestibility of Ca, P, Mg and K.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Intake, g/d					
Ca	181	177	176	8.2	0.24
P	123^{A}	119^{AB}	118^{B}	5.4	0.08
Mg	89^{b}	88^{b}	117^{a}	4.3	< 0.01
K	312	305	303	13.7	0.14
Fecal excretion, g/d					
Ca	130^{a}	122^{b}	113^{c}	6.3	< 0.01
P	84^{a}	79^{ab}	74^{b}	5.2	0.03
Mg	67^{b}	69^{b}	86^{a}	3.6	< 0.01
K	51^{a}	46^{b}	36^{c}	2.6	< 0.01
Total-tract apparent digestibility, %					
Ca	28^{b}	31^{ab}	36^{a}	2.2	< 0.01
P	32^{B}	34^{AB}	38^{A}	2.5	0.07
Mg	24^{AB}	21^{B}	27^{A}	2.2	0.07
K	84^{b}	85^{b}	88^{a}	0.7	< 0.01

^{a,b} Means in a row with different lowercase superscripts differs ($P < 0.05$; Tukey test).

^{A,B} Means in a row with different uppercase superscripts differs ($0.05 \leq P < 0.10$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

Table 9. Effect of treatments[1] on meal behavior, activities, proportion of daily intake during periods of the day, feed particles, and nutrients sorting.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Meal behavior					
Meal frequency, meals/d	9.1	9.6	9.0	0.32	0.29
Meal size, kg DM/meal	2.6	2.4	2.5	0.15	0.19
Meal time, min/d	320	331	344	23.9	0.71
Meal duration, min/meal	36.3	36.0	39.3	3.27	0.65
First meal duration, min/meal	33.6^{b}	32.9^{b}	37.2^{a}	3.43	0.01
Activities, min/d					
Eating	271	276	266	9.79	0.31
Ruminating	418	419	419	17.50	0.99
Chewing	689	695	685	23.89	0.94
Idle	416	417	421	23.19	0.90
Proportion of daily intake, %					
0700-1200h	34.3^{ab}	33.8^{b}	36.8^{a}	1.39	0.05
1200-1900h	39.0	39.0	39.3	1.40	0.95
1900-0700h	26.7^{ab}	27.3^{a}	23.9^{b}	1.52	0.04
Feed particles sorting					
0700-1200 h					
> 19 mm	92^{b}	122^{a}	118^{ab}	9.8	0.04
8-19 mm	110	110	107	15.0	0.30
< 8 mm	90^{A}	85^{B}	89^{AB}	17.9	0.07
1200-1900 h					
> 19 mm	91	88	79	5.6	0.25
8-19 mm	100	103	101	1.2	0.16
< 8mm	100^{AB}	98^{B}	101^{A}	1.2	0.07
1900-0700 h					
> 19 mm	82	87	80	5.3	0.57

8-19 mm	103	102	103	11.3	0.59
< 8 mm	99	100	101	13.9	0.61
Nutrient sorting					
NDF	96.7	96.8	96.7	0.43	0.91
Starch	102.3	102.6	103.1	0.54	0.53
P	99.5	100.1	100.1	0.21	0.21
Mg	99.7[b]	100.9[a]	100.5[ab]	0.34	0.03

[a,b] Means in a row with different lowercase superscripts differs ($P < 0.05$; Tukey test).

[A,B] Means in a row with different uppercase superscripts differs ($0.05 \leq P < 0.10$; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

Table 10. Effect of treatments[1] on Mg, P and Ca in blood serum, jugular blood acid-base balance, serum haptoglobin, serum LBP, and plasma D-lactate.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Minerals					
Mg, mg/dL	1.85^{b}	1.94^{a}	1.83^{b}	0.03	< 0.01
P, mg/dL	7.33	7.30	7.28	0.26	0.93
Ca, mg/dL	9.70	10.05	9.84	0.14	0.19
Acid-base balance					
pH	7.44	7.43	7.44	0.007	0.59
pCO2, mm Hg	37.95	39.05	38.46	0.80	0.47
pO2, mm Hg	28.98	31.76	29.42	1.41	0.42
$HCO3^{-}$, mM	25.09^{B}	25.67^{AB}	26.09^{A}	0.54	0.10
Total CO2, mM	26.24^{B}	26.81^{AB}	27.33^{A}	0.57	0.09
Base excess, mM	1.57	1.86	2.48	0.58	0.22
SatO2[3], %	59.10	61.33	56.57	2.96	0.51
SARA related markers					
Haptoglobin, mg/dL	20.36	20.64	19.31	1.61	0.70
LBP, µg/mL	1.55	1.74	1.54	0.09	0.15
D-lactate, mM	1.22^{AB}	1.23^{A}	1.08^{B}	0.06	0.06

^{a,b} Means in a row with different lowercase superscripts differs (P < 0.05; Tukey test).

^{A,B} Means in a row with different uppercase superscripts differs (0.05 ≤ P < 0.10; Tukey test).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

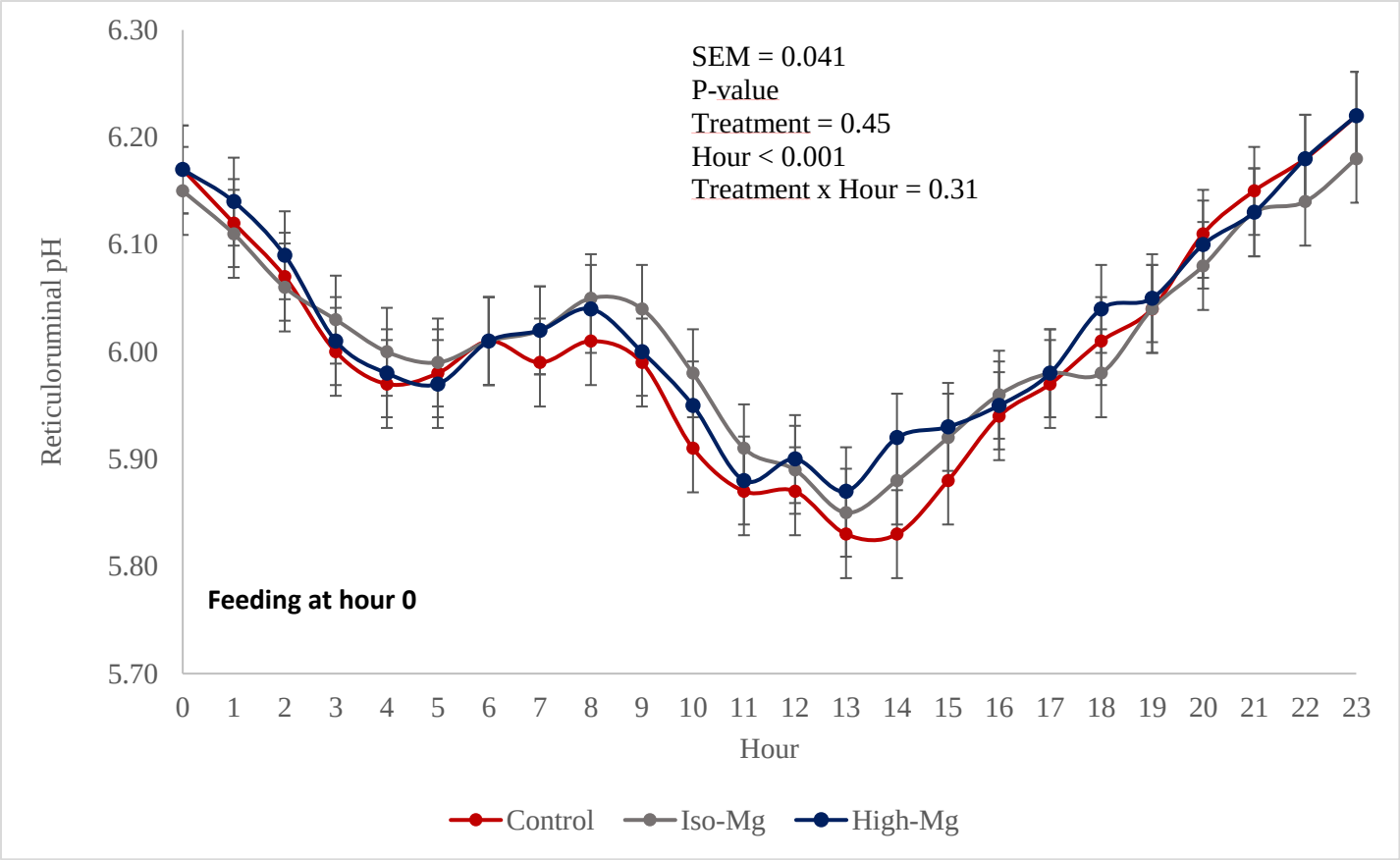
Table 11. Effect of treatments[1] on fecal pH, viscosity, urine and allantoin excretion.

Item	Control	Iso-Mg	High-Mg	SEM	P-value
Fecal pH	7.44[b]	7.47[ab]	7.53[a]	0.02	0.03
Fecal viscosity, cP	53.8	57.0	55.3	3.65	0.73
Urine excretion, L/d	27.3	29.5	28.1	3.64	0.48
Allantoin in urine, mmol/d	336	374	356	30	0.27

[a,b] Means in a row with different superscripts differs at Tukey test ($P \leq 0.05$).

[1]Control = supplementation of 0.41% dicalcium phosphate and 0.23% magnesium oxide (0.54% P, 0.39% Mg in the diet). Iso-Mg = supplementation of 0.50% magnesium phosphate (0.54% P, 0.40% Mg). High-Mg = supplementation of 0.50% magnesium phosphate and 0.23% magnesium oxide (0.54% P, 0.53% Mg).

Figure 1. Effects of treatment, hour and treatment x hour interaction on reticuloruminal pH.



ARTIGO 2 - Associations among chewing behavior, ruminal pH, performance and metabolism in primiparous and multiparous dairy cows

Artigo formatado de acordo com as normas para submissão ao Journal of Dairy Science.

ABSTRACT

Ruminal pH is central to productivity and health in lactating dairy cows, and its regulation arises from multiple interacting dietary, behavioral, and animal factors. This study evaluated associations between dietary carbohydrate intake, sorting behavior, chewing activity, and ruminal pH dynamics, and determined whether these relationships differed between primiparous and multiparous cows. Data were compiled from two experiments, totaling 108 cow-period observations from 36 cows (15 primiparous and 21 multiparous). Ruminal pH was continuously recorded using bolus sensors, and chewing behavior was assessed by visual observation. Associations were evaluated using linear mixed-effects models including experiment, parity, the continuous predictor, and their interaction as fixed effects, with cow nested within experiment as a random effect and repeated measures modeled using an autoregressive AR(1) structure. Predictors were retained when $P \leq 0.05$ and model fit improved relative to a basal model ($\Delta AIC < 0$). Partial R^2 was defined as the increase in marginal R^2 after inclusion of the predictor in the model, corresponding to the additional proportion of variance explained by the predictor relative to a model containing the same fixed and random effects but excluding that predictor. Fiber intake, particularly forage NDF and physically effective NDF (peNDF>8) expressed relative to BW, was positively associated with rumination (partial R^2 = 0.18–0.19) and chewing time (partial R^2 = 0.13–0.14), and with indicators of greater ruminal pH stability, including reduced time below pH 6.0 (partial R^2 = 0.04–0.09) and increased maximum pH (partial R^2 = 0.08–0.09). Chewing behavior did not consistently explain variation in ruminal pH as an independent predictor. However, rumination modified the association between fiber intake and pH, strengthening the reduction in time below pH 6.0 as fiber increased. Several associations were influenced by parity, with multiparous cows showing greater increases in rumination with increasing fiber intake, whereas primiparous cows

exhibited a more attenuated response and greater susceptibility to unfavorable pH dynamics. Ruminal pH metrics were associated with DMI, milk yield, milk fatty acid profile, serum haptoglobin, ruminal VFA profile, fecal pH, and jugular blood pCO_2, supporting their biological relevance. Overall, ruminal pH stability reflected the interaction between fiber intake, chewing behavior, and animal characteristics.

INTRODUCTION

Ruminal pH is a key determinant of productivity and health in lactating dairy cows. When fermentable carbohydrate supply exceeds the buffering and absorptive capacity of the rumen, pH may decline for prolonged periods, leading to SARA, a condition associated with reduced performance, impaired reproduction, and compromised animal health (Plaizier et al., 2008; Aschenbach et a., 2019). Although widely studied, SARA is a multifactorial and complex disorder whose effects are not always consistently expressed or easily detected under practical feeding conditions, and it may also be linked to other digestive disorders (Plaizier et al., 2022). Therefore, understanding the determinants of ruminal pH dynamics remains a central objective in dairy nutrition research.

Dietary fiber intake plays a central role in maintaining ruminal stability. Physically effective NDF (peNDF>8) promotes the formation of the ruminal mat and stimulates chewing activity, including eating and rumination, thereby increasing saliva secretion and enhancing ruminal buffering through bicarbonate and phosphate (Mertens, 1997). In this context, several studies have shown that increasing dietary peNDF>8 or forage NDF intake increases rumination and total chewing time, supporting ruminal function and reducing the risk of excessive pH depression (Allen, 1997; White et al., 2017). These relationships have led to the widespread use of chewing behavior as an indirect indicator of ruminal health in dairy cows. Despite these well-established associations, ruminal pH regulation arises from multiple interacting mechanisms. Ruminal pH reflects the dynamic balance among fermentation acid

production, buffering capacity, and the absorption of VFA across the rumen epithelium (Penner et al., 2009; Aschenbach et al., 2011). Consequently, chewing activity alone may not consistently predict ruminal pH dynamics.

Animal characteristics may further modulate these relationships. Differences between primiparous and multiparous cows in chewing behavior have been reported. Primiparous cows typically consume less feed and may exhibit distinct feeding patterns compared with multiparous cows (Maekawa et al., 2002), which may alter the relationships among dietary fiber intake, chewing behavior, and ruminal pH stability (Humer et al., 2015; Khorrami et al., 2021). However, parity effects are not always considered in models evaluating ruminal pH dynamics.

Alterations in ruminal pH are also linked to broader digestive and systemic responses. Prolonged exposure to low ruminal pH has been associated with reduced fiber digestibility (Krajcarski-Hunt et al., 2002), shifts in ruminal fermentation patterns (Saleem et al., 2012), changes in biohydrogenation pathways (Bauman and Griinari, 2001), and alterations in milk component synthesis, particularly milk fat production (Razzaghi et al., 2022; Fukumori et al., 2021). In addition, low ruminal pH has been associated with increased risk of systemic inflammation and changes in circulating metabolites and acid–base balance (Aschenbach et al., 2019; Hu and Murphy, 2004). Evaluating associations between ruminal pH metrics and productive or metabolic responses may therefore provide further insight into the biological relevance of ruminal pH dynamics.

Although dietary fiber intake and chewing behavior are recognized as key determinants of ruminal function, they are typically evaluated independently. Limited information is available on how these factors interact to influence ruminal pH dynamics within the same analytical framework, particularly when accounting for variation in animal characteristics such as parity. Understanding whether chewing behavior modifies the relationship between fiber

intake and ruminal pH stability may help refine current concepts of ruminal buffering and improve the interpretation of behavioral indicators.

Therefore, the objectives of this study were to evaluate associations between dietary carbohydrate intake, sorting behavior, and ruminal pH dynamics in lactating dairy cows; determine whether these associations differ between primiparous and multiparous cows; investigate relationships between ruminal pH metrics and production or metabolic variables; and assess whether chewing behavior modifies the association between fiber intake and ruminal pH stability.

MATERIAL AND METHODS

Animals, experiments, and general design

Data used in this study were compiled from two experiments conducted at the Better Nature Research Center (Ijaci, MG, Brazil). Both experiments were designed to evaluate mineral sources. Dietary energy, protein, and carbohydrate profiles were not altered among treatments within each experiment. All animal procedures were approved by the Animal Use Ethics Committee of the Federal University of Lavras (protocols 015/2020 and 3443040923). In both experiments, lactating Holstein cows were housed in the same open-sided, sand-bedded tie-stall barn equipped with fans and sprinklers and milked in an adjacent parlor.

Experiment 1 was conducted with 15 cows distributed into five 3 × 3 Latin squares blocked by parity (6 primiparous and 9 multiparous), milk yield, and days in milk (193 ± 120 at the beginning of the experiment). Each experimental period lasted 21 d, including 13 d of diet adaptation followed by 8 d of data collection. The experiment included a phase of normal feeding followed by a short-term SARA challenge designed to evaluate treatment responses. For the present study, only data collected during the normal feeding phase (d 14 to 17 of each period) were used, when cows were fed the experimental diets under standard management

conditions. This approach was adopted because the objective was not to evaluate treatment responses to the SARA challenge, but rather to assemble a dataset with variation in diet composition, chewing behavior, and ruminal pH under practical feeding conditions.

Experiment 2 was conducted with 21 cows distributed into seven 3 × 3 Latin squares also blocked by parity (9 primiparous and 12 multiparous), milk yield, and DIM (144 ± 77 at the beginning of the experiment). Each experimental period lasted 21 d, including 14 d of diet adaptation followed by 7 d of data collection, and data from d 15 to 21 of each period were used in the present study. No cow was used in both experiments. Therefore, the compiled dataset included 108 cow-period observations obtained from 36 cows, comprising 21 multiparous and 15 primiparous animals. Descriptive statistics of cows in both experiments are presented in Table 1.

Feeding and diet characterization

Cows were individually fed a TMR once daily at 0700 h in both experiments, formulated to allow for 10 to 15% orts. The DM concentration of forages and rehydrated corn grain was monitored weekly and diets were adjusted accordingly. For the present study, predictor variables of dietary interest included DMI and the intake of NDF, forage NDF, physically effective NDF (>8 mm; peNDF>8), forage NDF and peNDF>8 expressed as percentage of BW, starch, NFC, and non-starch NFC. Diet composition and nutrient intake were calculated from the chemical composition of feed ingredients and orts collected during the sampling phase of each experiment (d 14 to 16 for Experiment 1 and d 15 to 21 for Experiment 2). Ingredient and nutrient concentrations of diets in each experiment are presented in Table 1.

Feed and ort samples were collected and composited by experimental period, dried in a forced-air oven at 55°C for 72 h, and ground to pass a 1-mm screen for chemical analyses. The DM concentration was determined at 105°C for 24 h. In Experiment 1, NDF concentration was

determined using the filter bag technique with heat-stable α-amylase and without sodium sulfite (Schlau et al., 2021). Starch plus free glucose was determined using α-amylase and amyloglucosidase with colorimetric determination of glucose as described by Fernandes et al. (2022). Crude protein concentration was determined using micro-Kjeldahl steam distillation (method 990.03; AOAC International, 2006), ether extract according to AOAC method 2003.05 (AOAC International, 2006), and ash by incineration at 550°C for 8 h.

In Experiment 2, NDF concentration was determined using heat-stable α-amylase and sodium sulfite according to Detmann et al. (2012), based on AOAC method 2002.04 (AOAC International, 2006) and adapted for autoclave procedures using filter bags. Starch plus free glucose was determined using α-amylase and amyloglucosidase with colorimetric quantification of glucose as described by Silva et al. (2019). Ether extract was determined using Soxhlet extraction with diethyl ether (method 920.39; AOAC, 2005), CP concentration was determined by combustion and detection of N_2 (Leco Analyzer; Leco Corp., St. Joseph, MI, USA), and ash concentration was determined by incineration at 600°C for 4 h. The NFC concentration was calculated as 100 − (CP + NDF + EE + Ash), and non-starch NFC as NFC − starch.

Particle size distribution of forages was evaluated using the Penn State Particle Separator (PSPS) with 19-mm and 8-mm sieves and the bottom pan during the sampling days of each experimental period. The DM and NDF concentration of each particle fraction were determined in composite samples formed by sieve fraction within each period. The peNDF>8 was calculated as the sum of NDF contributions from particles retained on sieves >8 mm, obtained by multiplying the proportion of DM retained on the 19- and 8-mm sieves by the NDF concentration of the respective fractions and summing these values. For concentrate ingredients, only the NDF from whole cottonseed was considered and assumed to be fully retained above 8 mm.

Chewing and sorting behavior

Chewing behavior was recorded by continuous visual observation at 5-min intervals over a 24-h period on d 16 of Experiment 1 and on d 15, 16, and 17 of Experiment 2. For Experiment 2, the average of the three days evaluated within each period was used in the statistical analyses. Activities were classified as eating, rumination, drinking, or idle. Chewing time was calculated as eating + rumination time. The duration of the first daily meal was also recorded using a stopwatch.

Particle size sorting behavior was evaluated using the Penn State Particle Separator (PSPS; Lammers et al., 1996) with 19- and 8-mm sieves and a pan. In Experiment 1, sorting behavior was evaluated during d 16 of each experimental period by measuring the particle distribution and weight of the offered TMR and orts from each cow at 0700, 1230, 1830, and 0500 h (orts collected on the following day). In Experiment 2, sorting behavior was evaluated from d 15 to 17 of each period by measuring the particle distribution of the TMR available to each cow at 0700 h (offered TMR), 1200 h, 1900 h, and 0500 h of the following day. For this experiment, the average of the three days evaluated within each period was used in the statistical analyses.

Predicted particle intake (as-fed basis) was calculated as the proportion of TMR retained on each sieve multiplied by TMR intake. Observed particle intake was calculated as the proportion of TMR retained on each sieve multiplied by TMR offered minus the proportion of orts retained on each sieve multiplied by orts. The sorting index was calculated as 100 × (observed intake / predicted intake), according to Leonardi and Armentano (2003), where values <100% indicate selective refusal, values >100% indicate preferential intake, and 100% indicates no sorting.

Ruminal pH

In both experiments, wireless ruminal pH boluses (smaXtec, Graz, Austria) were inserted into the reticulum of each cow, and pH was recorded at 10-min intervals throughout the experimental periods. Daily summaries of ruminal pH were calculated from these data, including mean pH, minimum pH, maximum pH, pH amplitude (maximum − minimum), and time below pH 6.0, 5.8, and 5.5 (min/d). For the present study, ruminal pH data from d 14 to 16 of each period were used for Experiment 1, whereas data from d 15 to 21 of each period were used for Experiment 2.

Milk yield, milk composition, and milk fatty acids

In Experiment 1, cows were milked twice daily at 0500 and 1600 h, whereas in Experiment 2 cows were milked three times daily at 0500, 1200, and 1900 h. Milk yield was recorded daily in both experiments, and period averages from the sampling phase were used for the present study. Milk samples were collected proportionally to yield at each milking and analyzed by mid-infrared spectroscopy for fat, protein, lactose, milk urea nitrogen (MUN), and fatty acid (FA) profile.

In Experiment 1, milk samples were analyzed using a Nexgen FTS/FCM analyzer (Bentley Instruments Inc., Chaska, MN, USA) at the Laboratory of the Paraná State Holstein Breeders Association (APCBRH; Curitiba, PR, Brazil). In Experiment 2, milk samples were analyzed using a MilkoScan FT (Foss Inc., Hillerød, Denmark) at the Clínica do Leite (Piracicaba, SP, Brazil). Fatty acids were grouped according to carbon chain length as de novo FA (C4 to C15) or preformed FA (≥ C17). Mixed FA were not included in the analyses because MIR data for this fraction were not available for Experiment 2. Body weight was recorded during the sampling period after the morning milking and was used to express fiber intake variables relative to BW.

Ruminal fermentation and blood analysis

Ruminal fluid was collected by orogastric tube during the sampling phase of each experiment (d 17 in Experiment 1 and d 21 in Experiment 2). Samples were analyzed for VFA profile by gas chromatography (CP 3800 Gas Chromatograph, Varian Chromatography Systems, Walnut Creek, CA, USA) and total protozoa count using an optical microscope with a Neubauer chamber.

In Experiment 1, jugular blood samples were collected on d 17 and 18 at 1300 h (6 h after feeding) for evaluation of blood acid–base balance in a commercial laboratory (Laboratório Santa Cecília, Lavras, Brazil). Coccygeal blood samples were collected on d 17 to 21 at 1700 h (10 h after feeding). Serum haptoglobin concentration was determined according to Cooke and Arthington (2012). Plasma D-lactate concentration was measured using a commercial colorimetric assay kit (D-Lactate Colorimetric Assay Kit, MAK058; Sigma-Aldrich, St. Louis, MO, USA).

In Experiment 2, jugular blood samples were collected on d 18 at 1400 h (7 h after feeding) to evaluate blood acid–base balance using a blood gas analyzer at a commercial laboratory (Hemocell, Lavras, Brazil. Additional coccygeal blood samples were collected on d 18 at 1800 h (11 h after feeding) for determination of D-lactate and haptoglobin concentrations. Plasma D-lactate concentration was determined using an enzymatic assay kit (EnzyChrom D-Lactate Assay Kit, BioAssay Systems). Serum haptoglobin concentration was determined by polyacrylamide gel electrophoresis according to Weber and Osborn (1969).

Fecal and digestibility measurements

Total fecal and urine collections were conducted in both experiments using three 8-h collection periods with 8-h intervals, starting 8 h later on each consecutive day to represent a complete 24-h sampling cycle. In Experiment 1, fecal and urine collections were conducted

from d 14 to 16 of each experimental period, whereas in Experiment 2 collections were conducted from d 19 to 21 of each experimental period.

Feces and urine were collected directly into buckets during excretion. Fecal samples were immediately weighed at each defecation, and subsamples corresponding to 1% of the fresh weight were collected and frozen throughout the collection period to form one composite sample per cow per period. Composite samples were dried in a forced-air oven at 55°C for 72 h. Concentrations of DM, ash, NDF, starch, ether extract, and CP were determined as previously described for feed analysis. Total-tract apparent digestibility of nutrients was calculated using intake and fecal excretion data.

Urine volume was measured immediately after each urination using a graduated beaker, and a subsample corresponding to 3% of the total volume was collected and stored under refrigeration throughout the collection period. Period composites were acidified with a 4% sulfuric acid solution and frozen for allantoin determination according to Chen and Gomes (1992). Daily urinary allantoin excretion was used as an indirect estimate of ruminal microbial yield (Valadares et al., 1999).

Fecal pH was evaluated in both experiments. In Experiment 1, fecal samples from the first defecation of each cow on d 16 of each experimental period were collected and frozen at −20°C until analysis. After thawing, samples were homogenized and a 15-g subsample was diluted in 100 mL of distilled water, homogenized for 30 s, and pH was measured using a pH meter (Digimed DM-20, Digicrom Analítica, Brazil). In Experiment 2, fecal pH was determined from composite fecal samples obtained during the collection period. After thawing and before drying, a 10-g subsample of feces was diluted in 100 mL of distilled water and pH was measured using a pH meter after 30 min of equilibration.

Statistical analysis

Statistical analyses were conducted in R using the nlme package. Associations between candidate predictors and response variables were evaluated using linear mixed-effects models. Fixed effects included experiment, parity, the continuous predictor of interest, and the interaction between predictor and parity. Cow nested within experiment was included as a random effect to account for repeated observations from the same animal across experimental periods. Repeated measurements within cow were modeled using a first-order autoregressive covariance structure [AR(1)]. Continuous predictors were mean-centered prior to analysis by subtracting the overall mean of the variable from each observation. This approach facilitates interpretation of regression coefficients and reduces collinearity between main effects and interaction terms.

Model outputs reported in tables include regression coefficients (β) and their standard errors (SE), P-values obtained from the model ANOVA table, and Akaike's Information Criterion (AIC). Model fit was further described using marginal and conditional coefficients of determination (R^2), calculated with the MuMIn package, representing the proportion of variance explained by fixed effects alone and by the full model (fixed plus random effects), respectively. Partial R^2 values were calculated as the increase in marginal R^2 when the predictor of interest was added to the model. The reduced model had the same structure as the full model but excluded the predictor while retaining experiment, parity, and the same random and repeated structure. Thus, partial R^2 represents the additional variance explained by including the predictor in the model.

Associations were retained for presentation when the predictor effect was significant ($P \leq 0.05$) and model fit improved relative to the basal model ($\Delta AIC < 0$). Statistical significance was declared at $P \leq 0.05$, and interactions were discussed when $P \leq 0.10$.

To evaluate whether chewing behavior modified the association between fiber-related variables and ruminal pH, additional models were fitted including the centered fiber variable,

the centered chewing behavior variable, and their interaction as fixed effects, while maintaining experiment and parity in the model structure. A hierarchical modeling approach was adopted, in which chewing behavior variables were evaluated both as independent predictors and as potential effect modifiers of fiber-related variables. An initial screening step based on partial R^2 values (Table 2) was used to identify fiber-related predictors with greater explanatory power for ruminal pH outcomes, which were subsequently selected for interaction analyses. Specifically, interactions between forage NDF (kg/d and % of BW) and rumination, as well as between peNDF>8 (kg/d and % of BW) and rumination, were tested in models predicting variables of ruminal pH. Model selection followed the same criteria described above, and fiber × chewing interactions were considered relevant when the interaction term was significant ($P \leq 0.05$) and improved model fit relative to the corresponding main-effects model ($\Delta AIC < 0$).

RESULTS

Descriptive statistics

Descriptive statistics for cows, diet composition, chewing behavior, and ruminal pH variables are presented in Table 1. Across experiments, cows averaged (average ± SD) 193 ± 120 and 144 ± 77 DIM in Experiments 1 and 2, respectively, with milk yield averaging 27.7 ± 4.2 and 34.6 ± 5.2 kg/d. The DMI averaged 22.5 ± 2.7 and 22.4 ± 3.2 kg/d across experiments. Mean ruminal pH averaged 6.26 ± 0.11 in Experiment 1 and 6.01 ± 0.17 in Experiment 2, whereas time below ruminal pH 5.8 averaged 46 ± 63 and 292 ± 279 min/d. Rumination, eating, and total chewing time averaged 471 ± 59, 370 ± 45, and 841 ± 79 min/d in Experiment 1 and 422 ± 68, 255 ± 36, and 677 ± 86 min/d in Experiment 2.

Total NDF concentration was 36.2% of DM in Experiment 1 and 29.4% of DM in Experiment 2, with forage NDF averaging 23.9 and 17.3% of DM, respectively. The peNDF>8 averaged 23.4% of DM in Experiment 1 and 20.1% of DM in Experiment 2. Ingredient

composition differed between experiments, with alfalfa haylage included in Experiment 1 and a corn fibrous byproduct (Comidão, Cargill Agrícola, Uberlândia, Brazil; Silva et al., 2022) included in Experiment 2. Starch concentration was relatively similar between experiments (22.6 vs. 24.2% of DM), whereas NFC averaged 35.4 and 42.4% of DM, respectively. The higher NFC concentration in Experiment 2 was largely associated with the greater proportion of non-starch NFC provided by the corn fibrous byproduct (21.4% in the present dataset).

Fiber intake variables and chewing behavior

Rumination time was positively associated with several fiber intake variables, with the strongest effects observed for peNDF>8 and forage NDF expressed as a percentage of BW. These predictors showed the greatest explanatory power among variables evaluated (partial R^2 = 0.19 and 0.18, respectively). Based on model coefficients, rumination increased by approximately 20 to 21 min/d for each 0.1-unit increase in peNDF>8 and forage NDF (% of BW), respectively, within the observed data range. Marginal R^2 values ranged from 0.45 to 0.46, whereas conditional R^2 values ranged from 0.79 to 0.83, indicating that both fixed effects and cow-level variation contributed substantially to explaining rumination variability. Additional positive associations were observed for peNDF>8 intake (kg/d), forage NDF intake (kg/d), and DMI, although these predictors explained a smaller proportion of the variance (partial R^2 ranging from 0.09 to 0.14).

Eating time showed similar directional associations with fiber intake. Both peNDF>8 (% of BW) and forage NDF (% of BW) were positively associated with eating time ($P < 0.01$), although their explanatory power was modest (partial $R^2 \approx 0.04$). Despite the low partial R^2, marginal R^2 values were high (~0.71), indicating that fixed effects collectively explained a large proportion of the variation in eating time, although the specific contribution of fiber variables was small. Conditional R^2 values were slightly higher (~0.84), suggesting a comparatively smaller contribution of random effects.

Total chewing time also increased with increasing fiber intake. The strongest associations were observed for peNDF>8 (% of BW) and forage NDF (% of BW), with partial R^2 values of 0.14 and 0.13, respectively. For these predictors, marginal R^2 values ranged from 0.66 to 0.67 and conditional R^2 values from 0.83 to 0.87. Positive associations with chewing time were also observed for peNDF>8 intake (kg/d), forage NDF intake (kg/d), and DMI, although these predictors explained a smaller proportion of the variance (partial R^2 between 0.05 and 0.09).

Idle time was negatively associated with fiber intake. The strongest relationship was observed for forage NDF expressed as a percentage of BW (partial $R^2 = 0.15$), followed by peNDF>8 (% of BW) (partial $R^2 = 0.10$). First meal duration was also positively associated with fiber intake, particularly forage NDF (% of BW) and peNDF>8 (% of BW), although these predictors explained a small proportion of the variance (partial $R^2 = 0.06$ and 0.04, respectively).

Several associations between fiber-related variables and chewing behavior were influenced by parity (Figure 1). Significant predictor × parity interactions were observed for rumination in relation to forage NDF (% of BW; $P = 0.02$) and chewing time in relation to forage NDF (% of BW; $P = 0.005$), with tendencies for peNDF>8 (% of BW; $P = 0.06$) and forage NDF intake (kg/d; $P = 0.06$). In these cases, multiparous cows exhibited a steeper increase in rumination and chewing time as fiber increased compared with primiparous cows. Parity interactions were also observed for idle time in relation to peNDF>8 (% of BW; $P = 0.03$) and forage NDF (% of BW; $P = 0.04$), with idle time decreasing more markedly in multiparous cows as fiber increased. Additionally, tendencies for interaction were detected between DMI and parity for time below pH 6.0 ($P = 0.06$), between peNDF>8 (% of BW) and parity for ruminal pH amplitude ($P = 0.07$), and for eating time in relation to forage NDF (% of BW; $P = 0.10$).

Fiber intake, sorting behavior, and ruminal pH

Associations between DMI, fiber intake, sorting behavior, and ruminal pH are presented in Table 3. Time below pH 6.0 was negatively associated with several intake-related variables, including DMI ($P = 0.05$; partial $R^2 = 0.09$), NDF intake (kg/d; $P = 0.009$; partial $R^2 = 0.08$), forage NDF (% of BW; $P = 0.008$; partial $R^2 = 0.06$), forage NDF intake (kg/d; $P = 0.05$; partial $R^2 = 0.06$), and peNDF>8 (% of BW; $P = 0.04$; partial $R^2 = 0.04$). For forage NDF (% of BW), the model coefficient indicates that time below pH 6.0 decreased by approximately 118 min/d for each 0.1-unit increase within the range observed in the dataset. Across these models, marginal and conditional R^2 values were low and nearly identical (≈0.05–0.10), indicating that most of the explained variation was attributable to fixed effects rather than cow-level random effects.

Feed sorting behavior was also associated with ruminal pH dynamics. Greater sorting of medium particles (8–19 mm) and fines (pan fraction) in the morning was negatively associated with time below pH 6.0 ($P = 0.01$ and $P = 0.03$, respectively), although the explanatory power of these predictors was modest (partial $R^2 \leq 0.04$). Notably, the model including sorting of medium particles showed a substantially higher conditional R^2 (0.39), indicating a greater contribution of random effects relative to fixed effects in this case. In addition, sorting behavior of long particles (>19 mm) in the afternoon was positively associated with time below pH 5.5 ($P = 0.04$), although the magnitude of this effect was small ($\beta = 0.36 \pm 0.36$) and explained little of the variance (partial $R^2 = 0.02$), with moderate marginal and conditional R^2 values (0.12 and 0.23, respectively).

Maximum ruminal pH increased with increasing fiber supply. Both forage NDF (% of BW) and peNDF>8 (% of BW) were positively associated with maximum ruminal pH ($P < 0.01$), explaining 8–9% of the variation in this variable (partial $R^2 = 0.09$ and 0.08, respectively). In these models, marginal R^2 values ranged from 0.51 to 0.53 and conditional R^2 from 0.67 to

0.72. Ruminal pH amplitude was also associated with peNDF>8 (% of BW), with a tendency for a positive interaction with parity (P = 0.07). This model explained a moderate proportion of the variation (partial R^2 = 0.08), with marginal and conditional R^2 values of 0.19 and 0.42, respectively.

Interactions between parity and peNDF>8 (% of BW) on ruminal pH amplitude, and between parity and DMI on time below pH 6.0, were detected ($P \leq 0.07$) and are presented in Figure 1 (panels G and H). The increase in ruminal pH amplitude with increasing peNDF>8 (% of BW) was more pronounced in multiparous cows than in primiparous cows. In contrast, the decrease in time below pH 6.0 with increasing DMI was more pronounced in primiparous cows than in multiparous cows.

Fiber x chewing interactions and ruminal pH

Interactions between fiber intake and rumination affecting time below ruminal pH 6.0 are presented in Table 4 and illustrated in Figure 2. Initial models evaluating direct associations between chewing behavior variables and ruminal pH outcomes did not meet the predefined criteria for retention. Additionally, rumination was evaluated as a modifier of the association between fiber intake and ruminal pH.

Significant fiber × rumination interactions were detected for forage NDF (kg/d and % of BW; $P < 0.01$) and peNDF>8 (kg/d and % of BW; $P \leq 0.05$). Across these models, greater rumination strengthened the negative association between fiber intake and time below pH 6.0 (Figure 2). Cows with higher rumination time exhibited a marked reduction in time below pH 6.0 as fiber intake increased, whereas cows with lower rumination showed a weak or minimal response across the same range of fiber supply.

Production and physiological variables associated with ruminal pH

Associations between ruminal pH dynamics and production, fermentation, and physiological variables are presented in Table 5. Milk-related variables were associated with ruminal pH dynamics, particularly time below pH 6.0. Milk de novo FA (% of total FA) showed the strongest association among milk variables (partial $R^2 = 0.12$; $P < 0.01$). Based on model estimates, a reduction of 1 percentage unit in milk de novo FA was associated with an increase of approximately 70.6 min/d in time below pH 6.0. Even smaller changes were biologically relevant. For example, a decrease of 0.5 percentage units corresponded to an increase of approximately 35 min/d below this threshold.

Milk yield (kg/d) was also negatively associated with time below pH 6.0 ($P = 0.01$; partial $R^2 = 0.07$). Model estimates indicate that a reduction of 1 kg/d in milk yield was associated with an increase of approximately 22.4 min/d below pH 6.0. Milk preformed FA (% and g/d) were also negatively associated with time below pH 6.0 ($P \leq 0.04$), although they explained a smaller proportion of the variance (partial $R^2 \leq 0.05$). Milk fat concentration (%) was positively associated with time below pH 6.0 ($P = 0.03$), but with low explanatory power (partial $R^2 = 0.05$). Jugular blood pCO_2 was also negatively associated with time below pH 6.0 ($P = 0.05$), explaining a small proportion of the variance (partial $R^2 = 0.02$). Across these models, marginal R^2 values were low (0.03–0.13), whereas conditional R^2 values ranged from 0.16 to 0.56.

Variables related to ruminal fermentation and physiological status were also associated with ruminal pH thresholds. For time below pH 5.8, serum haptoglobin was positively associated ($P < 0.01$), whereas fecal pH was negatively associated ($P < 0.01$). Although partial R^2 values were low (≤ 0.02), marginal and conditional R^2 values were relatively high (0.24–0.25 and 0.80–0.86, respectively), indicating a substantial contribution of cow-level variation.

For time below pH 5.5, serum haptoglobin remained positively associated ($P < 0.01$; partial $R^2 = 0.10$). Ruminal fluid propionate (% of total VFA) was positively associated with

this variable (P = 0.02), whereas ruminal fluid isovalerate and isobutyrate (% of total VFA) were negatively associated (P ≤ 0.03). Fecal pH was also negatively associated (P = 0.02). Partial R^2 values for these models ranged from 0.03 to 0.10, with marginal R^2 values between 0.12 and 0.21 and conditional R^2 values between 0.23 and 0.72.

Minimum ruminal pH was negatively associated with ECM/DMI (P = 0.04; partial R^2 = 0.08), and this relationship differed between parity groups (P = 0.02; Figure 1I), with a steeper decline in primiparous cows compared with multiparous cows. In addition, ruminal fluid isobutyrate (% of total VFA) was positively associated with minimum ruminal pH (P = 0.01; partial R^2 = 0.05).

Ruminal pH amplitude was negatively associated with ruminal fluid isobutyrate (% of total VFA; P < 0.01; partial R^2 = 0.11), and this association differed between parity groups (P = 0.04; Figure 1J), with a more pronounced response in multiparous cows. Fecal pH was also negatively associated with ruminal pH amplitude (P < 0.01; partial R^2 = 0.07). Marginal and conditional R^2 values for these models ranged from 0.18 to 0.21 and from 0.48 to 0.52, respectively.

DISCUSSION

This study was based on a combined dataset from two experiments in which dietary carbohydrate concentrations were not systematically manipulated within experiment. Therefore, the analyses focused on variation in carbohydrate intake rather than dietary composition per se. Because multiple predictors were evaluated individually, the results should be interpreted as hypothesis-generating rather than strictly confirmatory.

Linking fiber intake, feeding behavior, and ruminal pH dynamics

Associations between fiber intake and chewing behavior were consistent across the evaluated metrics, with both peNDF>8 and forage NDF being positively related to rumination,

eating time, total chewing, and first meal duration (Table 2). Notably, these associations were stronger when fiber variables were expressed relative to BW rather than as absolute intake, highlighting the relevance of rumen fill in regulating ingestive and rumination behavior. The relatively low mean values of peNDF>8 and forage NDF expressed as % of BW (0.78 and 0.72, respectively) suggest that physical fill was not excessive under the conditions evaluated. This indicates that chewing activity responds to relatively small changes in fiber intake, even when rumen fill is not limiting.

When evaluating the association between carbohydrate intake and sorting behavior with ruminal pH dynamics, the most informative pH-related variables were time below pH 6.0, maximum ruminal pH, and ruminal pH amplitude (Table 3). Among the evaluated predictors, DMI showed the strongest association with time below pH 6.0, with a negative coefficient. This pattern may reflect reduced feed intake in response to prolonged periods of low ruminal pH, rather than increased intake driving pH depression. Although greater DMI is often associated with increased risk of SARA due to higher intake of fermentable carbohydrates (Khorrami et al., 2021), elevated concentrations of VFA in the rumen can suppress intake through satiety-related mechanisms (Aschenbach et al., 2019).

The stabilizing effect of fiber intake on ruminal pH was evident, as greater intake of NDF, forage NDF (kg/d or % of BW), and peNDF (% of BW) was associated with reduced time below pH 6.0, lower ruminal pH amplitude, and greater maximum ruminal pH. In contrast, starch or NFC intake was not associated with ruminal pH dynamics in this dataset, which likely reflects the limited variation in dietary starch concentration across diets rather than the absence of a biological effect. Indeed, studies using datasets with greater variation in starch or NFC content have reported clear effects of starch or NFC on ruminal pH (White et al., 2017; Khorrami et al., 2021).

Interestingly, sorting behavior was also associated with ruminal pH dynamics. Greater selection for medium particles (8–19 mm) during the morning was associated with reduced time below pH 6.0, which is consistent with the role of peNDF>8 in stimulating chewing activity, enhancing saliva buffering, and contributing to rumen mat formation and motility (Mertens, 1997). Unexpectedly, greater selection for fine particles (<8 mm) during the morning was also associated with reduced time below pH 6.0. However, the average sorting index for this fraction was below 100, indicating that cows generally sorted against fine particles in this period. Therefore, these results likely reflect variation within a context of overall avoidance of fines and may not extrapolate to situations where cows actively select fine particles. In such scenarios, previous studies have reported negative impacts on ruminal pH (Piran-Filho et al., 2023).

Other finding was the positive association between sorting for long particles (>19 mm) in the afternoon and time below pH 5.5. Although the magnitude of this effect was small and explained little of the variance, this pattern may reflect a behavioral response to ruminal pH disturbances rather than a direct causal effect of sorting on pH. Previous studies have suggested that cows may adjust feeding behavior in response to acidotic conditions, increasing the selection or consumption of peNDF>8 as a compensatory mechanism to stimulate chewing activity and enhance ruminal buffering (Miller-Cushon and DeVries, 2017). In this context, greater selection of long particles may indicate an attempt by cows to restore ruminal stability following periods of low pH. Because the present analysis does not allow temporal resolution between changes in ruminal pH and sorting behavior, it is not possible to determine directionality. Nevertheless, the association suggests that feeding behavior may dynamically respond to ruminal conditions, highlighting the importance of considering behavioral adaptations when interpreting relationships between diet characteristics and ruminal pH dynamics.

Fiber intake × parity interactions associated with chewing behavior and ruminal pH

Parity-dependent responses in chewing behavior and ruminal pH dynamics (Figure 1) suggest that multiparous and primiparous cows differ in their responsiveness to fiber intake. Importantly, the range and distribution of fiber intake and chewing behavior variables differed between parity groups. Multiparous cows spanned a wider range of values across several predictors, whereas primiparous cows were more concentrated within an intermediate portion of the observed range. Despite these differences in distribution, significant parity interactions were still detected, indicating that the observed effects were not solely driven by unequal representation of predictor values between groups.

In the present study, multiparous cows showed a greater increase in rumination and chewing time as fiber intake increased, whereas primiparous cows exhibited a more attenuated response. Although some studies have reported greater absolute rumination time in multiparous cows, these differences are often explained by greater DMI, as rumination relative to intake tends to be similar across parity groups (Beauchemin and Rode, 1997; Kowsar et al., 2008). In contrast, the present results indicate a difference in behavioral responsiveness rather than intake level per se.

Consistent with these findings, primiparous cows appeared more susceptible to unfavorable ruminal pH dynamics, as indicated by the stronger reduction in time below pH 6.0 with increasing DMI (Figure 1). This pattern suggests that lower intake was more strongly associated with prolonged exposure to low ruminal pH in primiparous cows, whereas multiparous cows exhibited a more stable response across the observed range of intake. In the same direction, the steeper decline in minimum ruminal pH with increasing ECM/DMI in primiparous cows likely reflects differences in intake dynamics between parity groups. The interaction between peNDF>8 and parity for ruminal pH amplitude should be interpreted

cautiously. Although amplitude reflects pH fluctuation, it does not directly capture duration of exposure to low pH, and its biological interpretation in this context is less straightforward.

Several mechanisms may underlie this reduced responsiveness in primiparous cows. In addition to differences in chewing activity already discussed, which may limit saliva secretion and buffering capacity, limited prior exposure to high-concentrate diets may result in a less adapted ruminal epithelium and reduced capacity to cope with fermentative challenges (Humer et al., 2018). Furthermore, lower BW and rumen volume may increase susceptibility to rapid changes in ruminal conditions, contributing to less stable pH dynamics (Nasrollahi et al., 2017). Differences in social hierarchy could also influence feeding patterns and access to feed. However, this mechanism is unlikely to explain the present results, as cows were housed in tie-stalls and fed individually.

Fiber intake × chewing behavior interactions associated with ruminal pH

When chewing behavior variables were evaluated as independent predictors of ruminal pH dynamics, none met the predefined criteria for model retention. This indicates that chewing behavior did not consistently explain ruminal pH variation as an independent driver. Instead, chewing behavior likely acts as a mediator of dietary effects, particularly those related to fiber intake.

Accordingly, additional models were fitted to evaluate whether rumination time modified the association between fiber intake and ruminal pH (Table 4; Figure 2). In these models, greater rumination strengthened the negative association between fiber intake and time below pH 6.0. Cows with higher rumination time exhibited a marked reduction in time below pH 6.0 as fiber intake increased, whereas cows with lower rumination showed a weaker response. These results reinforce the concept that the effectiveness of dietary fiber depends not

only on intake, but also on its ability to stimulate chewing activity and saliva-mediated buffering.

Linking ruminal pH with production, milk composition, and physiology

Associations between ruminal pH dynamics and milk composition were primarily driven by milk FA profile (Table 5). Milk de novo FA (% of total FA) showed the strongest association with time below pH 6.0, which is consistent with the well-established relationship between ruminal conditions and milk fat synthesis. Milk fat depression occurs when ruminal conditions favor an alternative biohydrogenation pathway of unsaturated fatty acids, particularly under low ruminal pH and increased supply of unsaturated FA (Bauman and Griinari, 2001). This pathway leads to the formation of bioactive intermediates, such as trans-10, cis-12 CLA, which inhibit mammary lipogenesis. As a result, de novo fatty acid synthesis in the mammary gland is reduced, particularly affecting fatty acids with fewer than 16 carbons (Barbano, 2018; Razzaghi et al., 2022). The strong negative association between milk de novo FA and time below pH 6.0 supports its role as a sensitive indicator of ruminal pH disturbances.

Milk preformed FA (% of total FA) were also negatively associated with time below pH 6.0, although with smaller magnitude compared with de novo FA. In contrast, milk fat concentration (%) was positively associated with time below pH 6.0, which was unexpected. This result may reflect a dilution effect driven by the negative association between milk yield and time below pH 6.0, whereby reduced milk production leads to a relative increase in fat concentration. In line with this interpretation, models including fat yield (g/d) were not retained. Overall, these results reinforce the value of milk FA profile, particularly de novo FA, as a practical tool to assess SARA risk (Woolpert et al., 2017).

The negative association between time below pH 6.0 and jugular blood pCO_2 may reflect a compensatory response to increased acid load, with reduced pCO_2 potentially

indicating increased respiratory CO_2 elimination (Constable, 1999). However, this association explained little of the variance. In addition, blood pH, bicarbonate, base excess, and total CO_2 were not retained in the models, suggesting limited sensitivity of these systemic variables under the conditions evaluated.

Serum haptoglobin was not associated with time below pH 6.0, but was positively associated with time below pH 5.8 and more strongly with time below pH 5.5, indicating that the inflammatory response to ruminal pH depression was linked to more severe ruminal pH conditions. This finding suggests that mild ruminal pH depression may not be sufficient to trigger a detectable systemic inflammatory response. This distinction is relevant for practical applications, as many experimental SARA models rely on more extreme dietary challenges that may not reflect typical commercial conditions (Plaizier et al., 2022).

Greater time below pH 5.5 was associated with increased propionate and reduced branched-chain VFA (isobutyrate and isovalerate), consistent with a shift toward more amylolytic and less fibrolytic fermentation under more acidogenic conditions (Russell, 1998). Because branched-chain VFA are derived from microbial deamination of branched-chain amino acids (Russell, 2002), their reduction suggests alterations in ruminal nitrogen metabolism and overall microbial activity under these conditions. These results should be interpreted as changes in fermentation profile rather than total acid production, as VFA were expressed as proportions of total VFA. In this context, the stronger association between isobutyrate and reduced pH amplitude in multiparous cows suggests that, in these animals, this metabolite more consistently reflects microbial conditions associated with ruminal stability, whereas the weaker relationship in primiparous cows may indicate a less consistent coupling between microbial activity and pH dynamics.

Fecal pH was associated with ruminal pH dynamics, particularly with time below pH 5.8 and 5.5, and showed the strongest association with ruminal pH amplitude. This may reflect

increased flow of fermentable substrates to the hindgut when ruminal conditions are more acidogenic. Under such conditions, hindgut acidification can occur (Neubauer et al., 2020; Pederzolli et al., 2018), which has been proposed as a potential contributor to systemic responses through mechanisms similar to those described for the rumen (Aschenbach et al., 2019). However, recent studies inducing hindgut acidosis via abomasal starch infusion did not consistently detect inflammatory or performance responses, although fecal pH decreased consistently (Piantoni et al., 2022; Abeyta et al., 2022; Abeyta et al., 2023). Therefore, although fecal pH was associated with ruminal pH dynamics in the present study, the mechanisms underlying this relationship remain unclear, and its practical utility as an indicator of ruminal pH status requires further investigation.

CONCLUSION

In conclusion, ruminal pH in lactating dairy cows were associated not only with fiber intake, but also with the extent to which fiber stimulated rumination, and these relationships were modified by parity. Primiparous cows showed a more attenuated behavioral response to increasing fiber intake and appeared more susceptible to unfavorable ruminal pH dynamics than multiparous cows. In addition, associations of ruminal pH metrics with milk FA profile, serum haptoglobin, ruminal VFA profile, fecal pH, and jugular blood pCO_2 support the biological relevance of these responses. These findings reinforce that ruminal pH stability should be interpreted as the product of interacting dietary, behavioral, and animal-related factors rather than any single indicator in isolation.

REFERENCES

Abeyta, M.A., E.A. Horst, B.M. Goetz, S. Rodriguez-Jimenez, E.J. Mayorga, M. Al-Qaisi, and L.H. Baumgard. 2023. Effects of hindgut acidosis on inflammation, metabolism, and productivity in lactating dairy cows fed a high-fiber diet. Journal of Dairy Science 106:2879–2889. doi:10.3168/jds.2022-22680.

Abeyta, M.A., E.A. Horst, E.J. Mayorga, B.M. Goetz, M. Al-Qaisi, C.S. Mccarthy, M.R. O'Neil, B.C. Dooley, P. Piantoni, G.F. Schroeder, and L.H. Baumgard. 2022. Effects of hindgut acidosis on metabolism, inflammation, and production in dairy cows consuming a standard lactation diet. Journal of Dairy Science 106:1429–1440. doi:10.3168/jds.2022-22303.

Allen, M.S. 1997. Relationship Between Fermentation Acid Production in the Rumen and the Requirement for Physically Effective Fiber. Journal of dairy science 80:1447–1462. doi:10.3168/jds.s0022-0302(97)76074-0.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Arlington, Va.

Aschenbach, J.R., G.B. Penner, F. Stumpff, and G. Gäbel. 2010. RUMINANT NUTRITION SYMPOSIUM: Role of fermentation acid absorption in the regulation of ruminal pH12. Journal of Animal Science 89:1092–1107. doi:10.2527/jas.2010-3301.

Aschenbach, J.R., Q. Zebeli, A.K. Patra, G. Greco, S. Amasheh, and G.B. Penner. 2019. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. Journal of Dairy Science 102:1866–1882. doi:10.3168/jds.2018-15243.

Barbano, D. et al. New Milk Analysis Metrics for Dairy Herd Management. 2018.

Bauman, D.E., and J.M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science 70:15–29. doi:10.1016/s0301-6226(01)00195-6.

Beauchemin, K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. Journal of Dairy Science, 101(6), 4762–4784. https://doi.org/10.3168/jds.2017-13706

Beauchemin, K.A., and L.M. Rode. 1997. Minimum Versus Optimum Concentrations of Fiber in Dairy Cow Diets Based on Barley Silage and Concentrates of Barley or Corn. Journal of Dairy Science 80:1629–1639. doi:10.3168/jds.s0022-0302(97)76094-6.

Constable, P.D. 1999. Clinical Assessment of Acid-Base Status: Strong Ion Difference Theory. Veterinary Clinics of North America: Food Animal Practice 15:447–471. doi:10.1016/s0749-0720(15)30158-4.

Cooke, R. F., and J. D. Arthington. 2012. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. 97:531-536. https://doi.org/10.1111/j.1439-0396.2012.01298.x.

Detmann, E. 2012. Methods for feed analysis. INCT–Ciência Animal, Visconde do Rio Branco, Brazil.

Fernandes, T., K. T. da Silva, B. F. Carvalho, R. F. Schwan, R. A. N. Pereira, M. N. Pereira, and C. L. S. Ávila. 2022. Effect of amylases and storage length on losses, nutritional value, fermentation, and microbiology of silages of corn and sorghum kernels. Anim. Feed Sci. Technol. 285:115227. https://doi.org/10.1016/j.anifeedsci.2022.115227.

Fukumori, R., W. Shi, S. Oikawa, and M. Oba. 2021. Evaluation of relationship between ruminal pH and the proportion of de novo fatty acids in milk. JDS Communications 2:123–126. doi:10.3168/jdsc.2020-0042.

Gregory B, P., J.R. Aschenbach, G. Gäbel, R. Rackwitz, and M. Oba. 2009. Epithelial Capacity for Apical Uptake of Short Chain Fatty Acids Is a Key Determinant for Intraruminal pH and the Susceptibility to Subacute Ruminal Acidosis in Sheep ,. The Journal of Nutrition 139:1714–1720. doi:10.3945/jn.109.108506.

Hu, W., and M.R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: a meta-analysis.. Journal of Dairy Science 87:2222–2229. doi:10.3168/jds.s0022-0302(04)70042-9.

Humer, E., A. Khol-Parisini, L. Gruber, J. Gasteiner, S.M. Abdel-Raheem, and Q. Zebeli. 2015. Long-term reticuloruminal pH dynamics and markers of liver health in early-lactating cows of various parities fed diets differing in grain processing. Journal of Dairy Science 98:6433–6448. doi:10.3168/jds.2015-9522.

Humer, E., R.M. Petri, J.R. Aschenbach, B.J. Bradford, G.B. Penner, M. Tafaj, K.-H. Südekum, and Q. Zebeli. 2017. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of Dairy Science 101:872–888. doi:10.3168/jds.2017-13191.

Khorrami, B., R. Khiaosa-Ard, and Q. Zebeli. 2021. Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: A meta-analysis. Journal of Dairy Science 104:7761–7780. doi:10.3168/jds.2020-19890.

Kowsar, R., G.R. Ghorbani, M. Alikhani, M. Khorvash, and A. Nikkhah. 2008. Corn Silage Partially Replacing Short Alfalfa Hay to Optimize Forage Use in Total Mixed Rations for Lactating Cows. Journal of Dairy Science 91:4755–4764. doi:10.3168/jds.2008-1302.

Krajcarski-Hunt, H., J.C. Plaizier, J.-P. Walton, R. Spratt, and B.W. Mcbride. 2002. Short Communication: Effect of Subacute Ruminal Acidosis on In Situ Fiber Digestion in Lactating Dairy Cows. Journal of dairy science 85:570–573. doi:10.3168/jds.s0022-0302(02)74110-6.

Leonardi, C., and L.E. Armentano. 2003. Effect of Quantity, Quality, and Length of Alfalfa Hay on Selective Consumption by Dairy Cows. J. Dairy Sci. 86:557–564. doi:10.3168/jds.s0022-0302(03)73634-0.

Maekawa, M., K.A. Beauchemin, and D.A. Christensen. 2002. Chewing Activity, Saliva Production, and Ruminal pH of Primiparous and Multiparous Lactating Dairy Cows. Journal of Dairy Science 85:1176–1182. doi:10.3168/jds.s0022-0302(02)74180-5.

Mertens, D.R. 1997. Creating a System for Meeting the Fiber Requirements of Dairy Cows. Journal of dairy science 80:1463–1481. doi:10.3168/jds.s0022-0302(97)76075-2.

Miller-Cushon, E.K., and T.J. Devries. 2016. Feed sorting in dairy cattle: Causes, consequences, and management. Journal of Dairy Science 100:4172–4183. doi:10.3168/jds.2016-11983.

Nasrollahi, S.M., A. Zali, G.R. Ghorbani, M. Moradi Shahrbabak, and M. Heydari Soltan Abadi. 2017. Variability in susceptibility to acidosis among high producing mid-lactation dairy cows is associated with rumen pH, fermentation, feed intake, sorting activity, and milk fat percentage. Animal Feed Science and Technology 228:72–82. doi:10.1016/j.anifeedsci.2017.03.007.

Neubauer, V., R.M. Petri, E. Humer, I. Kröger, N. Reisinger, W. Baumgartner, M. Wagner, and Q. Zebeli. 2020. Starch-Rich Diet Induced Rumen Acidosis and Hindgut Dysbiosis in Dairy Cows of Different Lactations.. Animals 10:1727. doi:10.3390/ani10101727.

Pederzolli, R.-L.A., A.G. Van Kessel, J. Campbell, S. Hendrick, K.M. Wood, and G.B. Penner. 2018. Effect of ruminal acidosis and short-term low feed intake on indicators of gastrointestinal barrier function in Holstein steers. Journal of Animal Science 96:108–125. doi:10.1093/jas/skx049.
Piantoni, P., M.A. Abeyta, G.F. Schroeder, H.A. Tucker, and L.H. Baumgard. 2022. Evaluation of feed restriction and abomasal infusion of resistant starch as models to induce intestinal barrier dysfunction in healthy lactating cows. Journal of Dairy Science 106:1453–1463. doi:10.3168/jds.2022-22376.

Piran Filho, F.A., J.M. Bragatto, C.S. Parra, S.M.S. Silva, P.J. Roco, L.F. Ferraretto, M.N. Pereira, and J.L.P. Daniel. 2023. Physical effectiveness of corn silage fractions stratified with the Penn State Particle Separator for lactating dairy cows. Journal of Dairy Science 106:6041–6059. doi:10.3168/jds.2022-23063.

Plaizier, J.C., D.O. Krause, G.N. Gozho, and B.W. Mcbride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Veterinary Journal 176:21–31. doi:10.1016/j.tvjl.2007.12.016.

Plaizier, J.C., F.J. Mulligan, E.W. Neville, L.L. Guan, M.A. Steele, and G.B. Penner. 2022. Invited review: Effect of subacute ruminal acidosis on gut health of dairy cows.. Journal of dairy science 105:7141–7160. doi:10.3168/jds.2022-21960.

Razzaghi, A., A.R. Vakili, B. Khorrami, M.H. Ghaffari, and D.E. Rico. 2022. Effect of dietary supplementation or cessation of magnesium-based alkalizers on milk fat output in dairy cows under milk fat depression conditions. Journal of Dairy Science 105:2275–2287. doi:10.3168/jds.2021-20457.

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. Cornell Univ., Ithaca, NY.

Saleem, F., B.N. Ametaj, S. Bouatra, R. Mandal, Q. Zebeli, S.M. Dunn, and D.S. Wishart. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. Journal of Dairy Science 95:6606–6623. doi:10.3168/jds.2012-5403.

Schlau, N., D. R. Mertens, K. Taysom, and D. Taysom. 2021. Technical note: effects of filter bags on neutral detergent fiber recovery and fiber digestion in vitro. J. Dairy Sci. 104:1846-1854. https://doi.org/10.3168/jds.2020-18731.

Silva, B.D.C., L.A. Godoi, S.D.C. Valadares Filho, D. Zanetti, P.D.B. Benedeti, and E. Detmann. 2019. A suitable enzymatic method for starch quantification in different organic matrices. MethodsX 6:2322–2328. doi:10.1016/j.mex.2019.09.040.

Valadares, R.F.D., G.A. Broderick, S.C.V. Filho, and M.K. Clayton. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of Total Purine Derivatives. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.s0022-0302(99)75525-6.

Weber, K., and M. Osborn. 1969. The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. J. Biol. Chem. 244:4406–4412. doi:10.1016/s0021-9258(18)94333-4.

White, R.R., M.B. Hall, J.L. Firkins, and P.J. Kononoff. 2017. Physically adjusted neutral detergent fiber system for lactating dairy cow rations. II: Development of feeding recommendations. Journal of Dairy Science 100:9569–9584. doi:10.3168/jds.2017-12766.

Woolpert, M.E., H.M. Dann, K.W. Cotanch, C. Melilli, L.E. Chase, R.J. Grant, and D.M. Barbano. 2017. Management practices, physically effective fiber, and ether extract are related to bulk tank milk de novo fatty acid concentration on Holstein dairy farms. Journal of Dairy Science 100:5097–5106. doi:10.3168/jds.2016-12046.

Table 1. Cows, ingredients, and nutrient composition of diets in experiments 1 and 2 (colocar mínimo e máximo).

Item	Experiment 1	Experiment 2
Number of cows	15	21
Primiparous	6	9
Multiparous	9	12
DIM	193 ± 120	144 ± 77
Body weight	643 ± 62	611 ± 93
DMI, kg/d	22.5 ± 2.7	22.4 ± 3.2
Milk yield, kg/d	27.7 ± 4.2	34.6 ± 5.2
Milk fat, %	3.68 ± 0.45	3.49 ± 0.42
Milk protein, %	3.37 ± 0.28	3.22 ± 0.26
Mean ruminal pH[1]	6.26 ± 0.11	6.01 ± 0.17
Time below ruminal pH 6.0, min/d[1]		
Time below ruminal pH 5.8, min/d[1]	46 ± 63	292 ± 279
Rumination, min/d[2]	471 ± 59	422 ± 68
Eating, min/d[2]	370 ± 45	255 ± 36
Chewing, min/d[2]	841 ± 79	677 ± 86
Ingredient, % of DM		
Whole-plant corn silage	36.6	36.1
Alfafa haylage	9.3	-
Corn fibrous byproduct[3]	-	12.5
Rehydrated ensiled corn grain	14.5	11.5
Citrus pulp pellets	12.8	11.8
Whole cottonseeds	11.5	12.5
Soybean meal	11.0	13.2
Distillers dried grains[4]	2.2	-
Minerals and vitamins	1.8	2.4
Nutrient composition, % of DM		
CP	17.0	17.5
NDF	36.2	29.4
Forage NDF	23.9	17.3
peNDF>8[5]	23.4	20.1
Starch	22.6	24.2
NFC	35.4	42.4
Nonstarch NFC	12.8	18.2
Ether extract	4.2	5.0
Ashes	7.2	5.7

[1]Measured with wireless boluses (Smaxtec, Austria).

[2]Measured by visual observation.

[3]Comidão (Cargill Agrícola, Brazil; Silva et al., 2022).

[4]FlexyPro (SJC Energia, Brazil).

[5]Physically effective NDF. Particle size was evaluated using the Penn State Particle Separator with 19- and 8-mm sieves and a bottom pan. NDF concentration was determined for each sieve fraction, and only forage NDF retained on particles >8 mm was considered physically effective. Among concentrates, only whole cottonseed was included, with all of its NDF assumed to be physically effective.

Table 2. Associations between fiber intake, DMI, and chewing behavior.

				P-value						
Outcome	Predictor	Mean predictor	β ± SE	Predictor	Parity	Predictor x Parity	ΔAIC	R^2 Partial	R^2 Marginal	R^2 Conditional
Rumination, min/d	peNDF>8, % of BW	0.77	205.8 ± 60.4	<0.01	0.04	0.14	-52.0	0.19	0.46	0.83
	Forage NDF, % of BW	0.72	196.2 ± 78.9	<0.01	0.07	0.02	-40.0	0.18	0.45	0.79
	peNDF>8, kg/d	4.80	37.9 ± 12.1	<0.01	<0.01	0.56	-37.1	0.14	0.41	0.83
	DMI, kg/d	22.4	7.20 ± 3.91	<0.01	<0.01	0.71	-15.5	0.09	0.36	0.73
	Forage NDF, kg/d	4.46	27.0 ± 14.5	<0.01	<0.01	0.16	-22.0	0.10	0.37	0.73
Eating, min/d	peNDF>8, % of BW	0.78	94.0 ± 53.4	<0.01	0.24	0.17	-34.2	0.04	0.71	0.84
	Forage NDF, % of BW	0.72	140.0 ± 59.6	<0.01	0.13	0.04	-37.4	0.04	0.71	0.84
	peNDF>8, kg/d	4.81	15.3 ± 9.7	<0.01	0.35	0.28	-24.7	0.03	0.70	0.83
	Forage NDF, kg/d	4.47	20.8 ± 10.3	<0.01	0.31	0.11	-24.6	0.02	0.69	0.82
	DMI, kg/d	22.4	3.77 ± 2.82	<0.01	0.27	0.28	-17.9	0.02	0.69	0.81
Chewing, min/d	peNDF>8, % of BW	0.77	299.9 ± 87.1	<0.01	0.39	0.06	-62.1	0.14	0.67	0.87
	Forage NDF, % of BW	0.72	329.5 ± 107.4	<0.01	0.60	<0.01	-54.1	0.13	0.66	0.83
	peNDF>8, kg/d	4.80	56.9 ± 17.2	<0.01	<0.01	0.40	-47.9	0.09	0.62	0.84
	DMI, kg/d	22.4	12.1 ± 5.2	<0.01	<0.01	0.55	-27.4	0.05	0.57	0.68
	Forage NDF, kg/d	4.47	49.4 ± 19.6	<0.01	<0.01	0.10	-34.2	0.07	0.59	0.72
Idle, min/d	Forage NDF, % of BW	0.72	-143.2 ± 101.2	<0.01	0.29	0.01	-39.6	0.15	0.29	0.53
	peNDF>8, % of BW	0.78	-46.6 ± 92.7	<0.01	0.15	0.03	-34.1	0.10	0.24	0.29
	Forage NDF, kg/d	4.47	-26.1 ± 18.0	0.01	0.01	0.11	-25.7	0.09	0.23	0.24
	DMI, kg/d	22.4	-7.11 ± 4.74	0.01	0.01	0.57	-18.0	0.07	0.21	0.22
	peNDF>8, kg/d	4.81	-13.1 ± 17.3	0.01	0.01	0.17	-23.9	0.07	0.21	0.23
First meal duration, min	Forage NDF, % of BW	0.72	60.0 ± 24.7	<0.01	0.36	0.43	-25.0	0.06	0.62	0.83
	peNDF>8, % of BW	0.78	39.2 ± 22.5	<0.01	0.60	0.75	-21.3	0.04	0.61	0.81

Table 3. Associations between DMI, fiber intake, sorting behavior, and ruminal pH.

				P-value						
Outcome	Predictor	Mean predictor	β ± SE	Predictor	Parity	Predictor x Parity	ΔAIC	R^2 Partial	R^2 Marginal	R^2 Conditional
Time below pH 6.0, min/d	DMI, kg/d	22.4	-64.3 ± 23.3	0.05	0.37	0.06	-19.4	0.09	0.10	0.10
	NDF intake, kg/d	7.19	-135.3 ± 54.2	0.009	0.34	0.37	-23.8	0.08	0.09	0.09
	Forage NDF, % of BW	0.72	-1176 ± 528	0.008	0.64	0.86	-32.0	0.06	0.08	0.08
	Forage NDF intake, kg/d	4.46	-194 ± 90	0.05	0.40	0.40	-22.6	0.06	0.07	0.07
	Morning sorting index, 8-19 mm	103.3	-8.89 ± 3.82	0.01	0.74	0.86	-13.2	0.04	0.06	0.39
	peNDF>8, % of BW	0.77	-808 ± 484	0.04	0.91	0.60	-28.2	0.04	0.05	0.05
	Morning sorting index, bottom	90.2	-5.90 ± 3.70	0.03	0.64	0.85	-9.5	0.02	0.03	0.04
Time below pH 5.5, min/d	Sorting index >19 mm, afternoon	87.1	0.36 ± 0.36	0.04	0.46	0.60	-0.08	0.02	0.12	0.23
Maximum ruminal pH	Forage NDF, % of BW	0.72	0.47 ± 0.24	<0.01	0.92	0.19	-5.8	0.09	0.53	0.72
	peNDF>8, % of BW	0.77	0.42 ± 0.22	<0.01	0.90	0.62	-6.1	0.08	0.51	0.67
Ruminal pH amplitude	peNDF>8, % of BW	0.77	0.05 ± 0.34	0.01	0.72	0.07	-3.5	0.08	0.19	0.42

Table 4. Interactions between fiber intake and rumination time over time below ruminal pH 6.0.

Fiber variable	Mean fiber	Mean rumination	β fiber ± SE	β rumination ± SE	β fiber x rumination ± SE	Fiber x Rumination P-value	ΔAIC
Forage NDF, kg/d	4.45	442.6	-94.8 ± 63.5	-0.85 ± 0.56	-1.64 ± 0.52	<0.01	-6.4
Forage NDF, % of BW	0.72	442.6	-759 ± 405	-0.84 ± 0.59	-9.14 ± 3.28	<0.01	-8.8
peNDF>8, kg/d	4.80	442.6	-55.6 ± 50.1	-0.77 ± 0.62	-1.33 ± 0.56	0.02	-3.5
peNDF>8, % of BW	0.77	442.6	-380.0 ± 312.4	-0.78 ± 0.65	-6.60 ± 3.32	0.05	-6.0

Table 5. Association between milk yield, feed efficiency, milk composition, ruminal fermentation, blood parameters, fecal pH and ruminal pH variables.

				P-value						
Outcome	Predictor	Mean predictor	β ± SE	Predictor	Parity	Predictor x Parity	ΔAIC	R^2 Partial	R^2 Marginal	R^2 Conditional
Time below pH 6.0, min/d	Milk de novo FA, % of FA	22.9	-70.6 ± 19.4	<0.01	0.67	0.41	-29.2	0.12	0.13	0.56
	Milk yield, kg/d	32	-22.4 ± 15.5	0.01	0.66	0.95	-15.2	0.07	0.08	0.20
	Milk preformed FA, % of FA	41.4	-22.3 ± 12.5	<0.01	0.71	0.56	-18.6	0.05	0.06	0.28
	Milk fat, %	3.57	51.1 ± 152.8	0.03	0.70	0.27	-25.1	0.05	0.06	0.18
	Milk preformed FA, g/d	439.0	-1.45 ± 1.02	0.04	0.67	0.75	-3.1	0.03	0.04	0.17
	Jugular blood pCO2	40.3	-2.05 ± 10.4	0.05	0.82	0.20	-14.0	0.02	0.03	0.16
Time below pH 5.8, min/d	Serum haptoglobin, mg/dL	12.43	8.70 ± 3.18	<0.01	0.87	0.52	-13.8	0.02	0.25	0.86
	Fecal pH	7.63	-149 ± 61	<0.01	0.89	0.87	-27.1	0.01	0.24	0.80
Time below pH 5.5, min/d	Serum haptoglobin, mg/dL	12.43	4.72 ± 1.18	<0.01	0.42	0.30	-16.6	0.10	0.21	0.72
	Ruminal fluid propionate % of VFA	21.5	8.38 ± 3.72	0.02	0.50	0.42	-10.7	0.05	0.15	0.25
	Ruminal fluid isovalerate, % of VFA	1.90	-87.1 ± 44.4	0.03	0.50	0.40	-19.3	0.04	0.14	0.25
	Ruminal fluid isobutyrate, % of VFA	0.96	-160 ± 99	0.02	0.50	0.83	-22.6	0.03	0.12	0.23
	Fecal pH	7.63	-73.7 ± 28.6	0.02	0.46	0.23	-20.2	0.03	0.13	0.23
Minimum ruminal pH	ECM/DMI	0.98	-1.13 ± 0.35	0.04	0.79	0.02	-4.6	0.08	0.17	0.60
	Ruminal fluid isobutyrate, % of VFA	0.96	0.17 ± 0.31	0.01	0.79	0.19	-1.7	0.05	0.14	0.62
Ruminal pH amplitude	Ruminal fluid isobutyrate, % of VFA	0.96	-0.10 ± 0.38	<0.01	0.29	0.04	-6.3	0.11	0.21	0.52
	Fecal pH	7.63	-0.24 ± 0.11	<0.01	0.31	0.60	-3.3	0.07	0.18	0.48

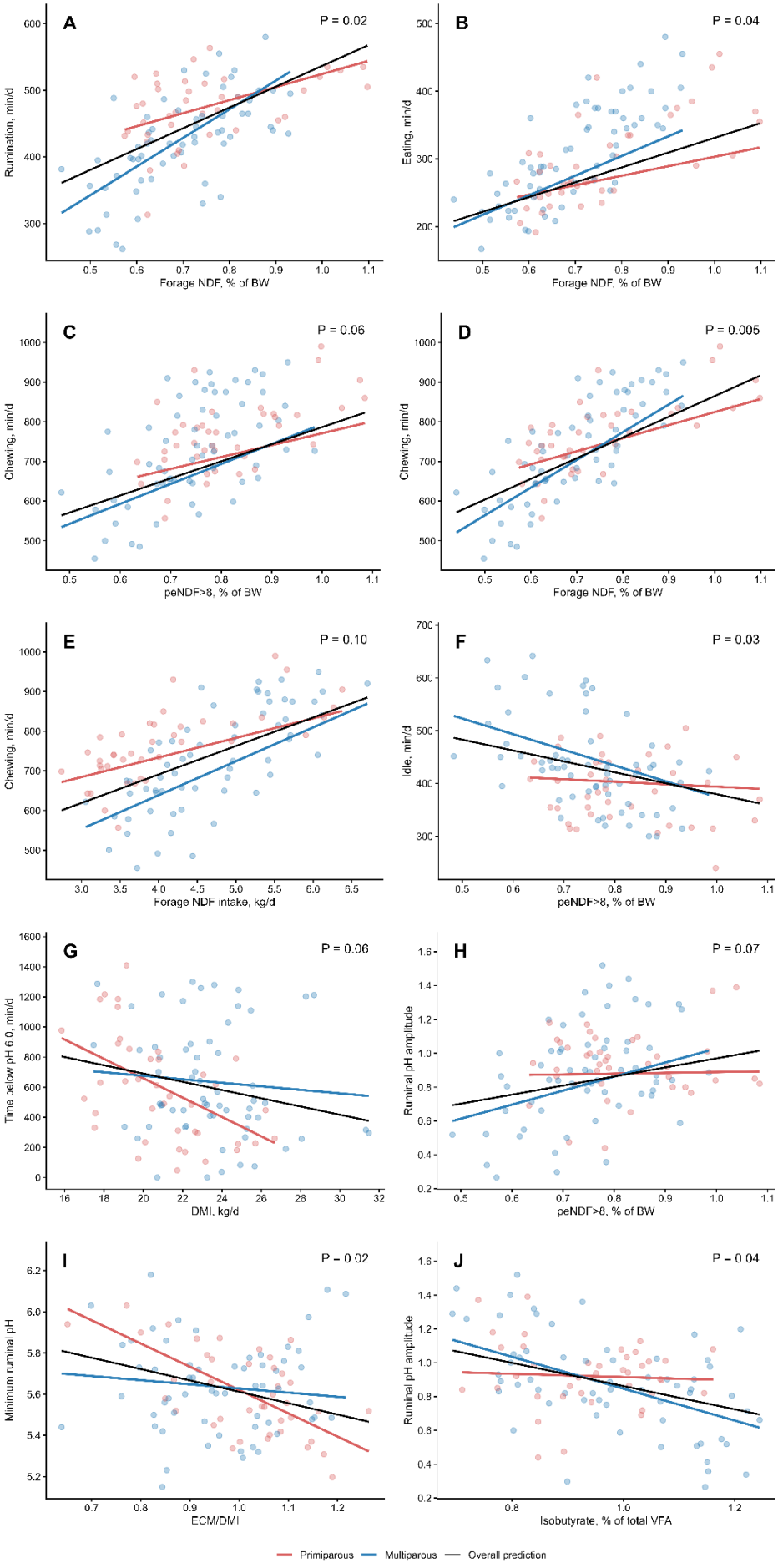


Figure 1.

Interaction between parity and fiber intake variables or DMI in relation to chewing behavior and ruminal pH dynamics. Panels A–B depict the association of forage NDF (% of BW) with

rumination and eating time. Panels C–E show the effects of peNDF>8 (% of BW) and forage NDF (expressed as % of BW and kg/d) on chewing time. Panel F presents the association between peNDF>8 (% of BW) and idle time. Panel G illustrates the relationship between DMI and time below ruminal pH 6.0, whereas panel H shows the association between peNDF>8 (% of BW) and ruminal pH amplitude. Panels I and J present the relationships of ECM/DMI and isobutyrate (% of total VFA) with minimum ruminal pH and pH amplitude, respectively. Points represent individual observations, and lines correspond to model-predicted values for primiparous (red), multiparous (blue), and overall (black) cows. P-values refer to parity × predictor interactions.

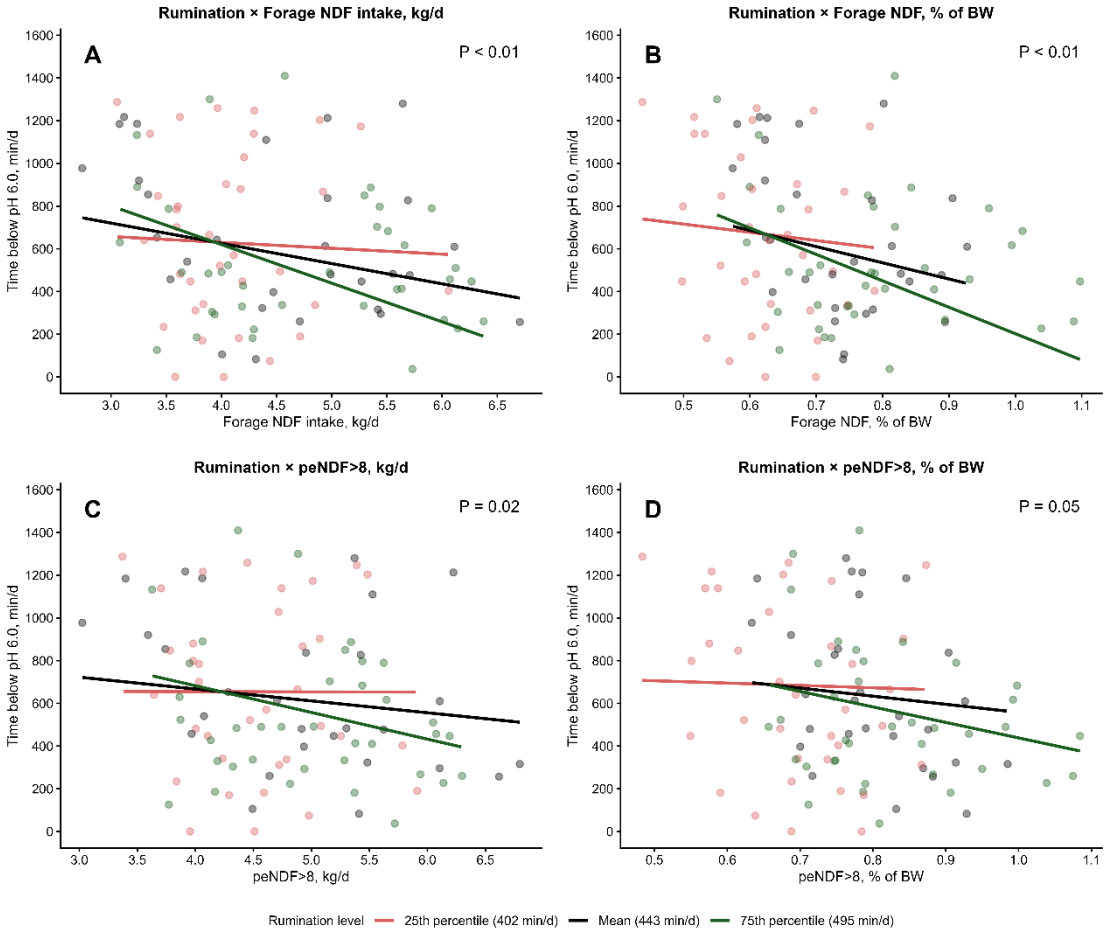


Figure 2. Interaction between fiber intake and rumination level associated with time below ruminal pH 6.0 (min/d). Panels A–D show the interaction between rumination level and forage NDF intake (kg/d; A), forage NDF (% of BW; B), peNDF>8 (kg/d; C), and peNDF>8 (% of BW; D). Lines represent model predictions at the 25th percentile (red), mean (black), and 75th percentile (green) of rumination time. Points represent individual observations, colored according to rumination level. P-values indicate the fiber × rumination interaction.

ARTIGO 3 – Association between collar-measured rumination time and milk yield, diet composition, and intake in groups of lactating dairy cows on commercial farms in Brazil.

Artigo formatado de acordo com as normas para submissão ao Journal of Dairy Science Communications.

ABSTRACT

Rumination is closely associated with feed intake, diet characteristics, and rumen function in dairy cows, and sensor-based technologies enable its continuous monitoring under commercial conditions. However, the relationship between collar-derived rumination time and nutritional variables at the pen level remains poorly characterized. This study evaluated associations between rumination time and milk yield, DMI, diet composition, and nutrient intake using data from 40 pens across five commercial dairy farms in Brazil (1,002 pen-week observations). Rumination time was derived from neck-mounted activity collars (Cowmed, Brazil) and analyzed using linear mixed-effects models including DIM, parity, and each predictor as fixed effects, with interactions between predictor and DIM and parity, and a random intercept for pen. Rumination time was positively associated with milk yield and DMI ($P < 0.01$), with increases of 1.92 and 3.15 min/d per kg/d, respectively (partial $R^2 = 0.17$ for both). These associations were stronger at lower DIM ($P \leq 0.01$), suggesting greater sensitivity of rumination patterns in early lactation. Among diet composition variables (% of DM), lignin showed the strongest association with rumination time ($\beta = 22.4$; $P < 0.01$; partial $R^2 = 0.12$), and forage plus whole cottonseed lignin showed a higher contribution to model fit than forage lignin alone. When nutrient intake (kg/d) was evaluated, all predictors were significant ($P < 0.01$), with lignin intake showing the largest contribution (partial $R^2 = 0.31$), corresponding to approximately 10 min/d increase in rumination per 0.1 kg/d increase in lignin intake. Associations between rumination time and most predictors varied with DIM, whereas interactions with parity were less consistent. Conditional R^2 values were consistently higher than marginal and partial R^2 values, indicating substantial variation attributable to differences among pens and unmeasured factors. Despite these limitations, observed associations were consistent with biological

expectations. These results suggest that collar-derived rumination time may provide useful information for nutritional monitoring under commercial conditions, particularly in early-lactation pens.

MAIN BODY

Rumination is an important process associated with feed particle breakdown, saliva production, and maintenance of rumen function in dairy cows. Because chewing activity is strongly stimulated by fiber intake, rumination time is related to dietary characteristics such as forage NDF intake and fiber digestibility (Beauchemin et al., 2018). Consequently, changes in rumination behavior may reflect variations in feed intake, diet composition, or rumen fill, making rumination an important indicator of nutritional status and feeding management in dairy herds. This physiological relevance has motivated the development of technologies capable of monitoring rumination under commercial conditions.

In dairy farms, behavior-monitoring collars can be used to track rumination activity over time. These technologies are widely applied to detect health and reproductive events (Steeneveld et al., 2015; Rutten et al., 2013), but their potential to support nutritional monitoring and feeding management at the pen level remains less explored. Lovatti et al. (2024) validated a commercial behavior-monitoring collar (Cowmed, Santa Maria, Brazil) and reported Pearson correlations between collar-derived and visually observed rumination of 0.50. In that study, precision was evaluated using correlation and determination coefficients, whereas accuracy was assessed using the slope of linear regression and Bland–Altman analysis. Although the collar enabled continuous behavioral monitoring, the authors concluded that its overall accuracy remained limited. Similarly, Modesto et al. (2024) evaluated the same collar system and reported low agreement between collar-derived and visually observed rumination. Nevertheless, despite the weak correlation, the sensor detected temporal changes in rumination associated with feed restriction in a pattern similar to that observed visually.

Even with limitations in precision and accuracy, the ability of sensors to detect temporal variation in rumination behavior may still provide useful information for monitoring nutritional and feeding management in dairy herds. However, the relationship between collar-derived rumination metrics and dietary composition, nutrient intake, and milk production in lactating dairy cows remains poorly characterized, particularly under commercial conditions.

Therefore, the objective of this study was to evaluate the associations between collar-derived rumination time and DMI, diet composition, nutrient intake, and milk yield in pens of lactating dairy cows. We hypothesized that rumination time measured by collars would be positively associated with DMI, particularly with fiber intake parameters. We further hypothesized that these associations would be stronger during early lactation, when greater variability in feed intake and forage NDF consumption may result in stronger rumination responses.

All data used in this study were extracted directly from farm management software and did not involve direct contact between researchers and animals or any changes in management, so approval by an ethics committee was not necessary. Farms were selected from a commercial dataset maintained by a consulting company (Dairy Inside, Castro, Brazil). Farms were included only if they had daily records of pen milk production and DMI, routine monitoring of forage DM on farm, frequent forage composition analyses, and at least 12 consecutive weeks of behavioral monitoring data from neck-mounted activity collars (Cowmed, Santa Maria, Brazil). Based on these criteria, five commercial dairy farms located in the Brazilian states of Paraná, São Paulo, and Minas Gerais were included in the study. All farms had Holstein cows housed in free-stall and/or compost barn systems and fed TMR. Data were collected from 40 pens, including 19 multiparous pens, 9 primiparous pens, and 12 pens housing both parity groups. The monitoring period ranged from 13 to 46 weeks across farms, resulting in 1002 pen-

week observations. Across the 40 pens, an average of 168 cows per pen was monitored, representing approximately 6720 lactating cows across all pens in a typical week.

Rumination time was calculated as the average daily rumination time of cows with within each pen and subsequently averaged by week for statistical analyses. In all observations, more than 95% of the cows within each pen were equipped with collars. Pen DMI was calculated daily based on the amount of TMR delivered to each pen and the quantity of refusals. The DM content of forage ingredients was monitored weekly on each farm and used to adjust TMR inclusion rates to maintain the targeted dietary formulation on a DM basis. Refusals were assumed to have the same DM concentration as the offered TMR. Milk production and pen DMI were averaged weekly for subsequent analyses. The chemical composition of forages, including CP, NDF, ADF, starch, total fatty acids, lignin, and undigested neutral detergent fiber after 240 h of incubation (uNDF240) was determined using near-infrared spectroscopy. Analyses were conducted using Dairyland Laboratories calibration curves at a commercial laboratory (MS.DC Lab, Ponta Grossa, Brazil). For each week of observation, the forage analysis temporally closest to the feeding period was used to represent the chemical composition of the forage fraction of the diet. On average, forage samples were analyzed every 2.4 weeks for corn silage harvested during the main season, 3.1 weeks for corn silage from the second crop, and 2.7 weeks for alfalfa haylage or hay. Across other forage sources included in the dataset, the average interval between analyses was 3.8 weeks. Among concentrate ingredients, brewery by-products were analyzed every 2.3 weeks on average using the same NIRS procedures. For the remaining concentrate ingredients, nutrient composition values were obtained from tabular data reported in NASEM (2021).

Associations between rumination time and milk yield, DMI, feed efficiency, diet composition, and nutrient intake were evaluated using linear mixed-effects models fitted with the nlme package in R. Rumination time was defined as the dependent variable, and each

predictor variable was evaluated in a separate model. For each predictor, a sequence of nested models was fitted. The baseline model included DIM and parity class (primiparous vs. multiparous) as fixed effects. The predictor was then added to the model to evaluate its main effect. Subsequently, interaction terms between the predictor and DIM and between the predictor and parity were included to account for potential effect modification. All continuous predictors, including DIM, were centered on their respective means prior to model fitting to improve interpretability of regression coefficients and reduce collinearity. For predictors representing diet composition variables expressed as percentage of DM, centered DMI was included in the model as a covariate to account for variation in feed intake. Models included a random intercept for pen nested within farm to account for non-independence of observations within pens.

Models were fitted using maximum likelihood estimation, and predictor contribution was assessed using the Akaike Information Criterion (AIC), with ΔAIC calculated as the difference between models with and without the predictor, and partial R^2, defined as the increase in marginal R^2 after inclusion of the predictor relative to a model with the same fixed effects but excluding that predictor. Marginal R^2 represents the proportion of variance explained by fixed effects, whereas conditional R^2 represents the proportion explained by both fixed and random effects. P-values for fixed effects, including interaction terms, were obtained from the final model using Wald tests. Statistical significance was declared at $P \leq 0.05$.

Descriptive statistics of the pens and diets included in the dataset are presented in Table 1. Cows were, on average, 169 ± 91 DIM and produced 41.9 ± 9.4 kg of milk per day, with a mean DMI of 25.3 ± 4.0 kg/d. Rumination averaged 466 ± 25 min/d and ranged from 376 to 531 min/d across observations. These values are consistent with those reported in previous studies evaluating rumination behavior in dairy cows. For example, White et al. (2017), in a compilation of published studies, reported an average rumination time of 436 min/d in lactating

dairy cows, with values ranging from 236 to 610 min/d. Although the values observed in the present dataset fall within this previously reported interval, extreme observations were less evident. This was expected because rumination time in the present study was calculated as the average of cows within each pen, which reduces the occurrence of very low or very high individual observations. In addition, differences among studies may also be partially explained by the method used to quantify rumination activity. Sensor-based monitoring systems provide continuous measurements but may vary in accuracy depending on the technology and calibration, which can contribute to differences in reported rumination values across studies.

The relatively high mean rumination time observed in the present dataset is consistent with the elevated milk production and DMI of the cows. Supporting this pattern, Rumination time was positively associated with both milk yield and DMI ($P < 0.01$). Each 1 kg/d increase in milk yield was associated with an increase of 1.92 min/d in rumination time, whereas each 1 kg/d increase in DMI was associated with an increase of 3.15 min/d. Both predictors substantially improved model fit ($\Delta AIC = -102.1$ and -90.5, respectively; partial $R^2 = 0.17$), indicating that production level and feed intake were strongly associated with rumination behavior. These results are consistent with previous studies reporting positive associations between rumination activity and productive performance (Stone et al., 2017; Kaufman et al., 2018). Significant interactions with DIM ($P \leq 0.01$) indicated that these associations were stronger at lower DIM and attenuated as lactation progressed (Figure 1A and 1B), suggesting that rumination monitoring using sensor-based systems may be particularly useful in early-lactation pens. In addition, the association between milk yield and rumination differed by parity ($P < 0.01$), with a steeper slope observed in primiparous cows (Figure 1E).

Among diet composition variables expressed as % of DM, several predictors were statistically significant ($P < 0.05$); however, most showed minimal contribution to explained variance. Partial R^2 values highlighted lignin as the most relevant variable within this group.

Lignin concentration was positively associated with rumination time ($\beta = 22.4$; $P < 0.01$; partial $R^2 = 0.12$), indicating that an increase of 0.5 percentage units in dietary lignin was associated with an increase of approximately 11.2 min/d in rumination time. Forage plus whole cottonseed (WCS) lignin also showed a positive association ($\beta = 6.81$; $P = 0.04$; partial $R^2 = 0.13$), corresponding to an increase of approximately 3.4 min/d in rumination time for each 0.5 percentage unit increase. Notably, the partial R^2 for forage plus WCS lignin was greater than that observed for forage lignin alone (partial $R^2 = 0.07$), reinforcing the contribution of WCS to the stimulation of rumination (Abel-Caines et al., 1997). Interactions with DIM were also observed for several of these variables ($P \leq 0.05$), with stronger positive associations at lower DIM and attenuated slopes at higher DIM (Figure 1C and 1D).

When nutrient intake (kg/d) was evaluated, all predictors were strongly associated with rumination time ($P < 0.01$). However, partial R^2 values provided additional insight into the magnitude of these associations. Lignin intake showed the largest contribution to model fit among all predictors evaluated ($\beta = 99.81$; partial $R^2 = 0.31$; $\Delta AIC = -272.8$), indicating that an increase of 0.1 kg/d in lignin intake was associated with an increase of approximately 10.0 min/d in rumination time. This finding is consistent with previous studies reporting associations between indigestible fiber and rumination activity. For example, Sá Neto et al. (2014) reported increased rumination time when corn silage was replaced with sugarcane silage, despite similar NDF content and particle size, with differences attributed to lower fiber digestibility of sugarcane. The magnitude of the association varied across nutrients, with lignin intake showing the strongest association, followed by ADF, NFC, and NDF (partial R^2 ranging from 0.10 to 0.14). Notably, the association with lignin was greater than that observed for uNDF240 intake, although the reasons for this difference are not clear. Together, these results indicate that while overall intake was strongly associated with rumination, the indigestible fraction of fiber showed a comparatively stronger association. Interactions with DIM were also frequently observed for

intake variables ($P \leq 0.01$), indicating that these associations were not constant across lactation, whereas interactions with parity were less consistent, with a notable association observed for forage plus WCS lignin (Figure 1F).

Across models, conditional R^2 values were consistently higher than marginal and partial R^2 values, indicating that a substantial proportion of the total variance in rumination time was explained by differences among pens. This pattern also suggests that relevant sources of variation were not fully captured by the predictors included in the models. In this context, factors known to influence rumination, such as particle size and physically effective NDF (Mertens, 1997), could not be accounted for in the present study, despite their well-established associations with chewing activity (White et al., 2017; Beauchemin et al., 2018). Although particle size is commonly monitored at the farm level, these data were not available in a sufficiently standardized format to be included in the analysis. Additional factors, including feeding management, diet mixing quality, sorting behavior, and feed delivery practices, may also influence the diet actually consumed by cows (Carneiro et al., 2021) and, consequently, rumination time. Even so, several predictors showed statistically significant associations with rumination time and moderate contributions to model fit, with patterns that are consistent with biological expectations. Together, these results suggest that rumination time measured using sensor-based systems may provide useful information for nutritional monitoring under commercial conditions, particularly in early-lactation pens.

REFERENCES

Abel-Caines, S.F., R.J. Grant, and S.G. Haddad. 1997. Whole Cottonseeds or a Combination of Soybeans and Soybean Hulls in the Diets of Lactating Dairy Cows. Journal of Dairy Science 80:1353–1357. doi:10.3168/jds.s0022-0302(97)76063-6.

Beauchemin, K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. Journal of Dairy Science, 101(6), 4762–4784. https://doi.org/10.3168/jds.2017-13706

Carneiro, J.H., J.F.D. Santos, and R.D. Almeida. 2021. Accuracy and physical characteristics of total mixed rations and feeding sorting behavior in dairy herds of Castro, Paraná. Revista Brasileira de Zootecnia 50. doi:10.37496/rbz5020200174.

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

Lovatti, J.V.R., K.A. Dijkinga, J.F. Aires, L.F.C. Garrido, J.H.C. Costa, and R.R. Daros. 2024. Validation and interdevice reliability of a behavior monitoring collar to measure rumination, feeding activity, and idle time of lactating dairy cows. JDS Communications 5:602–607. doi:10.3168/jdsc.2023-0467.

Mertens, D.R. 1997. Creating a System for Meeting the Fiber Requirements of Dairy Cows. Journal of dairy science 80:1463–1481. doi:10.3168/jds.s0022-0302(97)76075-2.

Modesto, J. L. S., C. D. S. Oliveira, R. B. Silva, R. A. N. Pereira, T. J. DeVries, and M. N. Pereira. 2024. Rumination time as a tool to monitor feed bunk management and slug-feeding in dairy cows. Proc. 58th Annu. Meet. Soc. Bras. Zootec., Cuiabá, MT, Brazil. (Abstr.)

Rutten, C.J., A.G.J. Velthuis, W. Steeneveld, and H. Hogeveen. 2013. Invited review: Sensors to support health management on dairy farms. Journal of Dairy Science 96:1928–1952. doi:10.3168/jds.2012-6107.

Sá Neto, A., A.W. Bispo, D. Junges, A.K. Bercht, M. Zopollatto, J.L.P. Daniel, and L.G. Nussio. 2014. Exchanging physically effective neutral detergent fiber does not affect chewing activity and performance of late-lactation dairy cows fed corn and sugarcane silages. Journal of dairy science 97:7012–7020. doi:10.3168/jds.2013-7856.

Steeneveld, W., Vernooij, J. C. M., & Hogeveen, H. (2015). Effect of sensor systems for cow management on milk production, somatic cell count, and reproduction. Journal of Dairy Science, 98(6), 3896–3905. https://doi.org/10.3168/jds.2014-9101

Stone, A.E., B.W. Jones, C.A. Becker, and J.M. Bewley. 2017. Influence of breed, milk yield, and temperature-humidity index on dairy cow lying time, neck activity, reticulorumen temperature, and rumination behavior. Journal of Dairy Science 100:2395–2403. doi:10.3168/jds.2016-11607.

White, R.R., M.B. Hall, J.L. Firkins, and P.J. Kononoff. 2017. Physically adjusted neutral detergent fiber system for lactating dairy cow rations. II: Development of feeding recommendations. Journal of Dairy Science 100:9569–9584. doi:10.3168/jds.2017-12766.

Table 1. Descriptive statistics of pens and diets in the dataset (1002 observations of 40 pens).

	Mean	SD	Min	Max
Number of cows	168	72	11	328
DIM	169	91	2	387
Milk yield, kg/d	41.9	9.4	19.1	68.8
DMI, kg/d	25.3	4.0	14.8	34.5
Milk/DMI	1.66	0.24	1.00	2.37
Rumination, min/d	466	25	376	531
Diet, % of DM				
Forage	48.33	7.67	34.46	81.57
CP	18.08	0.66	15.24	19.60
NDF	31.07	3.19	24.45	44.17
ADF	18.55	1.91	14.39	25.09
Lignin	3.09	0.51	1.59	4.56
uNDF240[1]	6.56	1.35	3.79	11.24
Forage NDF	18.27	3.77	12.96	35.43
Forage lignin	1.45	0.46	0.44	2.85
Forage uNDF240	5.56	1.38	2.98	10.97
Forage plus WCS[2] NDF	21.05	3.25	15.68	33.64
Forage plus WCS lignin	1.89	0.69	0.44	3.90
Forage plus WCS uNDF240	7.15	1.31	4.50	10.97
Starch	27.27	3.28	16.42	34.50
NFC	39.45	3.09	27.94	47.42
Nonstarch NFC	12.17	1.77	6.08	19.59
Total fatty acids	4.43	0.62	2.28	5.40
Ashes	6.97	0.49	5.50	7.72

[1]uNDF240 = undigested NDF 240 after hours of incubation (measured with near-infrared spectroscopy).

[2]WCS = whole cottonseed.

Predictor	Mean of predictor	β	SE	P-value Predictor	DIM	Parity	Predictor x DIM	Predictor x Parity[1]	ΔAIC	R^2 Partial	R^2 Marginal	R^2 Conditional
Milk yield, kg/d	41.9	1.92	0.23	<0.01	<0.01	<0.01	0.01	<0.01	-102.1	0.17	0.27	0.61
DMI, kg/d	25.3	3.15	0.51	<0.01	<0.01	<0.01	<0.01	0.72	-90.5	0.17	0.31	0.60
Milk yield/DMI	1.66	3.05	5.68	0.59	<0.01	0.02	<0.01	0.15	-8.1	0.01	0.13	0.51
Diet, % of DM												
Forage	48.3	-0.49	0.34	0.15	<0.01	<0.01	<0.01	<0.01	1.3	0.00	0.27	0.61
NDF	31.0	-0.26	0.74	0.72	<0.01	<0.01	0.85	0.21	2.0	0.00	0.22	0.55
ADF	18.5	2.96	1.32	0.02	<0.01	<0.01	0.44	0.32	-7.9	0.01	0.22	0.59
Lignin	3.09	22.4	3.13	<0.01	<0.01	<0.01	0.38	0.10	-175.5	0.12	0.36	0.62
uNDF240[2]	6.56	2.68	1.37	0.05	0.07	<0.01	<0.01	0.24	-7.8	0.00	0.21	0.60
Forage NDF	18.3	-1.35	0.65	0.04	<0.01	<0.01	0.01	<0.01	1.9	0.00	0.23	0.55
Forage lignin	1.45	14.4	3.95	<0.01	0.01	0.01	0.01	0.02	-104.5	0.07	0.29	0.55
Forage uNDF240	5.56	1.34	1.38	0.33	0.04	0.05	<0.01	0.12	-3.4	0.00	0.21	0.58
Forage plus WCS[3] NDF	21.0	0.65	0.67	0.34	<0.01	0.01	0.07	0.03	-15.3	0.02	0.24	0.54
Forage plus WCS lignin	1.89	6.81	3.35	0.04	<0.01	<0.01	0.82	<0.01	-85.1	0.13	0.36	0.55
Forage plus WCS uNDF240	7.15	6.05	1.27	<0.01	0.16	<0.01	0.26	0.70	-39.0	0.00	0.22	0.64
Starch	27.3	-1.45	0.66	0.03	<0.01	<0.01	0.89	0.40	-3.6	0.01	0.24	0.57
NFC	39.5	-0.64	0.72	0.38	<0.01	<0.01	0.12	0.81	1.9	0.00	0.23	0.58
Nonstarch NFC	12.2	1.97	0.90	0.03	<0.01	<0.01	<0.01	0.15	-11.5	0.03	0.29	0.52
Intake, kg/d												
Forage	12.1	2.41	0.82	<0.01	<0.01	<0.01	<0.01	<0.01	-38.0	0.05	0.31	0.65
NDF	7.82	8.49	1.68	<0.01	<0.01	<0.01	<0.01	0.64	-47.5	0.10	0.25	0.56
ADF	4.67	17.7	2.69	<0.01	<0.01	<0.01	<0.01	0.87	-85.0	0.14	0.27	0.61
Lignin	0.78	99.81	9.16	<0.01	<0.01	<0.01	<0.01	1.00	-272.8	0.31	0.40	0.60
uNDF240	1.65	21.55	4.70	<0.01	0.33	<0.01	<0.01	0.70	-57.9	0.07	0.19	0.63
Forage NDF	4.57	5.49	2.20	0.01	<0.01	0.04	<0.01	0.04	-4.8	0.02	0.23	0.52
Forage lignin	0.36	100.90	15.17	<0.01	0.02	<0.01	<0.01	0.62	-152.9	0.15	0.24	0.54
Forage uNDF240	1.39	16.50	5.21	<0.01	0.21	<0.01	<0.01	0.45	-33.4	0.04	0.17	0.60
Forage plus WCS NDF	5.28	11.25	2.04	<0.01	0.01	<0.01	<0.01	0.23	-62.0	0.09	0.24	0.53
Forage plus WCS lignin	0.47	67.92	13.43	<0.01	<0.01	0.03	0.21	0.26	-126.2	0.22	0.29	0.56

Forage plus WCS uNDF240	1.79	31.93	4.37	<0.01	0.32	<0.01	<0.01	0.68	-105.7	0.11	0.21	0.66
Starch	6.93	6.79	1.45	<0.01	<0.01	0.02	<0.01	0.09	-38.3	0.07	0.20	0.58
NFC	10.0	6.16	1.06	<0.01	<0.01	0.02	<0.01	0.53	-72.5	0.14	0.30	0.58
Nonstarch NFC	3.08	16.07	2.55	<0.01	<0.01	<0.01	<0.01	0.90	-75.1	0.14	0.29	0.51

Table 2. Associations between production variables, diet composition, and nutrient intake with rumination time.

[1]Predictor × parity denotes the interaction term between the predictor and parity, with parity included in the model as a 2-level categorical variable (primiparous and multiparous).

[2]uNDF240 = undigested NDF 240 after hours of incubation (measured with near-infrared spectroscopy).

[3]WCS = whole cottonseed.

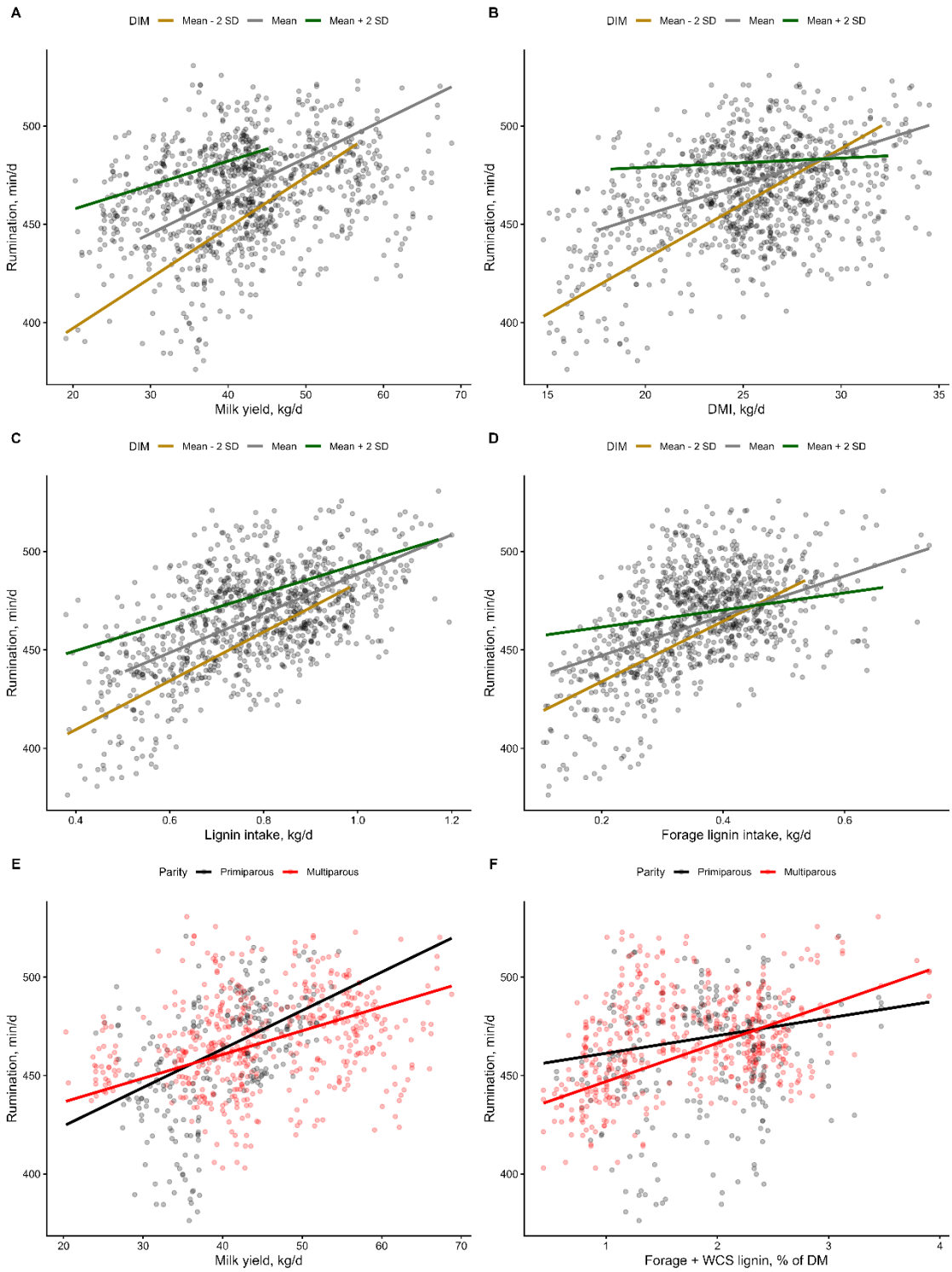


Figure 1. Relationships between rumination time and selected production and dietary variables. Points represent observed values and lines represent marginal predictions from mixed models. In panels A–D, predictions are shown at DIM equal to the mean (gray), mean − 2 SD (gold), and mean + 2 SD (green), and were restricted to the observed predictor range within each DIM stratum. In panels E–F, predictions are shown for primiparous (black) and multiparous (red) cows.