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INCLUSÃO DE PASTO NA DIETA E BALANÇO ENERGÉTICO DE VACAS LEITEIRAS: CONSUMO, EMISSÃO DE METANO, DESEMPENHO PRODUTIVO E FERTILIDADE

Herbage inclusion in the diet and energy balance of dairy cows: intake, methane emission, productive performance, and fertility

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade do Estado de Santa Catarina – UDESC, como requisito parcial à obtenção do título de Doutor em Ciência Animal, Área de Concentração: Produção Animal.

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Dedico esta obra a minha família, que sem conhecer seu conteúdo, entenderam qual seu significado.

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The ruminant animal: *"A small fermentation unit which gathers the raw material, transfers it to the fermentation chamber, and regulates its further passage, continuously absorbs the fermentation products, and transforms them into a few valuable substances such as meat and milk. To these advantages must be added the crowning adaptation: the unit replicates itself"*

Robert E. Hungate, 1950

"Os homens que tem alma para mim são como as madeira que tem cerne, embora que elas caiam o cerne continua servindo para outras coisas, os homens que também são iluminados, embora que muitas vezes nos deixem mas suas ideias continuam"

Mano Lima

Se dois homens vêm andando por uma estrada, cada um com um pão, e, ao se encontrarem, trocarem os pães, cada um vai embora com um.
Se dois homens vêm andando por uma estrada, cada um com uma ideia, e, ao se encontrarem, trocarem as ideias, cada um vai embora com duas.

Proverbio Chinês

RESUMO

CIVIERO, Maurício. **Inclusão de pasto na dieta e balanço energético de vacas leiteiras: consumo, emissão de metano, desempenho produtivo e fertilidade**. 2021. p.148 Tese (Doutorado em Ciência Animal – Área: Produção Animal). Universidade do Estado de Santa Catarina. Programa de Pós-graduação em Ciência Animal, Lages, 2021.

Estratégias nutricionais podem alterar o desempenho produtivo e reprodutivo de vacas leiteiras, além de interferir nas emissões de gases de efeito estufa. Objetivou-se avaliar o efeito da inclusão de pastos de clima tropical e temperado sobre a o consumo e a resposta produtiva de vacas leiteiras no terço médio de lactação, além da emissão de metano (CH_4) com a inclusão de pastos de clima tropical e o efeito do balanço energético (BE) no início da lactação sobre o desempenho produtivo e reprodutivo de vacas leiteiras. Para isto, foram realizados dois experimentos e um estudo de meta-análise. O delineamento dos experimentos foi em Quadrado latino 3 × 3 e os tratamentos foram 100% ração total misturada (RTM) oferecida *ad libitum* (controle)*,* 75% RTM *ad libitum* + acesso a pasto (7h/d) e 50% RTM *ad libitum* + acesso a pasto (7h/d). O pasto de clima tropical foi o capim milheto (*Pennisetum glaucum* 'Campeiro') e o de clima temperado o azevém anual *(Lolium multiflorum* 'Maximus'). A meta-análise foi realizada no Agri-Food Bioscìense Institut – AFBI (Reino Unido) e avaliou o efeito do BE entre os dias 4 e 21 de lactação no desempenho produtivo e reprodutivo de vacas primíparas (488) e multíparas (1020). Os animais foram divididos em quartis conforme o BE entre os dias 4 e 21 de lactação a partir de dados coletados durante um período de 20 anos (1996-2016), utilizando 27 experimentos e 79 tratamentos. No primeiro experimento, a inclusão progressiva de pasto de clima tropical na dieta reduziu a ingestão de matéria seca (IMS) total, mantendo a produção de leite acima de 90% do observado no tratamento controle sem alterar a intensidade de emissão de CH_4 (g/kg de leite corrigido para energia). No segundo experimento, a inclusão progressiva de pasto de clima temperado reduziu a IMS total. Entretanto, a produção e a composição do leite não se alteraram, levando os animais a um BE levemente negativo (92% das exigências diárias) no maior nível de ingestão de forragem. A meta-análise demonstrou que o BE negativo (BEN) entre os dias 4 e 21 de lactação é capaz de atrasar o retorno à atividade cíclica. Para cada 10 MJ de energia metabolizável em BEN o retorno ao cio foi atrasado em 1,2 e 0,8 dias em primíparas e multíparas, respectivamente. Contudo, o BEN não afetou as taxas de prenhez tanto nas primíparas como nas multíparas. A inclusão de pastos de clima tropical e temperado na dieta de vacas que recebem RTM mostrou-se capaz de manter o desempenho produtivo sem alterar a intensidade de emissão de CH_4 entérico. Melhorar o BE de vacas leiteiras durante início de lactação mostrou-se eficiente para reduzir o período entre o parto e o primeiro cio, mas não afetou a taxa de prenhez.

Palavras-chave: metano, dieta total misturada, pastoreio, produção de leite, GEE.

ABSTRACT

CIVIERO, Maurício. **Herbage inclusion in the diet and energy balance of dairy cows: intake, methane emission, productive performance, and fertility**. 2021. p.148. Thesis (Doctorate in Animal Science - Area: Animal Production) - Santa Catarina State University. Post Graduate Program in Animal Science, Lages, 2021.

Nutritional strategies may change productive and reproductive performance of dairy cows, as well as modify greenhouse gas emissions. It was evaluated the effect of including tropical and temperate herbages on DM intake and productive response of dairy cows in the middle third of lactation, as well as the methane (CH_4) emission with the inclusion of a tropical herbage and the effect of energy balance (EB) at the beginning of lactation on productive and reproductive performance of dairy cows. For this, two experiments and a meta-analysis study were carried out. The experiments were designed in 3 × 3 Latin square and the treatments were 100% total mixed ration (TMR) offered ad libitum (control), 75% TMR ad libitum + access to herbage (7h/d) and 50% TMR ad libitum + access to herbage (7h/d). The tropical herbage was pearl millet (*Pennisetum glaucum* 'Campeiro') and the temperate herbage was ryegrass (*Lolium multiflorum* 'Maximus'). The meta-analysis was carried out at the Agri-Food Biosciense Institute - AFBI (United Kingdom) and evaluated the effect of EB between days 4 and 21 of lactation on the productive and reproductive performance of primiparous (488) and multiparous (1020) cows. The animals were divided into quartiles according to the EB between days 4 and 21 of lactation based on data collected over a period of 20 years (1996-2016), using 27 experiments and 79 treatments. In the first experiment, the progressive inclusion of tropical herbage in the diet decreased the total dry matter intake (DMI), maintaining milk production above 90% of that observed in the control treatment, without changing the CH_4 intensity (g/kg of energy-corrected milk). In the second experiment, the progressive inclusion of temperate herbage decreased the total DMI. However, milk production and composition did not change, leading the animals to a slightly negative EB (92% of daily requirements) at the greatest level of herbage intake. The meta-analysis demonstrated that negative EB (NEB) between days 4 and 21 of lactation is able to delay the return to cyclic activity. For every 10 MJ of metabolizable energy in NEB, the interval from calving to first observed heat (FOH) was delayed by 1.2 and 0.8 days in primiparous and multiparous cows, respectively. Nevertheless, NEB did not affect the conception to the first service in both primiparous and multiparous cows. Including tropical and temperate herbages in the diet of dairy cows receiving TMR proved to be capable of maintaining productive performance without altering the CH_4 intensity. Improving EB of dairy cows during the beginning of lactation proved to be efficient in reducing the interval from calving to FOH, but had no effect on conception to the first service.

Keywords: methane, total mixed ration, grazing, milk production, GHG.

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LISTA DE ABREVIATURAS E SIGLAS

ABIEC – Associação brasileira das indústrias exportadoras de carnes

AFBI – AgriFood and Bioscience Institute

AGNE – Ácidos graxos não esterificados

AGV – Ácidos graxos voláteis

BE – Balanço energético

BEN - Balanço energético negativo

BHB – Beta-hidroxibutirato

C3 –Metabolismo fotossintético com três carbonos

C4 - Metabolismo fotossintético com quatro carbonos

CH_4 – Metano

CO_2 – Dióxido de carbono

CO2e – Equivalente de dióxido de carbono

DEL – Dias em lactação

ECC – Escore de condição corporal

FAO – Organização das Nações Unidas para Alimentação e Agricultura

FDN – Fibra em detergente neutro

FSH – Hormônio folículo estimulante

GEE – Gases de efeito estufa

GH – Hormônio do crescimento

GnRH – Hormônio liberador das gonadotrofinas

GTP – Potencial de mudança de temperatura em 100 anos

GWP – Potencial de aquecimento global em 100 anos

H_2 – Gás hidrogênio

IGF-I - Fator de crescimento semelhante à insulina-I

IMS – Ingestão de matéria seca

IPCC – Painel intergovernamental sobre mudanças climáticas

LCE – Leite corrigido para energia

LH – Hormônio luteinizante

MO – Matéria Orgânica

MS – Matéria seca

ppb – Parte por bilhão

ppm – Parte por milhão

PUFAs – Ácidos graxos poli-insaturados

RTM – Ração totalmente misturada

SNC – Sistema nervoso central

Tg – Teragramas

SUMÁRIO

1. INTRODUÇÃO

O Brasil, imponente por suas dimensões continentais de 8.516.000 km² e privilegiado pelas características edafoclimáticas, possui notoriedade na produção de alimentos e produção animal. A pecuária brasileira detêm o maior rebanho comercial mundial de bovinos, com 221,81 milhões de cabeças, sendo 39 milhões destinadas à exploração leiteira (ABIEC, 2018). Os três estados da região sul detêm 6,7% do território nacional, com aproximadamente 28 milhões de cabeças, correspondendo a 12,6% do rebanho.

A produção de proteína animal com eficiência e redução dos impactos ambientais são grandes desafios para a pecuária atual e futura, e a adoção de tecnologias depende do avanço do conhecimento e das exigências do mercado. Neste contexto, a produção de alimentos para os animais implica em 70% do custo total da atividade, e se resume no principal fator que influencia a eficiência produtiva. A maneira de colher, armazenar e fornecer alimento são divergentes em relação às regiões, dependendo dos recursos financeiros empregados e exigências nutricionais de cada categoria animal. A dieta total misturada (RTM) ou alimentação exclusiva com pasto estão aquém do ideal para a maioria das condições produtivas encontradas. O alto custo para o uso de RTM e o não atendimento das exigências nutricionais dos animais em dietas com uso exclusivo de pastos são os principais motivos para o uso de dietas mistas (parcialmente misturada + pasto). A utilização de dietas mistas objetiva unir as vantagens da RTM, reduzindo ou eliminando os problemas da sazonalidade da produção de forragem, com os benefícios do uso do pasto, como, diminuição dos custos de produção e do uso de mão-de-obra além de possibilitar o uso de áreas não mecanizáveis (KOLVER; MULLER, 1998; SCHINGOETHE, 2017). Não obstante, a busca por melhorias na produção, qualidade e custos reduzidos necessita levar em conta os aspectos ambientais.

A fermentação entérica emite de 2 a 12% da energia bruta ingerida na forma de metano (CH_4) (JOHNSON et al., 1994). A utilização de diferentes estratégias alimentares para mitigar CH_4 e manter a produção é conhecida em muitos países do hemisfério norte de clima temperado, mas não consolidada em regiões de clima subtropical, como o sul do Brasil, onde é possível utilizar pastos de clima tropical e temperado nas estações quente e fria do ano, respectivamente. Forragens tropicais possuem diferentes proporções de constituintes, principalmente fibra, em comparação às de clima temperado, podendo alterar a emissão de CH_4 entérico e a produtividade dos animais.

Dentre os desafios de maximizar a produção animal, minimizando os impactos ambientais, sobretudo aqueles advindos da emissão de CH_4, encontra-se a manutenção do elevado desempenho reprodutivo dos animais. Novilhas que iniciam a atividade reprodutiva na idade adequada e vacas que conseguem intervalos entre partos de um ano são mais produtivas e consequentemente tendem a diminuir a intensidade de emissão de CH_4 entérico (g/kg leite corrigido para energia (LCE)) advinda do rebanho. Entretanto, nas últimas décadas o potencial produtivo de vacas leiteiras aumentou dramaticamente em muitos países (MIGLIOR; MUIR; VAN DOORMAAL, 2005). Maiores produções de leite têm conduzido os animais a mobilizarem altos níveis de reservas energéticas, impondo maiores períodos em balanço energético negativo (BEN) (AGNEW et al., 1998). Diferentes severidades de BEN estão associadas a alterações no desempenho produtivo e nas concentrações de metabolitos sanguíneos, com alterações no '*status*' metabólico e na capacidade reprodutiva de vacas leiteiras.

Esta tese avaliou o efeito da inclusão progressiva de pastos anuais de inverno e verão na dieta sobre o balanço energético (BE) e o desempenho produtivo de vacas leiteiras no meio da lactação. A inclusão de pasto de clima tropical foi acompanhada de medidas da emissão de CH_4 entérico, visando suprir uma lacuna do conhecimento para regiões de clima tropical e subtropical. Ademais, foi realizada uma meta-análise com dados de 1508 lactações (488 primíparas e 1020 multíparas) onde foram avaliadas as relações entre BE no pós-parto com alterações no desempenho produtivo, concentração de metabolitos sanguíneos e principalmente com o desempenho reprodutivo de vacas leiteiras.

2. REVISÃO DE LITERATURA

2.1. USO EXCLUSIVO DE DIETA MISTURADA PARA VACAS LEITEIRAS

O emprego da RTM em rebanhos leiteiros, é uma prática utilizada nos últimos 100 anos, sendo que os principais avanços existentes neste tema são mencionados em artigos a partir da década de 1960. A alimentação dos rebanhos leiteiros durante um longo período era baseada em apenas forragem. Entretanto, quando os rebanhos começaram a crescer e a produção individual aumentar, ocorreu também o aumento do número de animais confinados e da utilização de RTM (COPPOCK; BATH; HARRIS, 1981; SCHINGOETHE, 2017).

Coppock, (1977) apresenta vantagens quantitativas e qualitativas superiores às desvantagens no uso de RTM na alimentação de vacas leiteiras. Os bocados dos animais são realizados em uma dieta nutricionalmente completa, o que seria em tese próximo a necessidade animal, permitindo dividir o rebanho em grupos de produção, adicionar ingredientes preteridos e melhorar a relação energia/proteína da dieta (RAKES, 1969).

A formulação da RTM baseia-se nas necessidades de mantença e produção, sendo calculadas em função de dados de entrada: peso vivo, idade, estágio de lactação, produção, gordura e proteína do leite. A adequada formulação além de atender as demandas dos animais, minimiza a eliminação de produtos ambientalmente prejudiciais e melhora os aspectos econômicos da atividade.

Kolver e Muller, (1998) observaram que animais de alta produção recebendo pasto de alta qualidade e RTM balanceada tiveram diferentes desempenhos (29.6 *vs.* 44.1 kg/d para pasto e RTM, respectivamente) e diferentes ingestões de matéria seca (IMS) (19.0 *vs*. 23.4 kg/d, para pasto e RTM, respectivamente) caracterizando a necessidade das RTM em rebanhos de alto mérito genético. O consumo de energia tem sido identificado como o primeiro limitante em dietas a base de pasto. Entretanto, a suplementação energética minimiza os impactos em desempenho e consumo, mas pode gerar problemas digestivos no rúmen, diminuir a gordura do leite e influenciar no escorre corporal das vacas leiteiras (BARGO et al., 2002a). No mesmo sentido, Mohammad et al. (2017) demonstraram que fornecer separadamente os ingredientes e em condições restritas pode diminuir a IMS, a produção de leite e os teores de gordura.

A escolha do tipo de alimentação depende do balanço entre vantagens e desvantagens, levando em conta o sistema econômico e técnico adotado na fazenda leiteira. Propriedades com relevos acidentados, rebanhos pequenos e com pouco investimento dificilmente conseguirão adotar o uso exclusivo da RTM. Além disso, aspectos relacionados ao bem-estar animal, à sanidade do rebanho e a maior demanda em mão-de-obra podem ser considerados como limitações ao uso exclusivo de dietas misturadas.

2.2. USO EXCLUSIVO DE DIETAS A BASE DE PASTO DE CLIMA TROPICAL E/OU CLIMA TEMPERADO

As pastagens recobrem a maior fração do ambiente terrestre do Planeta Terra (WILLIAMS et al., 1968). Várias espécies, cada uma com suas características próprias contemplam as pastagens, e sobre elas é praticada a herbivoria. Mais de 90% dos alimentos para os herbívoros são compostos por gramíneas (Poaceae) de metabolismo fotossintético C3 e C4 e por leguminosas (Fabaceae). As regiões de clima subtropical, sobretudo nas áreas próximas dos trópicos de Câncer e Capricórnio (latitudes 23,5° ao sul e ao norte da linha do Equador), possuem condições climáticas para o desenvolvimento de gramíneas de clima tropical e temperado nas estações quente e fria, respectivamente. Em uma faixa que localiza-se 10° para o Norte ou para Sul das latitudes 23,5° - em função de características locais de clima, microclimas, solos, altitudes e estações do ano - as gramíneas supracitadas desenvolvem-se e são denominadas também como gramíneas de “estação fria” C3 e “estação quente” C4 (BARBEHENN et al., 2004). Estas espécies adaptaram-se às regiões e estações do ano para diferentes intensidades de temperatura e luminosidade, sendo que alterações de características anatômicas e bioquímicas das plantas são capazes de alterar a maneira com que os animais respondem produtivamente a cada tipo de gramínea. Entretanto, mesmo com a alta capacidade de ambas as gramíneas se intercalarem durante as estações do ano, as alterações bromatológicas inerentes aos diferentes estádios fenológicos das plantas fazem com que a ingestão diária de nutrientes fique aquém das exigências nutricionais para animais de médio e alto potencial produtivo.

Sage e Monson, (1999) descreveram valores mais baixos de nutrientes digestíveis e altos teores de fibra em gramíneas tropicais comparadas às de clima temperado. Em contrapartida, as tropicais são em termos globais as que mais sofrem o processo de herbivoria no mundo. Os tecidos que compõe as folhas de gramíneas tropicais têm nervuras próximas, compostas por feixes vasculares cercados por camadas concêntricas de células da bainha e células do mesófilo possuindo capacidade aumentada de realizar fotossíntese, apresentando níveis mais baixos de enzimas fotossintéticas e menor teor de proteína que as gramíneas de clima temperado (BARBEHENN et al., 2004).

A diferença entre os teores e características químicas das fibras de gramíneas tropicais e temperadas ocorrem principalmente pelo efeito da temperatura na maturação e maior lignificação das espécies de clima tropical, influenciando diretamente na resistência física à degradação microbiana, tendo relação direta com a digestibilidade da forragem (WILSON, 1994). As características químicas das gramíneas de clima tropical podem influenciar negativamente a taxa de passagem do conteúdo ruminal quando comparadas às gramíneas de clima temperado.

Considerando dados obtidos de experimentos conduzidos com pastos de clima tropical, Boval; Edouard; Sauvant, (2015) realizaram uma meta-análise avaliando o consumo e o desempenho animal. Os autores reforçam a necessidade do conhecimento entre as interações forragem-animal visando à máxima produção, seja ganho de peso ou produção de leite, com melhor utilização da terra (taxa de lotação). São poucos os trabalhos que medem a relação produtiva do consumo de pasto de clima tropical com o oferecimento de suplementos a base de silagem de milho e concentrado, sendo de suma importância medir e avaliar as repostas de consumo e produtivas decorrentes dessa relação. Desta maneira, a forragem utilizada seu estádio fenológico e os animais que a consomem determinam diferentes potenciais produtivos.

Aguilar-Pérez et al. (2009) investigaram animais cruzados (Holandês × Zebuínos) em região tropical com ou sem suplementação de concentrado e observaram que os animais sem suplementação tinham produção inferior aos suplementados (7,8 e 11,1 kg/d, respectivamente).

Miguel et al. (2014) estudando diferentes níveis de suplementação (0, 4 e 8 kg/d) com silagem de milho + farelo de soja (7:1) para vacas leiteiras pastejando azevém observaram que as vacas com zero suplementação apresentaram menor produção de leite que os animais recebendo 4 e 8 kg/d de suplemento (18,7, 20,3 e 20,4 kg/d, respectivamente). Entretanto, Dall-Orsoletta (2019) estudando animais em exclusivo pastejo e dois tipos de suplementação (milho moído ou silagem de milho) observaram que animais sem suplementação obtiveram produções de leite similares aos que recebiam silagem de milho, mas inferiores aos que recebiam milho moído.

Bargo et al. (2003) descrevem que menor IMS total é identificada como o a principal causa limitante da produção de leite em vacas com acesso exclusivo a pasto. Os mesmos autores relatam 24% menos IMS, 22% menos produção de leite, 4 % menos proteína e 6 % mais gordura no leite de vacas ingerindo dietas exclusivas a pasto quando comparadas com dietas mistas com suplementação maior que 10 kg MS/d.

2.3. UTILIZAÇÃO DE DIETAS MISTAS

A adoção de uma dieta mista ao invés da utilização de somente forragem ou RTM diminui o custo de produção, reduz a mão-de-obra e torna o sistema menos vulnerável às variáveis edafoclimáticas que interferem sobre a produção forrageira e a obrigatoriedade de utilização de áreas mecanizáveis para produção de RTM. Isso ocorre porque ao decorrer do ano, com os diferentes índices de pluviosidade, temperaturas e luminosidade ocorrem diferentes taxas de acúmulo de forragem, além de proporcionar o desenvolvimento de diferentes espécies forrageiras.

A resposta animal quando lhe oferecido pasto para complementar a dieta, depende da capacidade genética dos animais, do balanço entre energia e proteína para degradação ruminal, das características químicas da forragem e principalmente do tempo, manejo e oferta de forragem disponíveis aos animais. Outro fator influenciado pelo manejo e oferta de forragem, que pode ajustar as respostas produtivas e afetar o consumo, é o comportamento ingestivo, o qual determina a ingestão de MS por bocado, a taxa de bocados e o tempo de pastejo (KOLVER; MULLER, 1998; O'NEILL et al., 2011; PEYRAUD; DELAGARDE, 2013).

As misturas utilizadas entre RTM e pasto com oferta e qualidade suficiente podem manter a produção de vacas do meio para o fim da lactação. No trabalho de Dall-Orsoletta et al., (2016) foi demonstrado que a utilização de 42% de pasto (avezém) na dieta de vacas leiteiras recebendo RTM parcial é possível manter produções de leite similares àquelas de vacas recebendo RTM de forma exclusiva

Quando Bargo et al. (2002b) investigaram o efeito de dietas mistas e RTM para vacas de alto potencial, observaram que os animais em dietas mistas apresentaram menores valores de IMS (25,2 *vs.* 26,7 kg/d) e produção de leite (32*vs.* 38 kg/d) quando comparados aos animais ingerindo exclusivamente RTM. A redução da IMS total observada nos animais recebendo dietas mistas foi explicada, em parte, pelo fato de a ingestão de pasto ter sido negativamente afetada pelas altas temperaturas nos meses de verão. As reduções na produção de leite foram relacionadas ao aumento das exigências em energia de mantença (relacionada a caminhada e atividade de pastejo) e pelas reduções na IMS.

Mendoza et al. (2016) investigando vacas leiteiras recebendo exclusiva RTM, RTM (20 h/d) + oferecimento de forragem verde (4 h/d) e RTM (16 h/d) + oferecimento de forragem verde (8 h/d) observaram IMS equivalentes a 24,5, 25,6 e 22,6 kg/d e produções de leite equivalentes a 34,4, 34,9 e 32,7 kg/d, respectivamente. As IMS de pasto foram relativamente pequenas para os tratamentos com 4 e 8 h/d de acesso à forragem verde, 2,8 e 3,6 kg/d, respectivamente. A possível explicação para a baixa IMS de forragem e consequentemente redução da IMS total é o baixo teor de MS da forragem que influencia o preenchimento ruminal e a capacidade de apreensão da forragem. A redução na produção de leite que ocorreu no tratamento com 8 h/d de acesso a forragem verde deu-se pela redução na IMS total. Nessas condições a composição do leite permaneceu inalterada para os diferentes tratamentos.

Wales et al. (2013) realizaram uma revisão sobre o uso de parcial RTM em sistemas a base de pasto nas regiões temperadas da Austrália e observaram que as respostas positivas na produção de leite estão mais relacionadas à capacidade que as dietas mistas têm de aumentarem a IMS total do que com alterações na digestibilidade total no trato gastrointestinal. Também, as diferenças mais expressivas na produção de leite aparecem quando o consumo de suplemento (RTM parcial) é maior que 10 kg/d. Entretanto, o percentual de gordura do leite manteve-se constante com o aumento do consumo de concentrado nas dietas mistas.

Bargo et al. (2003) em uma ampla revisão explicam inúmeros fatores que podem alterar o desempenho produtivo de vacas recebendo dietas a base de pasto com suplementação. Produções de leite aumentam linearmente com a inclusão de concentrado como suplemento (de 1,2 até 10 kg MS/d). Entretanto a magnitude de resposta ao concentrado depende do efeito do mesmo na taxa de substituição. A produção de leite esta inversamente relacionada à taxa de substituição, onde menor substituição se reflete em maiores magnitudes de resposta na produção de leite. Um dos fatores que podem alterar a resposta da suplementação é o estágio de lactação que os animais se encontram, sendo cada vez menor para os animais que avançam para o terço final de lactação. Trabalhos investigando os efeitos da adição de suplementos para vacas de médio e alto potencial quando ingerindo forragens de clima tropical ainda são escassos e apresentam uma lacuna do conhecimento para melhor entender as dinâmicas de respostas dos animais.

Alinhados as questões produtivas mencionadas sobre a utilização das dietas mistas, essas mesmas apresentam grande potencial para sequestro de carbono atmosférico. Dentre as espécies de clima tropical e metabolismo C4 utilizadas está o capim milheto (*Pennisetum glaucum*), que encontra-se na sexta posição em relação a cultura alimentar no mundo (FAO, 2017). Amplamente distribuído nos continentes asiático e africano e em países como Austrália e Brasil, desenvolve-se em regiões tropicais e semiáridas, recobrindo cerca de 5 milhões de hectares ao redor do mundo (DE ASSIS; DE FREITAS; MASON, 2018). Os mesmos autores ainda relatam que nas regiões sul do Brasil, com chuvas regulares, a capacidade produtiva do milheto fica entre 10 e 20 toneladas anuais de matéria seca, com altos teores de proteína e digestibilidade, ao mesmo tempo que aproximadamente 5,8 a 11,6 toneladas são do elemento carbono (58% da matéria orgânica é composta de carbono) que originou-se do processo fotossintético de redução do dióxido de carbono da atmosfera, importante gás de efeito estufa.

Principal representante das gramíneas temperadas de metabolismo C3 e conhecido mundialmente o azévem anual e perene do genêro *Lolium* são importantes fontes alimentares para a pecuária devido a sua alta qualidade bromatológica. No sul do Brasil estima-se que 2,1 milhões de hectares são cultivados com azévem anual com produções que variam em função de condições edafoclimáticas e adubação nitrogenada que estão entre 1,2 até 7,2 toneladas de matéria seca por hectare (VARELLA et al., 2010). É possível assim acumular de 0,7 a 4,2 toneladas de carbono através do processo fotossintético do azévem durante seu ciclo de vida.

Segundo a FAO (2006), as pastagens cultivadas e nativas possuem uma capacidade de drenar até 1,7 bilhões de toneladas de carbono por ano da atmosfera, além de que com o correto manejo das pastagens aumenta-se a fixação do carbono no solo, melhorando as condições de fertilidade, assim autopromovendo-se.

Desta forma, avaliar a influência de pastos de clima temperado e tropical numa mesma estratégia de alimentação, com condições de manejo pré-determinadas, faz-se necessário para a correta inferência sobre sistemas produtivos em ambientes de clima subtropical.

2.4. EMISSÃO DE METANO ENTÉRICO

É importante ressaltar neste tópico que a utilização de pastagens na alimentação de vacas leiteiras tem papel fundamental no sequestro de GEE. Segundo o IPCC (2014), o potencial de aquecimento global (GWP) do CH_4 em relação a equivalentes dióxido de carbono (CO_2e) é de 28 vezes. Assim, somente as pastagens anuais de milheto e azévem durante seu ciclo de alguns meses são capazes de retirar através da fotossíntese e utilização dióxido de carbono (CO_2) quantidades que variam de 207 a 414 kg e 25 a 150 kg de CH_4, respectivamente. O milheto e o azévem são gramíneas não competitivas pois desenvolvem-se em espaços temporais diferentes permitindo serem somadas suas capacidades de aprisionamento de GEE.

As concentrações atmosféricas de GEE de origem das atividades agropecuária, representadas por CO_2, CH_4 e óxido nitroso (N_2O) cresceram nos últimos anos superando a taxa de desaparecimento (IPCC, 2014). Segundo Sejian et al., (2015) o setor pecuário mundial contribui com 18% das emissões diretas ou indiretas dos GEE, totalizando 7,1 bilhões de toneladas de CO_2e.

Existem duas formas de mensurar os impactos ambientais dos GEE em CO_2e, sendo o potencial de aquecimento global (GWP) e o potencial de temperatura global (GTP). O GWP desenvolvido para o IPCC 1990, embasa-se na força radiativa do gás integrada no tempo, para 1 kg de emissão de determinado gás em relação ao gás de referência CO_2 (Tabela 1). O GTP é definido como a razão entre a mudança da temperatura média da superfície da Terra em um determinado espaço temporal de um gás para o gás de referência CO_2 (Tabela 1) (CHANG-KE; XIN-ZHENG; HUA, 2015).

Tabela 1. Tempo de vida, potencial de aquecimento global e potencial de temperatura global dos principais gases de efeito estufa para emissões de 2010.

Gases	Tempo de Vida (anos)	**GWP** Potencial de aquecimento global em 100 anos	**GTP** Potencial de mudança de temperatura em 100 anos
CO_2	-	1	1
CH_4	12,4	28	4
N_2O	121	265	234
CF_4	50000	6630	8040

Fonte: IPCC, 2014.

Segundo Chang-Ke, Xin-Zheng e Hua, (2015) países como União Europeia, Estados Unidos da América, Japão, Canadá e África do Sul teriam maior impacto nas mudanças de temperatura quando o mesmo for estimado por GTP. Brasil, Austrália, China, Índia, México e Rússia diminuiriam suas contribuições nos inventários se fossem utilizados o GTP. Ainda os mesmos autores citam que o Brasil e a Austrália sugeriram através de documento para que os próximos inventários fossem estimados através do GTP, porém ainda existe grande resistência principalmente por parte da União Europeia (EU), pelo fato de este critério aumentar significativamente suas contribuições no aumento de temperatura.

As emissões de CH_4 cresceram duas vezes e meia na era industrial, alcançando 1750 ppb (partes por bilhão ou nano moles por moles de ar seco) (LASSEY, 2007). O CH_4, mesmo sendo o segundo GEE, atrás do CO_2, possui uma grande capacidade de absorção do infravermelho, fazendo-o obter um GWP estimado em 28 kg CO_2e/kg CH_4, ou seja, cada kg de CH_4 que chega a atmosfera absorve a mesma quantidade que 28 kg CO_2 do espectro de radiação que sairia da Terra e um GTP estimado de 4 kg CO_2e/kg CH_4, ou seja, cada kg de CH_4 possui a capacidade de aquecer a temperatura em 4 vezes, ambos para um tempo de 100 anos (IPCC, 2014).

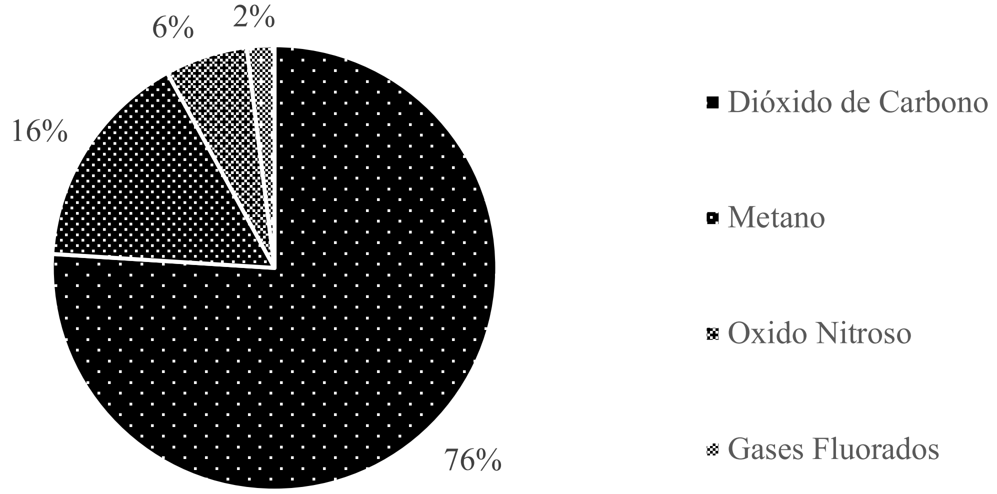
Segundo Knapp et al., (2014) as emissões de CH_4 com origem antropogênicas circundam os valores de 58%, sendo a fermentação entérica a maior geradora de CH_4 (17%). Estima-se que os ruminantes presentes ao redor do mundo produzam 86 milhões de toneladas de CH_4 por ano (SCHELLNHUBER et al., 2006). O setor agropecuário no Brasil contribui com 37% das emissões totais em CO_2e, onde 62% destes é advindo da emissão de CH_4. A bovinocultura de corte e leite estão em primeira e segunda colocação na emissão com 76 e 11%, respectivamente. A grande discrepância dos valores se dá em função do contingente de animais inclusos em cada atividade (MCTI, 2017).

O CH_4 tem origem de fontes bióticas e abióticas. As abióticas são emitidas através da queima de combustíveis fósseis (petróleo, gás natural e carvão), queima de biomassa e fontes geológicas (origem vulcânica). Entretanto, as fontes bióticas correspondem a mais de 70% da emissões globais, representadas por pântanos, arroz irrigado, ruminantes, aterros e cupins (DENMAN et al., 2007). Pode-se classificar também as emissões em, natural -160 teragramas (Tg) CH_4/ano (30%) correspondente as regiões úmidas, cupins e oceanos com 115, 20 e 10 Tg CH_4/ano, respectivamente - e antropogênicas 375 Tg CH_4/ano (70%) correspondente a fermentação entérica, arroz irrigado, queima de biomassa, aterros sanitários, gás natural, minas de carvão, resíduos de animais, esgoto domésticos, indústria petrolífera e queima de carvão, com 85, 60, 40, 40, 40, 30, 25, 25 15 e 15 Tg CH_4/ano, respectivamente (SEJIAN et al., 2015).

Segundo o IPCC (2014), de todos os GEE de origem antrópica o CH_4 corresponde ao total de 16% (Figura 1). Desses, 23% correspondem a fermentação entérica proveniente dos ruminantes (Figura 2; SEJIAM et al., 2015). Assim, podemos inferir através da multiplicação de 16% por 23% que 3,7% de todos GEE são oriundos da fermentação entérica dos ruminantes. Benchaar, Pomar e Chiquette, (2001) relataram em seus estudos que os fatores que influenciam a emissão de CH_4 total seguem uma ordem: aumento da ingestão de matéria seca, aumento da proporção de alimentos concentrados na dieta, fonte do concentrado, velocidade de degradação do amido, estágio vegetativo da forragem, método de conservação, espécie da forragem, processamento do alimento e suplementação de forragens de baixo valor bromatológico.

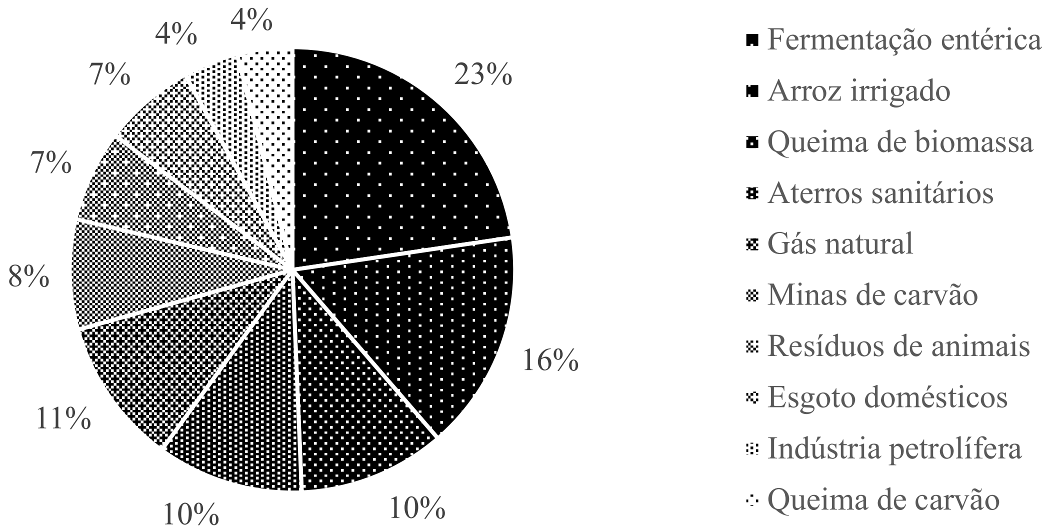
Niu et al. (2018) reportaram em um banco de dados intercontinental que existe uma grande variação na faixa de emissões de CH_4, estando entre 90 e 729 g/d, provavelmente refletindo o número de fatores que são capazes de alterar a emissão de CH_4 entérico.

Figura 1. Porcentagem dos gases de efeito estufa de origem antropogênica em CO_2e, que influenciam no aquecimento global.



Fonte: IPCC, 2014.

Figura 2. Porcentagem das contribuições de cada atividade antropogênica que emitem gás metano e contribui para o aquecimento global.



Fonte: Sejian et al., 2015.

As emissões de CH_4 alteram-se com a utilização de dietas mistas, uma vez que o substrato se torna diferente. Savian et al., (2014) trabalhando com ovelhas lactantes, Warner et al. (2016), Dall-Orsoletta et al. (2016) e Dall-Orsoletta et al. (2019) trabalhando com vacas lactantes em pastejo de gramíneas anuais de clima temperado obtiveram, respectivamente, emissões de 7,3, 6,4, 9,3 e 7,6% da energia bruta ingerida. As emissões de CH_4 para vacas leiteiras em pasto anual de clima temperado estão num intervalo de 251 a 656 g/d e 18 a 42 g/kg MS (DALL-ORSOLETTA et al., 2016, 2019; O'NEILL et al., 2011; WARNER et al., 2016). Entretanto, Alves et al. (2017) investigando o uso de dietas mistas com utilização de gramíneas de clima tropical (capim milheto - *Pennisetum glaucum*) com ou sem uso de suplementação com extrato tanífero obtiveram emissões e produções médias de CH_4 na ordem de 491 g/d e 30,2 g/kg MS, respectivamente. Nos estudos de O'Neill et al. (2011) e Cameron et al. (2018) foi possível observar que animais recebendo dietas mistas (pasto + RTM) em relação animais recebendo exclusivamente RTM obtiveram redução nas emissões diárias de CH_4 devido as reduções conjuntas de IMS.

2.4.1. Metanogênese Ruminal

A alimentação de animais com RTM ou forragem de alta qualidade pode contribuir para reduzir a emissão de CH_4 entérico. Isso ocorre pelo baixo teor de fibra e maiores quantidades de carboidratos solúveis, o que pode estar associado à adição de concentrado, ao estádio vegetativo da forragem e à inclusão de leguminosas (BEAUCHEMIN et al., 2008).

Existe correlação direta entre o consumo e a emissão de CH_4, principalmente para forragens tropicais, a qual é representada por uma resposta linear ($R^2=0,99$) (KURIHARA et al., 1999). A comparação entre gramíneas C3 e C4, com o mesmo teor de fibra e digestibilidade permite observar que as emissões de CH_4 são de 10 a 17% superiores para gramíneas C4, devido a característica da fibra presente e alterações na metanogênese (ARCHIMÈDE et al., 2011).

Ainda existe bastante discrepância entre os inventários das diferentes regiões do mundo, sendo necessárias algumas revisões nas formas de mensurar para melhorar as estimativas. Niu et al. (2018) enfatizam que é necessário informações sobre ingestão de matéria seca e teor de FDN da dieta, para junto com dados de produção e composição de leite predizer com segurança a intensidade das emissões.

O processo de degradação ruminal dos carboidratos em completa ausência de oxigênio, pelo processo de interação entre hospedeiro (ruminante) e o hospedado (bactérias, archeas, protozoários e fungos) tem como um dos produtos finais o CH_4 (MURRAY; BRYANT; LENG, 1976). A fermentação e as diferentes vias metabólicas ativadas pelos microrganismos do rúmen dependem do tipo de carboidrato da dieta, do ambiente ruminal e da taxa de passagem dos nutrientes entre os pré-estômagos e o estômago (BUCCIONI; CAPPUCCI; MELE, 2015).

No ecossistema ruminal, as fontes de energia após passarem por moléculas energéticas menores como glicose, frutose e pentoses são posteriormente transformadas em piruvato, necessário para ativar as vias dos ácidos graxos voláteis (AGV), originando acetato, propionato e butirato, com produção de CO_2 e CH_4 (1) (BANNINKA et al., 2006).

(1) $C_6H_{12}O_6 + 2\ NAD^+ + 2ADP + 2P_i \rightarrow 2\ NADH + 2\ \text{piruvato} + 2\ ATP + 2\ H_2O + 2\ H^+$

A produção do acetato a partir de 1 mol de glicose origina duas moléculas de piruvato, possui um balanço líquido de dois moles de acetato, dois moles de CO_2 e 4 moles de H_2 (2). O CO_2 e o H_2 são metabolizados a CH_4 por microrganismos do gênero Archaea (3) (RUSSELL; WALLACE, 1997).

(2) $C_6H_{12}O_6 \rightarrow C_2H_4O_2 + 2CO_2 + 4H_2$

(3) $CO_2 + 4H_2 = CH4 + 2H_2O$

A produção do butirato, terceiro AGV mais abundante no rúmen, tem como resultado da equação química, uma molécula de butirato, 1,5 moles de CO_2 e 0,5 mol de CH_4 por mol de glicose (4) (SAGE; MONSON, 1999). O propionato, segundo AGV mais abundante e altamente responsivo à pressão de H_2 no rúmen é originário também da quebra da glicose em piruvato, e pode seguir por duas rotas metabólicas distintas, a via do acrilato ou succionato (NIU et al., 2018). O balanço líquido resultante é duas moléculas de propionato e dois oxigênios por mol de glicose (5), não contribuindo este para emissão de CH_4.

(4) $C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2CO_2 + 2H_2$

(5) $C_6H_{12}O_6 \rightarrow 2C_3H_6O_2 + 2\ [O]$

Wolin, (1979) em seu livro “The rumen fermentation: a model for microbial interactions in anaerobic ecosystems” propôs uma equação química universal para fermentação da glicose ocorrendo em condições normais de alimentação a pasto, produzindo a partir de 57,5 moles de glicose, 65 moles de acetato, 20 moles de propionato, 15 moles de butirato, 35 moles de CH_4, 60 moles de dióxido de carbono e 25 moles de água (6).

(6) $57.5\ C_6H_{12}O_6 \rightarrow 65\ C_2H_4O_2 + 20\ C_4H_8O_2 + 15C_3H_6O_2 + 35\ CH4 + 60\ CO_2 + 25\ H_2O$

As relações acetato:propionato possuem correlação direta com o substrato ingerido pelo animal. Quando alterou-se a relação volumoso:concentrado de 68:32 para 39:61 houve uma relação de acetato:propionato de 4,09 para 2,41 (OLIJHOEK et al., 2018). Para os mesmos autores, a redução de CH_4 ocorreu de 22,8 para 16,6 g/kg MS ingerida para as dietas respectivas, sendo a emissão de CH_4 reduzida quando dietas ricas em grãos (amido) propiciam a utilização do H_2 para produção da molécula de AGV mais reduzida, o propionato. Isso resulta em menos H_2 disponível para a metanogênese. Portanto, a equação 6 citada acima é apenas uma demonstração hipotética de uma dieta a base de pasto, alterando-se a todo instante em função das características da dieta e padrões de consumo.

Várias reações podem competir com os substratos para metanogênese, como as realizadas por bactérias redutoras de sulfato e nitratos que são aceptores de íons de H_2, podendo em determinadas dietas e condições reduzir a emissão de CH_4 (MACFARLANE, 1993). A biohidrogenação que ocorre com os ácidos graxos poli-insaturados (PUFAs) no rúmem é outra rota que compete diretamente com a emissão de CH_4. Os PUFAs são tóxicos aos microrganismos ruminais, principalmente os gram-positivos, os quais são os maiores produtores de acetato e H_2, favorecendo a rota do propionato que é menos sensível aos PUFAs. Portanto, as mudanças das rotas e a utilização dos átomos de H_2 para saturar as ligações duplas dos PUFAs diminuem a emissão de CH_4 (GIGER-REVERDIN; MORAND-FEHR; TRAN, 2003;JENKINS et al., 2008).

Em um dos consolidados trabalhos sobre CH_4 de Johnson e Johnson, (1995) relatou-se que a perda de energia entre os processos de oxidação e redução pode ficar entre os intervalos de 2 e 12% da energia bruta ingerida pelos animais. As ativações das rotas dos AGV não são definidas geneticamente, mas sim por condições termodinâmicas, que se alteram em função do tipo de substrato ingerido e determinam o tempo de retenção do alimento. Várias cepas de microrganismos possuem capacidade de produzir acetato e/ou propionato, o que varia principalmente com a pressão de H_2 existente no interior da câmara fermentativa, variando de 0,1 a 50 µM (JANSSEN, 2010).

Tabela 2. Taxa de passagem, produção de metano, relação acetato:propionato, concentração de H_2 e pH do ambiente ruminal de animais recebendo alimentos de alta e baixa qualidade.

	Baixa degradabilidade do alimento e/ou longo tempo após a alimentação	Alta degradabilidade do alimento e/ou logo após a alimentação
Taxa de passagem dos sólidos	Baixa	Alta
Produção de CH_4 [1]	Alta	Baixa
Relação: Acetato: Propionato	Alta	Baixa
Concentração de H_2	Baixa	Alta
pH	Alto	Baixo

Fonte: JANSSEN, 2010. [1] Por unidade de energia no alimento.

Finalmente, pode-se dizer que a metanogênese é alterada à medida que se alteram as condições ruminais. Dessa forma, o conhecimento de como manipular a fermentação ruminal é fundamental para adotar estratégias alimentares que reduzam os impactos ambientais e as perdas de energia através da formação do gás CH_4.

2.5. BALANCO ENERGÉTICO E REPRODUÇÃO DE VACAS LEITEIRAS

A atividade leiteira depende da capacidade reprodutiva dos animais para dar início a secreção láctea bem como para produção de novilhas e consequentemente reposição de animais ao plantel. Nas últimas décadas, as vacas aumentaram significativamente a produção de leite devido a seleção genética, o que foi acompanhado de maior intensificação do manejo. Entretanto, o desempenho reprodutivo dos animais diminuiu e passou a ser um desafio maior nas propriedades leiteiras (MIGLIOR; MUIR; VAN DOORMAAL, 2005; ROYAL et al., 2000).

2.5.1. Balanço energético no terço inicial da lactação

A produção de leite está diretamente relacionada a controles endócrinos e metabólicos, os quais estão associados aos eventos reprodutivos que precisam estar em equilíbrio com o BE principalmente no início da lactação para que os animais consigam conceber e dar início ao próximo ciclo de lactação (SANTOS; BISINOTTO; RIBEIRO, 2016). Dentre os nutrientes demandados pelas vacas leiteiras, a energia é sem dúvidas o mais exigido para garantir o desempenho produtivo e reprodutivo. A maior proporção da energia ingerida é necessária para produção e mantença dos animais, mas também é utilizada para síntese e secreção de hormônios reprodutivos, crescimento folicular e manutenção do início do desenvolvimento embrionário que ocorre ou deveria ocorrer no primeiro terço de lactação para se alcançar bons índices zootécnicos. Logo, sabe-se que o '*status*' metabólico e endócrino do animal é dependente do BE, o qual é capaz de alterar a capacidade reprodutiva das vacas leiteiras. Os principais problemas decorrentes de um BEN no início da lactação, e que geram perdas econômicas são: atraso na involução uterina, atraso para início da atividade reprodutiva, redução das taxas de prenhez, aumento do intervalo entre partos, redução da produção de leite, maior uso de drogas e hormônios e redução do número de proles nascidas (WATHES, 2012).

O BE tem um importante papel na reprodução de vacas leiteiras sendo essas relações bem estabelecidas e demostrando que o *status* de subnutrição no período de transição é a chave de alguns problemas (BACH, 2019). As exigências nutricionais das vacas leiteiras mudam de acordo com o estágio de lactação e/ou gestação, mas são superiores durante o início da lactação. O *status* nutricional de vacas no período pré e pós-parto influencia subsequentemente no desempenho produtivo e reprodutivo. Em particular, o BE e proteico durante o início da lactação são fatores que estão associados e podem implicar em baixa fertilidade de vacas leiteiras. Vacas leiteiras que não possuem saúde no período de transição têm direta ou indiretamente (via BEN) seu desempenho reprodutivo afetado negativamente (GALVÃO et al., 2010a). A ingestão inadequada de nutrientes em relação à demanda metabólica é o maior fator contribuinte para prolongar o anestro pós-parto, o que ocorre particularmente para vacas em pastagens de baixo valor nutritivo ou dietas não balanceadas para atender as demandas metabólicas (BUTLER, 2003). De acordo com Santos et al. (2016) o início da atividade ovariana em vacas de alta produção é determinada pelo *status* energético do animal. A inadequada ingestão de energia ou proteína durante a prenhez ou início da lactação resulta em baixo escore de condição corporal ECC no parto e maior intervalo entre partos (PATTON et al., 2007).

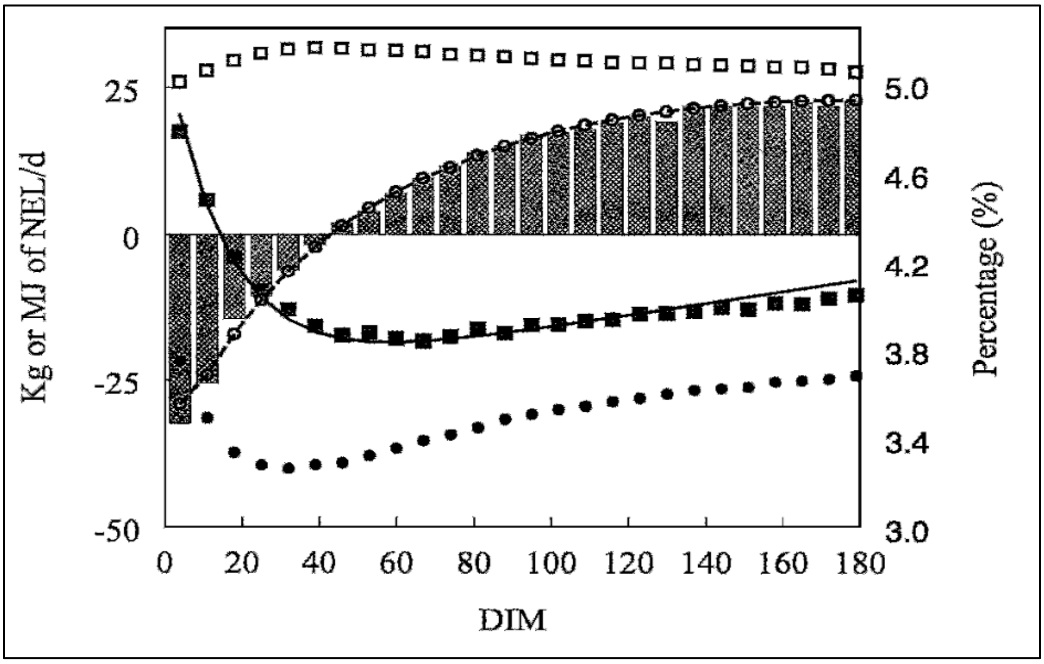
Preconiza-se que o manejo alimentar deve ser priorizado para minimizar perda de condição corporal durante o período pós-parto imediato e reduzir a incidência de desordens metabólicas durante começo da lactação, aumentando o número de vacas com a primeira ovulação durante 4 a 6 semanas pós-parto (BELL, 2008; SANTOS; RUTIGLIANO, 2009). Em vacas de alto mérito genético, a IMS e o BE começam a diminuir no período pré-parto, o que resulta em mobilização de tecido adiposo e acréscimo de ácidos graxos não esterificados (AGNE) e beta-hidroxibutirato (BHB) na corrente sanguínea (BUTLER; PELTON; BUTLER, 2006).

O início da lactação, é o momento em que ocorre evidente e abrupto aumento da produção de leite, enquanto a ingestão de alimento encontra-se atrasada ocasionando uma instabilidade no '*status* metabólico' (a produção de leite aumenta em taxas superiores à ingestão de matéria seca). Dessa forma, a demanda energética diária é superior a quantidade de energia ingerida. Sendo assim, animais de alto mérito genético para produção de leite passam a mobilizar reservas corporais e perdem peso (BEN). A conhecida capacidade limitada para ingerir a quantidade de energia demandada diariamente no início da lactação faz com que o processo de mobilização de gordura seja um processo 'normalizado' para vacas de alto mérito genético. Vacas de maior potencial genético para produção de leite irão mobilizar mais gordura por um período mais longo que vacas de mérito genético inferior. Normalmente o BEN alcança um mínimo de 80% do total de vacas do rebanho no início da lactação e pode resultar em perdas de até 0,7 kg/d (PATTON et al., 2007). A magnitude e a duração do BEN são mais dependentes da ingestão de alimento do que da produção de leite, ocorrendo os picos de produção de leite entre 30 e 90 dias e de IMS entre 70 e 140 dias (BEDERE et al., 2018).

É possível observar as alterações que ocorrem no desempenho produtivo das vacas em BEN no início da lactação, devido a redução da IMS e aumento da produção de leite (Figura 3) como aumento dos teores de gordura, redução dos teores de proteína e consequentemente alteração da relação gordura:proteína o leite (PATTON et al., 2007).

O período de transição foi definido por Drackley (1999) como aquele compreendido entre as três semanas que antecedem o parto e as três semanas após o parto, e é sem dúvidas a fase mais crítica na lactação das vacas. Uma vez que o BE é definido basicamente como a diferença entre a produção de leite e a IMS, sendo que a produção de leite é uma resposta do animal, e os manejos que permitem aumentar a IMS são as principais ferramentas para melhorar o seu *status* energético no início da lactação. Vacas leiteiras que tem maior IMS no início da lactação reduzem a intensidade e duração do BEN com impacto positivo no desempenho reprodutivo (BUTLER, 2003).

Figura 03. Balanço energético, produção de leite e composição do leite para os primeiros 180 dias de lactação (DIM). Balanço energético (círculo não preenchido), produção de leite (quadrado não preenchido), percentagem de gordura (quadrado preenchido) e percentagem de proteína (círculo preenchido).



Adaptado de (DE VRIES; VEERKAMP, 2000)

2.5.2. Balanço energético no terço médio e final da lactação

O terço médio e final de lactação corresponde ao período de 100 dias pós-parto até o dia da secagem e é acompanhado por declínio progressivo na produção de leite diária. No terço médio as vacas já alcançaram a máxima produção bem como o pico de ingestão de matéria seca, não havendo mais perdas de peso quando alimentadas com RTM. Nesta fase, as vacas leiteiras devem estar prenhes para atingirem intervalos de parto não superiores a 365 dias (um ano). O terço final de lactação, que dura até o momento em que os animais entram no período seco, caracteriza-se pela diminuição da produção leiteira além da redução da IMS. Entretanto, a vaca continua a ganhar peso nesse período para repor o tecido adiposo que foi mobilizado no início da lactação (PIÑEIRO et al., 2019).

Alguns países e/ou propriedades que adotam estratégias nutricionais como a retirada dos animais dos estábulos e de dietas baseadas em RTM para o fornecimento de pasto aceitam períodos de BEN no terço médio lactação desde que a falta de energia para as exigências nutricionais não exceda mais que 10%, pois neste período os animais apresentam capacidade de regulação homeostática reduzindo impactos negativos do BEN (GROSS et al., 2011).

Estudos anteriores observam que vacas com deficiência de energia no meio de lactação são capazes de recuperar sua condição corporal no terço final de lactação e primeiras quatro semanas do período seco sem qualquer efeito negativo no desempenho produtivo e reprodutivo durante as próximas lactações (ROCHE et al., 2017; ROCHE; BERRY; KOLVER, 2006). Além disso, quando o fornecimento de energia não é inferior a 90% das necessidades, há poucas possibilidades de ocorrerem distúrbios metabólicos (OVERTON; WALDRON, 2004).

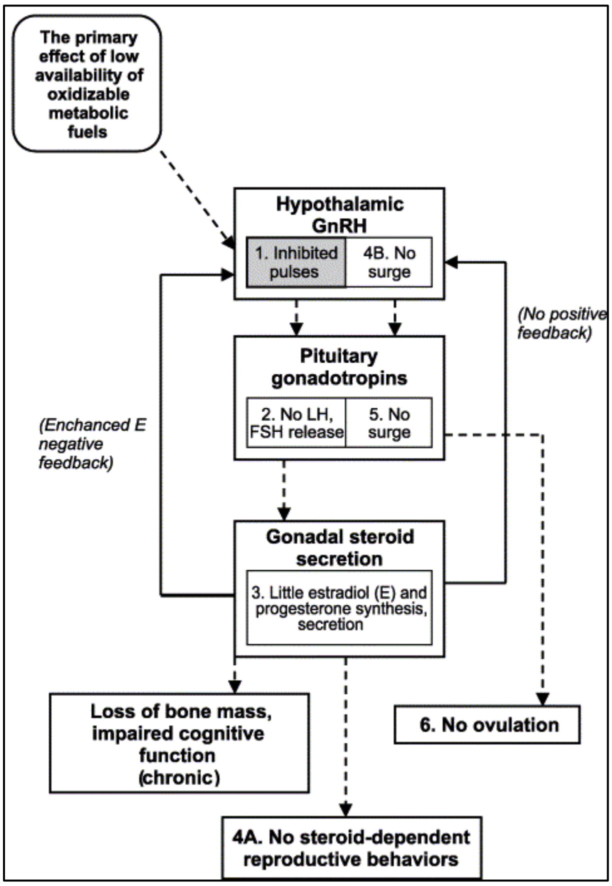
2.5.3. Fatores relacionados ao equilíbrio de energia que afetam a retomada da atividade ovariana em vacas no período de transição

O período de transição, onde ocorre o crescimento final do feto e inicia a produção de leite, também está associado a fatores estressantes, como, a separação do bezerro, desafios imunológicos e exposição a um novo regime alimentar com reagrupamento hierárquico do rebanho (LEBLANC, 2010; WALSH et al., 2007). Esses fatores, contribuintes para o BEN, interferem de maneira indireta no retorno à atividade cíclica dos animais (INGVARTSEN, 2006).

Durante a fase final de gestação os níveis de hormônios esteróides (estrógeno e progesterona) diminuem a liberação de gonadotrofinas hipofisárias (hormônio luteinizante (LH) e hormônio folículo estimulante (FSH)) e consequentemente a atividade folicular ovariana. Com o evento do parto os hormônios esteróides são reduzidos e a secreção dos pulsos de LH e FSH restabelece-se para estimular o retorno à atividade cíclica (BUTLER, 2003). O BEN durante as três semanas seguintes ao parto retarda a ovulação precoce e a recuperação da função reprodutiva pós-parto, tornando-se o principal *link* entre nutrição e fertilidade em vacas leiteiras. Durante o período de BEN as vacas leiteiras apresentam maiores concentrações de hormônio do crescimento (GH) e menores concentrações de AGNE e beta-hidroxibutirato (BHB) ao passo que as concentrações do fator de crescimento semelhante à insulina-I (IGF-I), glicose e insulina estão baixas (KARIS et al., 2020). As mudanças nos metabólitos e hormônios na circulação sanguínea podem comprometer a função ovariana e a fertilidade das vacas leiteiras. Durante o BEN o fígado deve suportar a carga metabólica crescente para sustentar os níveis de glicose e oxidação de ácidos graxos. Quando as vacas leiteiras de alto desempenho mobilizam as reservas corporais em excesso pode ocorrer o processo de infiltração de gordura no fígado, além de comprometer direta ou indiretamente a reprodução (BUTLER; SMITH, 1989). O fígado possui papel fundamental como local onde ocorre o metabolismo dos AGNE e BHB, além de atuar como fonte primária de IGF-I que estimula o desenvolvimento de folículos ovarianos.

A regulação da atividade cíclica está principalmente sob o controle do eixo hipotálamo-hipófise-ovário. Em uma extremidade deste eixo há a influência da quantidade de energia disponível para oxidação (glicose), a qual interfere diretamente nos pulsos de LH e FSH (Figura 04) e indiretamente na ovulação e manifestação do cio (SCHNEIDER, 2004). Segundo Galvão et al. (2010b), as interações e respostas do BEN são capazes de mudar o curso da atividade ovariana pós-parto influenciando fortemente a retomada aos ciclos estrais, pois o BEN age através de suas repostas de baixa concentração de glicose e insulina, elevadas concentrações de AGNE e BHB e acúmulos de triglicerídeos no fígado. Isso atrasa a ocorrência de aumentos nos pulsos de LH e FSH, os quais são necessários para estimular o desenvolvimento dos folículos que irão secretar estrógeno responsável por manifestar o comportamento do cio e a ovulação folicular possibilitando a fecundação posterior.

Figura 04. Fluxograma do efeito do déficit energético do início da lactação no início da atividade reprodutiva de vacas leiteira.



Adaptado (SCHNEIDER, 2004).

Vacas leiteiras que tem menores concentrações de glicose sanguínea têm também concentrações de progesterona reduzidas no plasma (VASCONCELOS et al., 2003). Além de baixas concentrações de insulina no sangue são responsáveis pela baixa produção de IGF-I pelo fígado (BUTLER et al., 2008; BUTLER, 2003). Estes fatores associados reduzem a capacidade de resposta dos folículos ovarianos às gonadotrofinas. O sinais oriundos de metabólitos e de gonadotrofina que são capazes de controlar o desenvolvimento folicular inicial estão inter-relacionados: o FSH estimula as células da granulosa nos folículos a desenvolverem receptores para insulina, hormônios do crescimento e IGF-I; a insulina e o IGF-I fornece o estimulo hormonal para o desenvolvimento completo dos folículos ovarianos pré-ovulatórios (SHIMIZU et al., 2008).

Entretanto, o grau de BEN altera diretamente a frequência de pulsos de LH durante na primeira onda folicular definindo se o mesmo é suficiente para estimular a alta produção de estradiol para ovulação (BEAM; BUTLER, 1999). O LH é um hormônio fundamental pelo fato de ser necessário para o restabelecimento da atividade ovariana, crescimento final e maturação do folículo ovulatório, ovulação, luteinização e secreção ovariana de progesterona (BUTLER et al., 2008). A diferença entre um folículo ovulatório para um não ovulatório resume-se na capacidade do primeiro produzir grandes quantidades de estrógeno e assim ser capaz de estimular os receptores no sistema nervoso central (SNC) para exteriorizar o comportamento do cio, bem como a ovulação do folículo.

Butler et. al. (2006) e Patton et al. (2007) relataram, em estudos que monitoraram vacas quanto ao seu desenvolvimento folicular após o parto, que a ovulação depende da IMS pré-parto, do BE, do perfil hormonal e da concentração de metabólitos sanguíneos. Ou seja, as vacas que comem menos, aumentam o BEN e as concentrações de AGNE e BHB, diminuindo os níveis de estradiol e a ocorrência de ovulação. Além dos efeitos em reduzir a proporção de vacas que não ovularam, as concentrações plasmáticas de progesterona estavam menores para aquelas que conseguiram ovular, interferindo no crescimento folicular do próximo ciclo estral ou na manutenção da gestação (VASCONCELOS et al., 2003).

O escore de condição corporal (ECC) é uma medida indireta do atual '*status*' energético da vaca leiteira, bem como é utilizado para conhecer a intensidade de remoção das reservas corporais entre os períodos pré- e pós-parto. Vacas que chegam no pré-parto com ECC superior a 3,5 (escala de 1 a 5 (EDMONSON et al., 1989)) ou perdem mais que uma condição de escore corporal (aprox. 8 a 10% do peso vivo) são animais com predisposição a reduzirem a IMS devido a teoria da oxidação hepática, tendo consequentemente a fertilidade reduzida (ROCHE et al., 2009).

Sabe-se que IMS e produção de leite são os dois principais 'drivers' do BE, entretanto, ainda não existem evidências concretas de qual desses fatores têm maior influência. BE. Segundo Villa-Godoy et al. (1988) a IMS parece ter o maior impacto sobre o '*status*' energético de vacas leiteiras no pós-parto que a produção de leite. Patton et al. (2007) indentificou que o BE durante o início da lactação está diretamente corelacionado à IMS e que os mesmos são capazes de alterar a performance reprodutiva. Vacas primíparas além da produção de leite e da IMS tem seu '*status*' energético afetado negativamente pela necessidade energética para concluir seu crescimento e chegar ao peso adulto (MACMILLAN et al., 2018; WATHES et al., 2007). Na última década cresceram os estudos demostrando o impacto do BEN no estresse oxidativo sobre a saúde e função imune dos animais, bem como seu impacto direto na fertilidade de vacas leiteiras.

2.5.4. Estratégias para melhorar o balanço energético de vacas leiteiras

Assumindo-se que o '*status*' de BEN é inerente a fisiologia de vacas de alto mérito leiteiro, discute-se assim, quais estratégias podem ser aplicadas para reduzir seus impactos negativos na saúde e fertilidade dessas vacas. Manejos aliados à maior capacidade de ingestão de energia diária (*input)* estão em vias preferenciais do que a manipulação da produção ou composição leiteira (*output)*. Manter a IMS no pré-parto e realizar manejos para acelerar o pico de IMS no pós-parto são as principais estratégias preconizadas para reduzir a intensidade e duração do BEN, minimizando os efeitos negativos sobre a função ovariana e hepática (PATTON et al., 2007). A IMS é primariamente afetada pela disponibilidade de alimento para o animal, sendo indispensável a oferta continua de dietas balanceadas (ALLEN, 2000). A utilização de corretas proporções de volumoso:concentrado, aditivos e suplementos energéticos em uma RTM associados a condições ambientais ideias, são capazes de garantir o melhor balanceamento possível de nutrientes além da sua máxima ingestão diária.

A ingestão adequada de nutrientes em vacas peri-parturientes reduz as alterações metabólicas decorrentes do BEN em vacas de alta produção sendo capaz de reduzir os efeitos prejudicias a reproducão dessas vacas. Considerando que o BEN durante o início da lactação está relacionado à diminuição da IMS pré e pós-parto, seria fundamental maximizar consumo de nutrientes para reduzir os efeitos deletérios do BEN na recuperação da atividade reprodutiva de vacas leiteiras.

3. QUESTÕES E ESTRATÉGIA DE PESQUISA

As relações entre a utilização de RTM e/ou dietas mistas (RTM + pasto) quanto ao desempenho produtivo e emissão de CH_4, além da relação do BE de vacas leiteiras com o desempenho produtivo e reprodutivo foram abordados anteriormente. As respostas de emissão (g/d), produção (g/kg MS ingerida) e intensidade de emissão de CH_4 (g/kg leite) para animais ingerindo RTM e/ou pasto de clima temperado são relativamente conhecidas quando comparadas com animais que recebem dietas mistas com a inclusão de pasto de clima tropical.

O sul do Brasil encontra-se em uma faixa de latitudes onde plantas de clima temperado e tropical desenvolvem-se nas estações fria e quente do ano, respectivamente. Desta maneira, discussões sobre como estratégias alimentares podem maximizar o desempenho, reduzindo a utilização de insumos e o impacto ambiental merecem estudos adicionais.

Além disso, a eficiência reprodutiva do rebanho no início ou reinício de uma lactação ocorre em consequência de um conjunto de alterações hormonais e metabólicas envolvidas no parto das vacas leiteiras. Assim sendo, o efeito de uma lactação sobre o BE e o seu impacto na atividade reprodutiva estão relacionados ao desempenho de vacas leiteiras.

Esta tese quantificou o efeito de estratégias alimentares que visam a inclusão de pastos de clima tropical e temperado sobre o desempenho produtivo de vacas leiteiras no meio da lactação, além da emissão de CH_4 entérico nos animais submetidos às diferentes estratégias alimentares durante a estação quente do ano. Junto a essa proposta, procurou-se identificar os impactos de diferentes intensidades de BE no pós-parto sobre o desempenho reprodutivo do rebanho.

Desta maneira, a tese foi dividida em três estudos:

I. Avaliação da utilização de três estratégias alimentares em vacas leiteiras no ciclo de gramíneas de estação quente. Foram avaliados o consumo, o desempenho produtivo, o BE e a emissão de CH_4 entérico. Como se alteram o consumo, a produção e a composição do leite, bem como as emissões de CH_4 com a inclusão progressiva de pasto nessa época do ano foram as principais perguntas a serem respondidas.

II. Avaliação da utilização de três estratégias alimentares em vacas leiteiras no ciclo de gramíneas de estação fria. Foram avaliados o consumo e o desempenho produtivo de vacas leiteiras. Como se alteram o consumo, a produção e a composição de leite com a inclusão progressiva de azevém anual tetraplóide para vacas leiteiras consumindo dieta misturada foram as principais perguntas a serem respondidas.

III. Avaliação de um grande banco de dados dos últimos 20 anos para examinar as relações entre o BE no início da lactação com a concentração de metabolitos sanguíneos, o retorno da atividade reprodutiva e o desempenho reprodutivo de primíparas e multíparas. Procurou-se responder quais as relações e impactos existem entre a intensidade do BE no início do período pós-parto com o retorno da atividade reprodutiva e as taxas de concepção na primeira inseminação.

O primeiro experimento foi conduzido em Lages, SC, Brasil, nos meses de janeiro, fevereiro e março de 2018. Foram utilizadas vacas com DEL de 136 dias e produção diária média de 25 kg de leite, as quais foram submetidas a três estratégias nutricionais: vacas consumindo 100% de RTM, 75% do consumo *ad libitum* de RTM + acesso a pasto de clima tropical (capim milheto) 7 h/d (das 8:30 h às 15:30 h) e 50% do consumo *ad libitum* de RTM + acesso a pasto de clima tropical (capim milheto) 7 h/d (das 8:30 h às 15:30 h). Utilizou-se a técnica de N-alcanos para estimativa do consumo de pasto e a diferença entre o oferecido menos sobras da RTM para estimar o consumo de RTM. Para medidas das emissões de CH_4 entérico utilizou-se a técnica do hexafluoreto de enxofre. A utilização de estratégias alimentares com inclusão de uma gramínea tropical (capim milheto) ocorreu para suprir uma carência de trabalhos na literatura que permitam identificar seus impactos no desempenho animal e emissão de CH_4 durante a estação quente em regiões subtropicais. Assim, torna-se possível através da interpretação das respostas propor estratégias alimentares que possibilitem a redução do uso de insumos externos, mantendo o desempenho produtivo sem aumentar a intensidade de emissão de CH_4, além de contribuir para a ampliação do banco de dados relativo à emissão de GEE em países de clima tropical e subtropical.

O segundo experimento também foi conduzido em Lages, SC, Brasil, nos meses de junho, julho e agosto de 2018. Foram utilizadas vacas com DEL de 129 dias e produção diária média de 26 kg de leite, as quais foram submetidas às mesmas estratégias nutricionais do primeiro experimento, com exceção que ao invés de capim de capim milheto o pasto utilizado foi o azevém anual. O consumo de forragem foi estimado pela diferença entre a biomassa pré-e pós-pastejo, e o consumo de RTM foi medido pela diferença entre a quantidade oferecida menos as sobras. A utilização de estratégias alimentares com a inclusão de pasto anual de clima temperado ocorreu para entender o efeito da inclusão do pasto na dieta de vacas recebendo RTM sobre o consumo de MS total quando o objetivo de manejo é um pastejo leniente (remoção de 50% da altura de entrada), mas com baixa massa de forragem por hectare (característica comum nos primeiros ciclos de pastejo do azevém anual). Dada a importância do consumo na vida produtiva das vacas leiteiras, as respostas tornam-se importantes para avaliar os efeitos da suplementação com pasto anual inverno na produção e composição do leite, bem como no BE de vacas no terço médio de lactação.

O terceiro estudo foi conduzido no Agri-food Biosciense Institut – AFBI em Hillsborough – Irlanda do Norte durante os meses de janeiro à junho de 2020. Avaliou-se as relações entre BE diário no início do pós parto com o desempenho produtivo, a concentração de metabólitos sanguíneos e o desempenho reprodutivo de 1508 vacas (488 primíparas e 1020 multíparas) leiteiras que participaram de 79 tratamentos em 27 experimentos durante os anos de 1996 a 2016 (20 anos). As primíparas e multíparas foram divididas inicialmente devido a necessidade energética que primíparas possuem para completar a fase de crescimento. Ambos os grupos foram divididos em quartis de acordo com o BE para que fosse possível identificar as relações/respostas em função da intensidade de BE negativo que esses animais se encontravam. Este estudo visa através da avaliação diária de um grande número de informações e animais diminuir as especulações que existem sobre o tema, onde em muitos casos essas relações são apresentadas a partir de medidas indiretas do BE dos animais.

4. OBJETIVOS

Avaliar o consumo e o desempenho produtivo de animais recebendo RTM com ou sem acesso a pastos anuais de clima tropical ou temperado, estimando as emissões de CH_4 entérico dos animais recebendo RTM com ou sem acesso a pastos de clima tropical.

Avaliar as relações que ocorrem entre o BE no início da lactação com o desempenho produtivo, a concentração de metabolitos sanguíneos e o desempenho reprodutivo.

5. HIPÓTESES

Quando o fornecimento de RTM é de no mínimo 50% do consumo *ad libitum* o acesso a pastos de clima temperado permite a manutenção do consumo total de MS e da produção de leite em função da elevada qualidade da forragem.

Nas condições citadas acima, o acesso a pastos de clima tropical diminui o consumo, a resposta produtiva e a emissão de CH_4 (g/d), mas a produção de CH_4 (g/kg MS) e a intensidade de emissão (g/kg leite) aumentam em função da elevação nos teores de fibra em detergente neutro (FDN) na matéria orgânica ingerida.

Vacas que sofrem maiores intensidades de BEN necessitam maior período de tempo para iniciar a atividade reprodutiva pós-parto e tem menores taxas de concepção.

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6. PROGRESSIVE INCLUSION OF PEARL MILLET HERBAGE AS A SUPPLEMENT FOR DAIRY COWS FED MIXED RATIONS: EFFECTS ON METHANE EMISSIONS, DRY MATTER INTAKE AND MILK PRODUCTION[1]

6.1. ABSTRACT

The inclusion of grazing in dairy feeding systems can improve animal welfare and reduce feed costs and labor for animal care and manure management. This work aimed to evaluate the effects of including pearl millet herbage (*Pennisetum glaucum* 'Campeiro') as a supplement for dairy cows fed total mixed rations (**TMR**). The treatments included 100% TMR offered *ad libitum* (control, **TMR_{100}**), 75% TMR *ad libitum* intake + access to grazing of a pearl millet pasture between the morning and afternoon milkings (7 h/d) (**$pTMR_{75}$**), and 50% TMR *ad libitum* intake + access to grazing of a pearl millet pasture between the morning and afternoon milkings (7 h/d) (**$pTMR_{50}$**). Nine multiparous Holstein and F1 Jersey × Holstein cows were distributed in a replicated 3×3 Latin square design with 3 periods of 21 d (a 16-d adaptation period and a 5-d measurement period). Cows in the TMR_{75} and TMR_{50} groups strip-grazed a pearl millet pasture with pre- and postgrazing sward height targets of 60 and 30 cm, respectively. The herbage dry matter intake (**DMI**) increased with decreasing mixed ration supplies, while the total DMI decreased linearly from 19.0 kg/d in the TMR_{100} group to 18.0 kg/d in the $pTMR_{50}$ group. Milk production decreased linearly from 24.0 kg/d in the TMR_{100} group to 22.4 kg/d in the $pTMR_{50}$ group, and energy-corrected milk (**ECM**) production decreased linearly from 26.0 kg/d to 23.6 kg/d. Enteric methane (**CH_4**) emissions decreased linearly from 540 g/d in the TMR_{100} group to 436 g/d in the $pTMR_{50}$ group, while CH_4 yields (g/kg DMI) tended to decrease linearly. The CH_4 intensity was similar between treatments, averaging 20 g CH_4/kg ECM. The inclusion of pearl millet herbage in the dairy cow diets decreased the total DMI and milk production to a small extent without affecting CH_4 intensity (g/kg ECM).

Keywords: dairy cow, grazing, *Pennisetum glaucum*, methane

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[1] Este capítulo apresenta o artigo publicado no periódico Journal of Dairy Science (doi: 10.3168/jds.2020-18894), seguindo as normas da revista.

6.2. INTRODUCTION

The use of grazing in dairy production systems can improve animal welfare and reduce health problems (Arnott et al., 2017) as well as reduce labor for animal care and manure management (White et al., 2002; Schingoethe, 2017). Grazing can also decrease feeding costs and improve income-over-feed costs (Soriano et al., 2001; White et al., 2002). In contrast, pastures alone are rarely able to meet the energy requirements of lactating dairy cows (Kolver and Muller, 1998; Delaby et al., 2001; O'Neill et al., 2011) and do not provide a constant herbage supply throughout the year (Wilkinson et al., 2019). Thus, mixed feeding systems involving grazing pastures and TMR have been proposed and used worldwide (Wales et al., 2013).

Investigations of dairy cow systems where cows are both grazing on temperate pastures and receiving a mixed ration have in some studies not affect total DMI and milk production (Vibart et al., 2008; Mendoza et al., 2016), and in other studies total DMI and milk production had been reduced up to 16% (Soriano et al., 2001; Bargo et al., 2002; White et al., 2002). Reductions in milk production have been observed in high-production cows (Bargo et al., 2002) and when the proportion of TMR is relatively low (Vibart et al., 2008; Mendoza et al., 2016). Relatively large reductions in DMI and milk production have also been observed when the quality of herbage decreases according to the season (White et al., 2002; Vibart et al., 2008). However, compared with investigations done on cows grazing temperate pastures, studies assessing the effect of including tropical herbage on diets of dairy cows receiving mixed rations are rare.

The effects of combining temperate pastures with mixed rations on enteric methane (**CH_4**) emissions have shown that CH_4 yields (g/kg DMI) may be lower in dairy cows exclusively grazing a high-quality perennial grass in the spring than in dairy cows receiving a TMR diet (O'Neill et al., 2011). Therefore, CH_4 intensity (g/kg energy-corrected milk (**ECM**)) did not change in cows exclusively grazing on a temperate pasture or receiving TMR supplementation, which was explained by the similarity between herbage and TMR quality (O'Neill et al., 2012). Additionally, CH_4 intensity decreased in cows receiving a mixture of corn silage and soybean meal with the inclusion of an annual temperate pasture because of the improved quality of herbage compared with that of corn silage (Dall-Orsoletta et al., 2016). However, the quality of herbage may be relatively low in tropical pastures such as those of pearl millet (*Pennisetum glaucum*), which has shown great potential for use in dairy cows;

compared with other tropical pastures, pearl millet pastures have been shown to increase DM yields from 10 to 20 t/ha and have both a high protein content and high digestibility (Assis et al., 2018). Thus, the effects of including pearl millet pasture on CH_4 emissions from dairy cows receiving TMR warrant further study.

We hypothesized that, due to greater herbage and diet NDF content, a progressive inclusion of pear millet in dairy cow diets would decrease total DMI, milk production and CH_4 emissions (g/d) but CH_4 yields (g/kg DMI) and CH_4 intensity (g/kg ECM) would increase. The aim of this work was to quantify the effects of partial TMR replacement by pearl millet on CH_4 emissions and production responses in dairy cows.

6.3. MATERIALS AND METHODS

The Ethics Committee of the University of Santa Catarina State (Brazil) approved all the procedures in this study, under protocol number 4373090816.

6.3.1. Treatments, Experimental Design, and Animals

The experiment was conducted according to a 3×3 Latin square design replicated three times. Nine multiparous cows were divided into three homogeneous groups (squares) of three animals, having one Holstein and two Holstein × Jersey cows as similar as possible in terms of milk production. Each square was then assigned a different treatment sequence. The following variables were determined during the week before the beginning of the experiment (means ± standard deviations): milk production (25.1 ± 3.93 kg/d), DIM (136 ± 40 d), BW (533 ± 41.7 kg) and number of lactations (2.6 ± 0.69). Each experimental period lasted 21 d, with a 16-d adaptation period and a 5-d measurement period.

The treatments were as follows: 100% TMR (**TMR_{100}**) offered *ad libitum* (control), 75% TMR *ad libitum* intake + access to grazing of a pearl millet pasture (**$pTMR_{75}$**) and 50% TMR *ad libitum* intake + access to grazing of a pearl millet pasture (**$pTMR_{50}$**). All treatments received the same mixed ration, which was balanced after chemical analysis of the ingredients to meet the net energy and metabolizable protein (**PDI**) requirements of the control treatment, according to equations developed by the INRA (2007). The TMR was composed of corn silage and a concentrate (60:40 ratio on a DM basis). The ingredients, chemical composition and nutritive value of the TMR are presented in Table 1.

Table 1. Chemical composition and nutritive value of mixed rations offered to dairy cows.[1]

Item	Content
Ingredients, g/kg DM	
Corn silage	600
Ground corn	260
Soybean meal	140
Chemical composition, g/kg DM	
DM, g/kg fresh	380
OM	962
CP	149
NDF	340
ADF	173
Nutritive value[2]	
GE, MJ/kg DM	18.7
OM digestibility	0.77
NE_L, MJ/kg DM	7.02
PDIN, g/kg DM	97.8
PDIE, g/kg DM	99.0

[1] Mineral supplement composition available in the feeding area and paddocks (on natural basis): 150 g/kg calcium, 78 g/kg phosphorus; 26 g/kg sulfur, 20 g/kg magnesium, 114 g/kg sodium, 100 mg/kg cobalt, 1500 mg/kg copper, 30 mg/kg chromium, 2000 mg/kg iron, 80 mg/kg iodine, 2300 mg/kg manganese, 30 mg/kg selenium, 5000 mg/kg zinc, and 780 mg/kg fluorine.

[2] Estimated from chemical analysis and via equations proposed by INRA (2007).

PDIN: metabolizable protein when N is limiting for microbial synthesis in the rumen.

PDIE: metabolizable protein when energy is limiting for microbial synthesis in the rumen.

The individual voluntary DM TMR intake was quantified before the experiment started in a 14-d preexperimental period, cows been housed and fed individually. The average DMI of the last 5 d was considered for calculating for each cow the amount of mixed ration to be offered in the $pTMR_{75}$ and $pTMR_{50}$ treatments throughout the experiment. During the experiment, the cows were housed individually where either TMR or pTMR were offered in covered outdoor feeders. The cows in the TMR_{100} group were fed twice a day after the morning and afternoon milkings; these cows received a daily quantity that was 20% greater than the voluntary DMI measured the prior day. In $pTMR_{75}$ and $pTMR_{50}$ treatments, cows received 75 and 50% of their TMR *ad libitum* intake measured during preexperimental period, respectively. They also had access to a pasture between the morning and afternoon milkings (7 h/d of access to the pasture, from 8:00 a.m. to 3:00 p.m.) and received pTMR after the afternoon milking (13 h/d of access to pTMR, from 5:00 p.m. to 6:00 a.m.). TMR and pTMR refusals were individually collected and weighed once a day during the morning milking. Water and mineral supplements (Bovigold®, DSM Tortuga, São Paulo, Brazil) were continuously available in the feeding area and paddocks.

6.3.2. Pasture and Grazing Management

The experiment was performed in Lages, SC, Brazil (50.18°W, 27.47°S; 920 m above sea level), from January 25 to March 29, 2019. An area of two hectares of pearl millet sown in 2017 was used. During the experimental period, the average temperature was 19.6°C, and the cumulative rainfall was 215 mm. The 10-year climatic average temperature and rainfall during the months of the experiment were 14.5°C and 161 mm, respectively. Before the first grazing cycle (after pear millet had developed the third-leaf stage) and after each experimental period, the experimental area was fertilized with 50 kg of N/ha, which was supplied as urea.

The area was divided into two paddocks, with one-third and two-thirds of the surface being assigned to the $pTMR_{75}$ and $pTMR_{50}$ groups, respectively. This size ratio of the surface was chosen because the expected herbage DMI for the $pTMR_{75}$ and $pTMR_{50}$ groups was 25% and 50% of the *ad libitum* DMI, respectively, so the TMR_{75} group was expected to require half the area of the $pTMR_{50}$ group. The paddocks were strip-grazed with pre- and postgrazing sward height targets of 60 and 30 cm, respectively. To achieve these targets, the areas allocated daily to the $pTMR_{75}$ and $pTMR_{50}$ groups were 41 and 82 m^2/cow, respectively, which was defined on the basis of a one-week pre-experimental period. As the actual pre- and postgrazing sward heights throughout the experiment were close to the pre- and postgrazing target heights, no other adjustments for area allocation were necessary. To minimize variations in herbage quality between periods, different areas were used during the last 14 d of each period. These areas were mowed 18 d before starting the measurement periods for controlling of pre-grazing sward height and chemical composition. Grazing management processes aimed to ensure that animals in the $pTMR_{75}$ and $pTMR_{50}$ groups removed the same proportion of forage in relation to the pregrazing height and that this removal did not exceed 50% of the initial height. The aim of 50% was chosen because it is the threshold at which the grazing management and structural characteristics of the herbage at the end of the occupation period can impose restrictions on herbage intake (Zanini et al., 2012; Mezzalira et al., 2013).

6.3.3. Animal Measurements

Individual milk production values were recorded twice daily (at 7:00 a.m. and 4:00 p.m.), and milk samples were collected at each milking via an electronic milk meter (Waikato Milking Systems, New Zealand) approved by the International Committee for Animal Recording (ICAR). The milk composition (fat, milk CP, and MUN concentrations) was individually measured for samples collected during each milking of the last 5 d of each period via infrared spectrophotometry (International Dairy Federation Standard 141C:2000). The ECM production standardized to 4.0% fat and 3.3% protein was calculated according to the equation proposed by Tyrrell and Reid (1965): ECM (kg/d) = milk production kg × (37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948) / 3,138.

The TMR and pTMR intake, as well OM, CP, NDF and ADF intake and diet concentration, were measured as the average difference between the supplied quantity and the remaining quantity from each of the last 5 d of each period, when the DM, OM, CP, NDF and ADF content of offered TMR, pTMR and refusals were measured separately. The individual herbage intake was measured according to the n-alkane technique (Mayes et al., 1986) via the C_{31} (naturally present in the forage):C_{32} (supplied to the animals) ratio. Animals received cellulose pellets (Carl Roth, GmbH, Karlsruhe, Germany) containing 186 mg of C_{32} twice a day after each milking from d 8 to d 21 of each experimental period. During the last 5 d of each experimental period, fecal grab samples were collected from each cow after each milking. The fecal samples were oven dried at 60°C for at least 72 h, composited by period and cow, and then ground (Solab SL-31, Piracicaba, Brazil) to pass through a 1-mm screen for subsequent chemical analyses.

The daily grazing time in the $pTMR_{75}$ and $pTMR_{50}$ groups was measured individually via visual observations every 5 min between 8:00 a.m. and 3:00 p.m. during the last 5 d of each period. The cows were previously accustomed to humans, and the time spent watching each individual animal was no more than 10 s, during which grazing behavior or no grazing was recorded (Penning and Rutter, 2004). No behavior was recorded indoors when the cows were milked or received the TMR. The herbage intake rate (g DM/min) was estimated per cow and period by dividing the average daily herbage intake by the average daily grazing time.

The gross energy (**GE**) of herbage was estimated as proposed by INRA (2018) as GE (kcal/kg OM) = 4531 + 1.731× CP (g/kg OM) – 71 (n = 166, $R^2 = 0.89$), whereas the GE for the mixed ration was calculated from tabulated values for the concentrates and corn silage

(INRA, 2018). The OM digestibility was estimated from the chemical composition of forages according to specific equations for corn silage, herbage and concentrates (INRA, 2018). The NE_L and PDI balances were estimated per cow and period according to the difference between the NE_L and PDI supply and requirements, according to the methods of the INRA (2007). The NE_L requirements were estimated considering BW (kg) and FCM (kg/d), as follows: NE_L requirements (Mcal/d) = $0.080 \times BW^{0.75} + FCM \times 0.7476$. The PDI requirements were estimated taking into account the BW (kg), actual milk production (kg/d) and CP content (g/kg milk), as follows: PDI requirements (g/d) = $3.25 \times BW^{0.75}$ + milk production × (CP × 0.93) × 10. The NE_L and PDI supplies were estimated considering herbage and TMR intakes and their NE_L and PDI contents, respectively.

Daily CH_4 emissions were measured individually according to the sulfur hexafluoride (**SF_6**) tracer gas technique described by Johnson et al. (1994). Each cow received one SF_6 capsule 21 d before beginning the experiment, with an average SF_6 release rate of 3.68 ± 0.10 mg/d. This average release rate was quantified by immersing the capsules in a 39°C water bath and then measuring weight loss during a period of 6 weeks. The gas samples were collected on the last 5 d of each period, from the afternoon milking of d 16 to the afternoon milking of d 21, which was possible due to the calibration of flow regulators and storage capacity of the air-sampling devices (Pinares-Patiño et al., 2012). Thus, from 5 d of gas sampling, a half-day was not concomitantly with intake measurements; however, both variables were measured for 120 hours consecutively.

Cows with or without access to pastures received the same kind of air-sampling devices concomitantly; each device was put on the head halters such that the sampling point was positioned above the nostrils. The air-sampling devices consisted of stainless steel cylinders (0.5-L volume) with the sample flow regulated by a brass ball bearing, (Gere and Gratton, 2010). The cylinders were cleaned with high-purity N gas and preevacuated prior to each sample collection. The flow regulators were calibrated to allow for an expected remaining vacuum of approximately 500 mb (which represents half of the total cylinder volume) in the cylinder at the end of the sample collection period (five consecutive d). In addition to breath samples, two identical apparatuses were placed 1.5 m above the soil in the paddocks, and two others were placed where the TMR was offered to measure the background concentrations of CH_4 and SF_6 in the environment.

To ensure the most successful individual gas samples, two gas-sampling cylinders were used simultaneously per animal. When two concomitant air samples per cow were collected successfully, the average was used. Operation of the gas-sampling apparatus was considered successful if the residual vacuum was between 350 and 650 mb, which correspond to 37.6 and 69.8% of the initial vacuum, respectively (Pinares-Patiño et al., 2012). The average residual vacuum in gas-sampling apparatus was similar between treatments, and overall collection of the 54 gas samples (2 gas-sampling cylinders × 9 cows × 3 periods) was 69% successful. In four situations, samples from both gas-sampling cylinders of the same cow within the same period were considered lost.

The CH_4 emissions (g/d) were calculated in relation to the known release rate of SF_6 by subtracting the background concentrations of CH_4 and SF_6 (Berndt et al., 2014) as follows:

$$\boxed{R_{CH4} = R_{SF6}\,\frac{[CH_4]_M - [CH_4]_{BG}}{[SF_6]_M - [SF_6]_{BG}} \times \frac{MW_{CH4}}{MW_{SF6}} \times 1000},$$

where R_{CH4} is the enteric CH_4 (g/cow/d), R_{SF6} is the release rate of SF_6 (mg/d), MW_{CH4} is the molecular mass of CH_4 (16 g), and MW_{SF6} is the molecular mass of SF_6 (146 g). $[CH_4]_{BG}$ and $[SF_6]_{BG}$ are the background concentrations of CH_4 (ppm) and SF_6 (ppt), respectively. The background CH_4 and SF_6 concentrations in the treatments with access to pearl millet pastures were calculated according to the weighted average of indoor and outdoor background concentrations, according to the length of time the animals spent in the pastures (7/24 h) or in confinement (17/24 h).

6.3.4. Feed and Pasture Measurements

Offered TMRs and pTMR were sampled twice daily from d 15 to d 20 of each period, and the samples were composited per period. Samples of the orts left by each cow were collected during the last 5 d of each period and were used to create a composite sample for each cow and period. All the samples were dried in an oven for 72 h at 60°C and then ground (Solab SL-31, Piracicaba, Brazil) to pass through a 1-mm screen for subsequent chemical analyses.

The pregrazing herbage mass was measured at ground level by cutting 4 1-m^2 squares of pearl millet with scissors per treatment every d during the last 5 d of each period. The herbage DM concentration was determined for each square from an 800-g subsample. The pre- and postgrazing sward heights were measured daily via a 1.0-m sward stick (Barthram, 1986) by averaging the first contact of 60 readings taken randomly throughout the area allocated for grazing by each group. Selected herbage samples were collected by the hand plucked method daily during the last 5 d of each experimental period. The samples were dried in a forced-ventilation oven for 72 h at 60°C then stored for chemical analyses. The morphological composition of the canopy was determined on d 18 and d 20. In each treatment, twenty handfuls of randomly selected herbage were cut at ground level. These samples were separated into leaf (lamina + sheath), stem and senescent material. Each component was oven dried at 60°C for 72 h and subsequently weighed.

6.3.5. Chemical Analyses

After the samples were ground, their DM content was determined by drying at 105°C for 24 h. The ash content was quantified by combustion in a muffle furnace at 550°C for 4 h, and the OM was quantified on the basis of the mass difference. The total N content was measured according to the Dumas combustion method 968.06 (AOAC International, 1998) via a Leco FP 528 instrument (LC, Leco Corporation, Saint Joseph, USA). The CP content was calculated as N content multiplied by 6.25. The NDF concentration was assessed according to the methods of Mertens (2002), with the exception that the samples were weighed in filter bags and treated with a neutral detergent in an ANKOM A220 system (ANKOM Technology, Macedon NY, USA). This analysis included alpha-amylase and residual ash but did not include sodium sulfite. The concentration of ADF was analyzed according to method 973.18 of the AOAC (AOAC, 1997).

The n-alkane content was quantified on the basis of the protocol described by Dove and Mayes (2006), which was adapted for the use of the columns, as proposed by Oliveira and Tedeschi (2010). The n-alkane content was analyzed via gas chromatography by a Clarus 580 instrument (PerkinElmer, Inc., Waltham, MA, USA) equipped with a flame ionization detector (**FID**) and capillary column (PerkinElmer Elite-1, 100% dimethyl polysiloxane; 30 m × 0.25 mm and a 0.25-µm film thickness).

The concentrations of CH_4 (ppm) and SF_6 (ppt) were determined via a GC-2014 gas chromatograph (Shimadzu, Japan). The chromatograph was equipped with an FID at 250°C and a 1/8” Shimalite Q packed column (0.7 m, 80/100 mesh) for the detection of CH_4 and was equipped with an electron capture detector (**ECD**) at 325°C and a 1/8” Porapak N packed column (1.5 m, 100/180 mesh) for the detection of SF_6. A mixture comprising 5% CH_4 and argon was used as the compositional gas in the SF_6 analysis (ECD). The gas chromatograph column was maintained at 80°C during the analysis, and N gas was used as a carrier, with a flow of 25 cm^3/min. Calibration curves were established by the use of certified standards (White Martins Development Laboratory), with CH_4 concentrations of 2.5, 5.0, 10 and 20 ppm and SF_6 concentrations of 11, 30 and 100 ppt. The minimum detection limit, which is usually critical because of the low concentration of background CH_4 and SF_6, were 0.15 ppm and 5.2 ppt, respectively.

6.3.6. Statistical Analyses

The dependent variables were subjected to analysis of variance via the PROC MIXED function of SAS software (2002, version 9.4, SAS Institute, Cary, NC). The animal variables, which were averaged per cow and per period (n=27), were analyzed using the model:

$$Y_{ijk} = \mu + \text{square}_i + \text{period}_j + \text{treatment}_k + \text{square}_i \times \text{treatment}_k + \text{cow}_{l(i)} + e_{\text{ijk}}$$

where Y_{ijk}, μ, square_i, period_j, treatment_k, $\text{square}_i \times \text{treatment}_k$, $\text{cow}_{l(i)}$ and e_{ijkl} represent the analyzed variable, the overall mean, the fixed effects of the square, the fixed effects of period, the fixed effects of treatment, the fixed effects of square × treatment interaction, the random effect of cow nested in square and the residual error, respectively. The fixed effect of treatment × period interaction was not significant for DMI, milk production and methane variables, being removed from the model.

The variables were tested via orthogonal polynomial contrasts to determine the linear and quadratic effects of the proportion of herbage inclusion in the diet. The least square means were considered as significantly different if $P<0.05$, P values between 0.05 and 0.10 were considered trends and SEM were reported to describe variations.

6.4. RESULTS

The pre- and postgrazing sward heights and the pregrazing herbage mass of pearl millet averaged 62 cm, 32 cm and 3500 kg DM/ha, respectively (Table 2). The CP, NDF and ADF contents of the ingested pearl millet averaged 201, 625 and 306 g/kg DM, respectively. The OM digestibility, energetic value and PDI content of selected herbage averaged 69.4%, 5.81 MJ EL_L/kg DM and 97 g/kg DM, respectively. Throughout the experiment, CP content of ingested pearl millet herbage were 198, 215 and 188 g/kg DM in periods 1, 2 and 3, respectively. The pearl millet NDF content were 611, 631 and 632 g/kg DM and OM digestibility 0.70, 0.69 and 0.69 in periods 1, 2 and 3, respectively.

Table 2. Herbage characteristics and grazing management of a pearl millet pasture (*Pennisetum glaucum* 'Campeiro') grazed by dairy cows receiving mixed rations.

	Treatment[1]	
	$pTMR_{75}$	$pTMR_{50}$
Herbage mass, kg DM/ha	3424	3592
Pregrazing sward height, cm	62.9	61.0
Postgrazing sward height, cm	31.8	31.7
Daily offered area, m^2/cow	41	82
Herbage allowance, kg DM/d		
Aboveground level	13.9	29.4
Living leaves	6.4	11.9
Pregrazing herbage morphological composition, g/kg DM		
Leaves (lamina + sheath)	470	403
Stems	516	576
Dead material	13	17
Herbage chemical composition, g/kg DM		
DM, g/kg	158	156
OM	915	917
CP	193	208
NDF	625	625
ADF	302	310
Herbage nutritive value		
GE, MJ/kg DM	18.5	18.6
OM digestibility	0.69	0.70
NE_L, MJ/kg DM	5.82	5.80
PDIN, g/kg DM[2]	128	134
PDIE, g/kg DM[3]	96	98

[1] pTMR75 = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); $pTMR_{50}$ = 50% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d).

[2] PDIN: metabolizable protein when N is limiting for microbial synthesis in the rumen (INRA. 2007).

[3] PDIE: metabolizable protein when energy is limiting for microbial synthesis in the rumen (INRA. 2007).

The CH_4 emissions (g/d) decreased linearly with the progressive inclusion of grazed herbage in the diet (linear effect: $P < 0.01$), while the CH_4 yield (g/kg DMI) tended to decrease linearly ($P < 0.07$) (Table 3). For each kg of pearl millet herbage inclusion there were a 13.3 g/d and 0.2 g/kg DMI of CH_4 reduction. The CH_4 intensity was similar between treatments, averaging 20 g CH_4/kg ECM. The herbage DMI increased with decreasing mixed ration supply, while the total DMI decreased linearly (Table 4). The mixed ration DMI decreased quadratically with decreasing mixed ration supply, with a greater reduction occurring between TMR_{100} and $pTMR_{75}$ than between $pTMR_{75}$ and $pTMR_{50}$. The concentrate DMI decreased from 7.6 kg/d in the TMR_{100} group to 5.5 and 4.1 kg/d in the $pTMR_{75}$ and $pTMR_{50}$ groups, respectively. The grazing time (+48 min/d) and herbage DMI rate (+ 9.6 g DM/min) were 22 and 42% greater ($P < 0.001$), respectively, in the $pTMR_{50}$ group than in the $pTMR_{75}$ group.

Table 3. Enteric methane emissions by dairy cows receiving mixed rations and with or without grazing access to a pearl millet pasture.[1]

	Treatment[2]				*P-value*		
	TMR_{100}	$pTMR_{75}$	$pTMR_{50}$	SEM	ANOVA	Linear	Quadratic
Methane							
g/d	540	481	436	16.3	0.005	0.001	0.59
g/kg DMI	26.9	26.9	25.6	1.11	0.09	0.07	0.45
g/kg ECM[3]	20.4	19.9	19.6	0.77	0.48	0.25	0.78
% gross energy intake	8.03	8.01	7.51	0.423	0.39	0.28	0.49

[1] Data from 23 observations.

[2] TMR_{100} = Total mixed ration *ad libitum*; $pTMR_{75}$ = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); $pTMR_{50}$ = 50% *ad libitum* intake TMR + grazing herbage after the morning milking (7 h/d).

[3] ECM, calculated as kg milk production × (37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948) / 3,138 (Tyrrell and Reid, 1965).

The milk production and ECM production decreased, in cows with access to grazed herbage compared with that of cows in the TMR_{100} group. For each kg of pearl millet herbage inclusion, there was a 0.2 kg of milk yield reduction. The milk fat and MUN concentrations were similar between treatments, and the milk protein content decreased linearly as the mixed ration supply was reduced.

6.5. DISCUSSION

6.5.1. Methane Emissions

The hypothesis concerning CH_4 production was confirmed in part because daily CH_4 emissions decreased linearly, but the CH_4 yield and CH_4 intensity did not increase with decreasing mixed ration supply. The linear reduction in daily CH_4 emissions with decreasing mixed ration intake and the inclusion of grazing herbage is consistent with the linear reduction in total DMI, which is well known as the main driver of enteric CH_4 emissions (Hristov et al., 2013). These results are also in agreement with those of other studies (O'Neill et al., 2011; Cameron et al., 2018) showing that reductions in daily CH_4 emissions occurred because of reductions in DMI in dairy cows receiving fresh herbage plus pTMR compared with that of cows receiving TMR exclusively. Moreover, the CH_4 emission values reported in the present study are within the range of values observed when cows consumed TMR (Dall-Orsoletta et al., 2016) or grazed on a pearl millet pasture (Alves et al., 2017).

Table 4. Dry matter intake, behavior and chemical composition of the diet of dairy cows receiving mixed rations and with or without grazing access to a pearl millet pasture.

	Treatment[1]				*P-value*		
	TMR$_{100}$	pTMR$_{75}$	pTMR$_{50}$	SEM	ANOVA	Linear	Quadratic
DMI, kg/d							
Total	19.0	18.4	18.0	0.27	0.04	0.02	0.51
Herbage	-	4.6	7.8	0.10	<0.001	-	-
TMR	19.0	13.8	10.2	0.19	<0.001	<0.001	0.007
Grazing time, min/d	-	216	264	6.6	0.03	-	-
Herbage DMI rate, g/min	-	23.0	32.6	2.34	0.001	-	-
Chemical composition of the diet (g/kg DM)							
OM	962	951	942	-	-	-	-
CP	155	169	175	-	-	-	-
NDF	348	429	463	-	-	-	-
ADF	177	215	232	-	-	-	-
NE$_L$ supply[2], MJ/d	136	124	118	-	-	-	-
NE$_L$ balance[3], MJ/d	20	13	10	-	-	-	-

[1] TMR$_{100}$ = Total mixed ration *ad libitum*; pTMR$_{75}$ = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d).

[2] Net energy for lactation supply.

[3] Net energy for lactation balance (NE$_L$ supply - NE$_L$ requirements).

The tendency for decreasing linearly CH$_4$ yields and the percentage with decreasing TMR supply was unexpected because decreasing the concentrate content may increase CH$_4$ emissions per unit of DMI (Hristov et al., 2013). However, both the NDF (Niu et al., 2018) and concentrate (INRA, 2018) contents of the diet have been shown to be strongly related to enteric CH$_4$ yields. While the dietary NDF content is linearly positively related to CH$_4$ yields, the dietary concentrate content exhibited a curvilinear relationship, where the maximum methanogenesis per kilogram of OM intake occurs with the inclusion of 35% concentrate (Sauvant and Giger-Reverdin, 2009). In the present study, the diet concentrate content averaged 22, 30 and 40% in the TMR$_{50}$, TMR$_{75}$ and TMR$_{100}$ groups, respectively. Thus, it is logical to assume that reductions in CH$_4$ yields due to a lower NDF content in the TMR$_{100}$ group compared with the other groups were offset by an increase amount of ruminal fermentable OM. This probably occurred because the concentrate content in the TMR$_{100}$ diet was not high enough to affect the CH$_4$ yield compared with that in the other treatments.

The similarity in CH$_4$ intensity (g/kg ECM) between treatments may be explained because the ECM production and DMI decreased linearly in similar proportions when the mixed ration supply decreased. The average value of CH$_4$ intensity (20 g/kg ECM) was close to the values estimated by Moate et al. (2016) as emissions from the Australian dairy industry (19.9 g/kg ECM) and values of studies assessing dairy cows receiving TMR or pTMR plus fresh temperate herbage grazing in Europe (16-24 g/kg ECM) (O'Neill et al., 2011, 2012). This is evidence that dairy cow diets including tropical herbage may have similar CH_4 intensities as do those of confined or temperate herbage-based diets.

Table 5. Milk production and milk composition of dairy cows receiving mixed rations and with or without grazing access to a pearl millet pasture.

	Treatment[1]				*P-value*		
	TMR$_{100}$	TMR$_{75}$	TMR$_{50}$	SEM	ANOVA	Linear	Quadratic
Milk production, kg/d	24.0	22.7	22.4	0.83	<0.001	<0.001	0.07
4% FCM production, kg/d[2]	26.3	24.8	24.0	0.73	<0.001	<0.001	0.12
ECM, kg/d[3]	26.0	24.5	23.6	0.68	<0.001	<0.001	0.16
Milk fat, g/kg	46.9	46.8	45.4	2.53	0.19	0.11	0.37
Milk protein, g/kg	33.8	33.4	32.3	0.63	0.009	0.003	0.33
Milk fat production, g/d	1112	1049	1008	12.7	<0.001	<0.001	0.34
Milk protein production, g/d	808	754	716	12.9	0.003	<0.001	0.50
MUN, mg/L	18.4	18.0	18.0	0.82	0.64	0.42	0.64

[1] TMR$_{100}$ = Total mixed ration *ad libitum*; pTMR$_{75}$ = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d).

[2] 4% fat-corrected milk production.

[3] ECM, calculated as kg milk production × (37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948) / 3,138 (Tyrrell and Reid, 1965).

6.5.2. Dry Matter Intake and Grazing Behavior

The linearly reduction in total DMI in cows grazing on the pearl millet pasture compared with that in cows in the TMR$_{100}$ group (-0.1 kg for each kg of pearl millet herbage inclusion) could be explained primarily by the reduction in mixed ration intake and thus the reduction in the concentrate intake. The substitution rate between forages and concentrates is typically in the range of 0.0 to 0.7 at grazing (Delagarde et al., 2011), leading to a reduction in total intake when the concentrate supply is reduced within a large range of herbage quality and herbage allowance values (Bargo et al., 2003; Faverdin et al., 2011). In this study, this reduction was no larger than 6%, which could be explained by the forage/concentrate substitution increasing with increasing concentrate intake (Faverdin et al., 2011), because high substitution rate values (approximately 1.0) have a little or no impact on total DMI. Additionally, the average percentage of reduction in total DMI observed when grazing partly replaced the mixed ration is also in agreement with the variation range (-4 to -7% of total DMI) observed for dairy cows when the concentrate proportion in the diet decreased in a similar range of roughage (corn silage or grass silage):concentrate (high starch) ratios (60:40 to 80:20) (Faverdin et al., 1991).

The similar reduction in total DMI in the $pTMR_{75}$ and $pTMR_{50}$ groups compared with the TMR_{100} groups may be explained by the reduction in the TMR supply from 75 to 50% of *ad libitum* intake being partly offset by an increase of 3.2 kg in herbage DMI. This increase was mediated through a greater grazing time (+ 48 min/d) and herbage intake rate (+ 9.6 g DM/min) in the $pTMR_{50}$ group than in the $pTMR_{75}$ group. These results are in agreement with those of other studies where dairy cows grazing on temperate herbage presented an increased herbage DMI as the feed supplement amount decreased (Pérez-Ramírez et al., 2008; Vibart et al., 2008). For instance, Pérez-Ramírez et al. (2008) reported that cows increased their herbage DMI (+ 3.0 kg/d) by increasing their daily grazing time (+ 36 min/d) and herbage intake rate (+ 7 g DM/min) when the feed supplement (corn silage + soybean meal) was reduced from 10 to 5 kg DM/d.

6.5.3. Milk Production and Milk Composition

The linearly reduction in ECM production in the $pTMR_{75}$ and $pTMR_{50}$ groups compared with that in the TMR_{100} group (-0.3 kg for each kg of pearl millet herbage inclusion) was a consequence of the concomitant linearly reductions in total DMI and in concentrate DMI, both of which reduced the net energy intake. When the reduction in milk production was calculated as a function of only the decrease in concentrate intake, the milk production decreased by only 0.5 kg/d for each kilogram of reduced concentrate intake. This reduction is lower than that described for classic milk production responses to concentrate supplementation (1 kg milk/kg concentrate intake) described in the literature (Peyraud and Delaby, 2001; Delagarde et al., 2011) and can be explained by the reduction in NE_L supply from the TMR being partly offset by the NE_L supply from the herbage intake. Finally, cows with access to the pearl millet pasture presented an important reduction in concentrate intake but produced more than 90% of the amount of milk produced by the TMR_{100} cows. However, owing to the potential of shifting herbage nutritive values throughout the growing season, long-term continuous studies with larger number of cows grazing both pearl millet and other tropical forage species are highly recommended.

The linearly reduction in milk fat production with the progressive reduction in mixed ration was a consequence of milk production being lower than that of cows without access to grazing of the pearl millet pasture, since the milk fat content was similar between the treatments. The high milk fat content observed in this study (46.2 g/kg) can be explained by the breed characteristics and is in agreement with the milk fat content reported in another study involving cows from the same herd (Dall-orsoletta et al., 2019). The reduction in milk protein content in cows with access to the grazing herbage compared with that in cows in the TMR_{100} group is in good agreement with variations in the energy supply. The role of the energy supply, rather than the protein or amino acid supply, in improving milk protein content has already been demonstrated in two comprehensive literature reviews (Coulon and Rémond, 1991; Beever et al., 2001).

6.6. CONCLUSION

Including pearl millet in dairy cow diets decreased the total DMI and milk production, but even at the greatest level of herbage inclusion, cows were able to achieve more than 90% of the total DMI and milk production recorded for cows that were fed only TMR. As the relative reduction in milk production was similar to that of the DMI, CH_4 emissions (g/d) decreased, but the CH_4 intensity (g/kg ECM) was unaffected by the progressive inclusion of herbage in the diet. Additional studies with dairy cows grazing tropical forages throughout the whole growing season are strongly encouraged.

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7. PROGRESSIVE INCLUSION OF ANNUAL RYEGRASS HERBAGE AS A SUPPLEMENT FOR DAIRY COWS FED MIXED RATIONS: EFFECTS ON DRY MATTER INTAKE, MILK PRODUCTION AND COMPOSITION[2]

7.1. ABSTRACT

The inclusion of herbage in the diet of medium yielding dairy cows offered a total mixed ration (TMR) may be beneficial. This study, which involved mid lactation dairy cows, examined the effect of partial replacement of a TMR with annual temperate pasture. Treatments were *ad libitum* TMR (TMR_{100}), 75% *ad libitum* TMR + ryegrass (*Lolium multiflorum* 'Maximus') ($pTMR_{75}$), and 50% *ad libitum* TMR + ryegrass ($pTMR_{50}$). Twelve multiparous Holstein and F1 Jersey × Holstein cows were divided into six homogeneous groups, taking account of milk production (26.6 ± 4.5 kg/day), days in milk (128 ± 50) and body weight (546 ± 31 kg). Treatments were compared in a replicated 3×3 Latin square design, comprising three 21-day periods (measurements during final 5 days). Cows on $pTMR_{75}$ and $pTMR_{50}$ strip grazed between morning and afternoon milking (7 h/day), with a target pre- and post-grazing sward height of 24 and 12 cm, respectively. Herbage DM intake was estimated as the difference between pre- and post-grazing herbage mass. The TMR and herbage had a crude protein content of 150 and 303 g/kg DM, and a NDF content of 366 and 495 g/kg DM, respectively. Herbage DM intake increased from 4.8 kg/day in $pTMR_{75}$ to 6.7 kg/day on $pTMR_{50}$. Total DM intake decreased from 19.4 kg/day (TMR_{100}), to 18.1 and 15.8 kg/day ($pTMR_{75}$ and $pTMR_{50}$, respectively). Milk production and milk fat content were similar between treatments, averaging 25.6 kg/day and 44.6 g/kg, respectively. Milk urea nitrogen increased, and milk protein content decreased with decreasing mixed ration supplies. The net energy for lactation (NE_L) supply was 113, 104 and 92% of NE_L requirements, for cows receiving TMR_{100}, $pTMR_{75}$ and $pTMR_{50}$, respectively. Ryegrass pastures were able to replace up to 50% of TMR offered to mid lactation dairy cows without any adverse effects on milk production and milk composition.

Key words: intake; milk production; milk composition; *Lolium multiflorum*

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[2] Este capítulo apresenta a versão preliminar do artigo que será submetido ao periódico Livestock Science, seguindo as normas da revista.

7.2. INTRODUCTION

Full time grazing systems are normally unable to supply all of the energy requirements of lactating dairy cows (Kolver and Muller 1998), and often do not provide a constant supply of herbage throughout the year (Wilkinson et al. 2020). As a result, systems involving full time housing, in which cows are often offered a total mixed ration (TMR), are common. However, giving housed cows access to grazing for part of the day may improve animal welfare (Arnott et al. 2017) and reduce feeding costs (White et al. 2002). Thus the adoption of 'mixed systems' ('part housing-part grazing') may provide a tool to help maintain individual cow total DM intakes and milk production, and overall stocking density (Wales et al. 2013).

Total DM intake amd milk production of dairy dairy cows in mixed systems are dependent of herbage characteristics, like herbage quality and herbage allowance. O'Neill et al. (2012) have put in evidence advantages of supplementing partial mixed rations (pTMR) for dairy cows grazing in low herbage allowance. Additionally, milk production did not change (Dall-Orsoletta et al., 2016) or decreased in a small quantity (Soriano et al., 2001) when cows receiving pTMR grazed high quality herbage without restrictions from herbage allowance. However, when herbage quality decreased, due to advanced grazing season, cows experience to decrease total DM intake and milk production.

Previous studies involving dairy cows grazing annual ryegrass (*Lolium multiflorum* Lam.) swards in a subtropical region have observed that even when herbage allowance and herbage quality were high, herbage DM intake was lower than expected due to low pre-grazing herbage mass (Miguel et al. 2014, 2019). The low herbage mass of annual ryegrass has been explained by its low tiller density, especially during the first grazing cycles, in comparison to perennial species (Miguel et al. 2014). Thus, herbage DM intake appears to be predominantly limited by sward structure. Nevertheless, to our best knowledge, the effect of including this kind of pasture in dairy cow diets which are predominantly comprised of a TMR have not been studied.

***Abreviattions:* TMR, total mixed ration; N, nitrogen; DM, dry matter; MUN, milk urea nitrogen; NE_L, net energy for lactation; BW, body weight; FCM, fat-corrected milk; BCS, body condition score; HM, herbage mass; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error mean; CP, crude protein; OM, organic matter.**

We hypothesized that when a TMR comprises at least 50% of *ad libitum* intake, annual ryegrass herbage would allow total DM intake and milk production to be maintained. The aim of this study was to assess the effect of including annual ryegrass herbage in the diets of TMR-fed dairy cows, on total DM intake and milk production.

7.3. MATERIAL AND METHODS

The Ethics Committee of University of Santa Catarina State approved all procedures, with protocol number 4373090816.

7.3.1. *Treatments, Experimental Design, and Animals*

The experiment was performed in Lages, SC, Brazil (50.18°W, 27.47°S; 920 m above sea level) from June 19 to August 21, 2019. Twelve multiparous Holstein and Holstein × Jersey cows were divided in six homogeneous groups, each of two cows (experimental unit), according to (means ± standard deviations) milk production (26.6 ± 4.55 kg/day), days-in-milk (129 ± 50.8 days) and body weight (546 ± 30.6 kg) measured during the week prior to the experiment starting. Each pair of cows were distributed within two 3 × 3 Latin squares, with each experimental period 21 days: a 16-day adaptation period and a 5-day measurement period.

The treatments comprised a TMR diet offered at 100% of *ad libitum* intake (TMR_{100}), TMR at 75% of *ad libitum* intake + access to an annual ryegrass (*Lolium multiflorum* 'Maximus') herbage for grazing ($pTMR_{75}$), and TMR at 50% of *ad libitum* intake + access to annual ryegrass pasture for grazing ($pTMR_{50}$). The TMR was balanced to meet the net energy and metabolizable protein requirements, according to INRA (2007). Ingredients, chemical composition and nutritive value of TMR are presented in Table 1.

The individual voluntary DM TMR intake was quantified before the experiment started in a 14-d pre-experimental period, cows been housed and fed by pairs. The average DMI of the last 5 d was considered for calculating, for each pair of cows, the amount of mixed ration to be offered in the $pTMR_{75}$ and $pTMR_{50}$ treatments throughout the experiment. During the experiment, the cows were housed by pairs where either TMR or pTMR were offered in covered outdoor feeders. The TMR group was fed twice a day after morning and afternoon milkings in a quantity 20% greater of the animal requirements. Cows on $pTMR_{75}$ and $pTMR_{50}$ had access to pasture for 7 h/day, between morning and afternoon milking (from 08:00 a.m. to

03:00 p.m.), and were offered the pTMR following afternoon milking (13 h/d of access to pTMR, from 05:00 p.m. to 6:00 a.m). TMR and pTMR refusals from each pair of cows were collected and weighed once a day, during the morning milking. Water and mineral supplements (Bovigold®, DSM Tortuga, São Paulo, Brazil) were continuously available in the feeding area and paddocks.

Table 1. Chemical composition and nutritive value of mixed rations offered to dairy cows.[a]

Item	Contend
Ingredients (g/kg DM)	
Corn silage	600
Corn ground	260
Soybean meal	140
Chemical composition, g/kg DM	
DM, g/kg fresh	380
OM	962
CP	149
NDF	340
ADF	173
Nutritive value[b]	
OM digestibility	76.8
NE_L, MJ/kg DM	7.02
PDIN, g/kg DM	97.8
PDIE, g/kg DM	99.0

[a] Mineral supplement composition available in the feeding area and paddocks1 (on natural basis): 150 g/kg calcium, 78 g/kg phosphorus; 26 g/kg sulfur, 20 g/kg magnesium, 114 g/kg sodium, 100 mg/kg cobalt, 1500 mg/kg copper, 30 mg/kg chromium, 2000 mg/kg iron, 80 mg/kg iodine, 2300 mg/kg manganese, 30 mg/kg selenium, 5000 mg/kg zinc, and 780 mg/kg fluorine.

[b] Estimated from chemical analysis and via equations proposed by INRA (2007).

NE_L: Net energy for lactation.

PDIN: metabolizable protein when N is limiting for microbial synthesis in the rumen.

PDIE: metabolizable protein when energy is limiting for microbial synthesis in the rumen.

7.3.2. *Herbage and Grazing Management*

An area of three hectares of annual ryegrass seeded in April 20019 was used. During the experimental period the average temperature was 11.8°C and the total rainfall was 197 mm (average = 3.1 mm/day). The 10-year climatic average temperature and rainfall during the months of the experiment were 13.9°C e 107 mm, respectively. Before the first grazing cycle (after ryegrass has emitted the third leaf) and after each experimental period, the experimental area was fertilized with 50 kg N/ha, supplied as urea.

The pasture area was divided into two halves (one half for each Latin square), with one third of each half assigned to $pTMR_{75}$ and two thirds assigned to $pTMR_{50}$ (4 paddocks in total, 2 cows per paddock). Paddocks were strip grazed, with target pre- and post-grazing sward height of 24 and 12 cm, respectively (a target reduction in sward height of 50%). Fresh pasture was allocated daily, with the area allocated based on allocations during the one-week pre-experimental period. As actual pre- and post-grazing sward heights were similar to the target heights, no other adjustment was necessary. To minimize variations in herbage quality between periods, different areas were used during each period. These areas were mowed 14 days before starting the measurement periods for controlling of pre-grazing sward height and chemical composition.

7.3.3. *Animal Measurements*

Individual milk production values were recorded twice a daily (at 7:00 a.m and 04:00 p.m.) and milk samples were collected at each milking via an electronic milk meter (Waikato Milking Systems, New Zealand) approved by the International Committee for Animal Recording (ICAR). The milk composition (fat, protein, and milk urea nitrogen [MUN] concentration) was individually measured for samples collected during each milking of the last 5 days of each period via infrared spectrophotometry (International Dairy Federation Standard 141C:2000).

The herbage DM intake was estimated as the difference between the total biomass at pre- and postgrazing (Lantinga et al., 2004) on each of the last 5 days of each period. Their TMR and pTMR intake were quantified daily as the difference between the quantity supplied and the quantity remaining from each of the last 5 days of each period and averaged per group and period.

The daily grazing time ($pTMR_{75}$ and $pTMR_{50}$ groups) was measured individually via visual observations every 5 min between 8:00 a.m. and 3:00 p.m. during the last 5 days of each period. Cows were previously conditioned with human company, and the time spent watching each individual animal was no more than 10 s, during which grazing behaviour or no grazing was recorded (Penning and Rutter, 2004). No behaviour was recorded indoors when the cows were milked or fed the supplement. The herbage intake rate (g DM/min) was estimated by dividing the daily herbage intake by the daily grazing time.

The net energy for lactation (NE_L) balance was estimated for each pair of cows according to the difference between the NE_L supply NE_L and requirements, according to the methods of the INRA (2007). Breafly, theoretical NE_L requirements were calculated from the actual BW and 4% fat-corrected milk (FCM) production during the experiment, and net energy supply was calculated from the intake of herbage, corn silage, corn ground, soybean meal and their concentrations of NE_L.

7.3.4. *Feed and Herbage Measurements*

Samples of TMR were collected twice daily from day 15 to day 20 of each period and combined to create a composite sample per period. Samples of the remaining feed from each pair of cows were collected during the last 5 days of each period and used to create composite samples for each group and period. All samples were dried in an oven for 72 h at 60°C and ground (Solab SL-31, Piracicaba, Brazil) by sieving through a 1-mm screen for subsequent chemical analyses.

Pre- and post-grazing compressed sward heights were measured daily using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand). In addition, the plate-meter was calibrated to predict herbage mass at the start of each experimental period, pre- and post-grazing. Samples were cut at ground level using scissors from 18×0.1 m^2 quadrats (4 and 5 in each of the $pTMR_{75}$ and $pTMR_{50}$ paddocks, respectively) in the 'footprint' of areas where the plate meter had been used to record herbage height. Individual samples were dried in an oven for 72 h at 60°C, and equations subsequently developed to predict the actual herbage mass that had been offered during the study. Owing to the relatively high R-squared value and lower residual standard deviation values, the pregrazing herbage mass was estimated according to equations generated per period and postgrazing herbage mass according to a general equation for all three periods (Table 2).

The pre- and postgrazing sward heights were measured daily via a 0.5-m sward stick (Barthram, 1986) by averaging the first contact of 100 readings taken randomly throughout the area allocated for grazing by each pair of cows. The pregrazing extended heights of the tallest leaf blade and sheath were measured on 100 tillers at random on days 16, 18, and 20. The postgrazing leaf and sheath extended heights were measured on days 18, 20, and 22 on 100 tillers per treatment. The morphological and chemical compositions of the sward were determined on days 17, 19, and 21. Twenty handfuls of randomly selected herbage (~1000 g fresh) were cut at ground level. This herbage sample was separated into two subsamples. One subsample was used to estimate the chemical composition of the herbage selected by grazing. For that purpose, the post-grazing extended tiller heights was considered to cut the herbage sample. Subsequently, the cut portion was dried in an oven for 72 h at 60°C with forced ventilation and stored for chemical analyses. The other subsample was used for morphological classification (ryegrass only). The ryegrass was separated into leaf (lamina + sheath), stems and senescent material. Each component was dried in an oven for 72 h at 60°C to determine the morphological composition of the pasture on a DM basis.

Table 2. Pre- and postgrazing herbage mass (HM) estimates as a function of compressed sward height on a annual ryegrass pasture (Lolium multiflorum 'Maximus') grazed by dairy cows receiving mixed rations.

Item	Equation	n	R^2	rsd
Pregrazing HM				
Period 1	y = 63 x - 54	18	0.82	237.6
Period 2	y = 83 x + 38	18	0.81	289.4
Period 3	y = 75 x + 151	18	0.78	336.8
General	y = 63 x + 286	54	0.71	356.2
Postgrazing HM				
Period 1	y = 105 x - 412	18	0.80	298.9
Period 2	y = 123 x - 371	18	0.83	294.7
Period 3	y = 122 x - 469	18	0.82	455.3
General	y = 118 x - 423	54	0.83	261.9

y = Pre- or postgrazing aboveground herbage mass (kg DM/ha); x = pre- or postgrazing compressed sward height measured using a rising plate metter (F200 model, Farmworks, Feilding, New Zealand); rsd = residual standard deviation.

7.3.5. *Chemical Analyses*

The DM content was determined by drying the samples at 105°C for 24 h. The ash was quantified by combustion in a muffle furnace at 550°C for 4 h, and the OM was quantified based on the mass difference. The total N content was measured by the Dumas combustion method 968,06 (AOAC International, 1998) using the Leco FP 528 equipment (LC, Leco Corporation, Saint Joseph, EUA). The aNDF concentration was assessed according to Mertens (2002), except that the samples were weighed in filter bags and treated with neutral detergent in an ANKOM A220 system (ANKOM Technology, Macedon NY, USA). This analysis included alpha-amylase and residual ash but did not include sodium sulfite. The concentration of acid detergent fibre (ADF) was analysed according to Method 973.18 of the AOAC (AOAC, 1997). Milk composition was analyzed by infrared spectrophotometry (International IDF Standard 141C:2000; IDF, 2000).

7.3.6. *Statistical Analyses*

The dependent variables were subjected to an analysis of variance using the function PROC MIXED in the software SAS (2002, version 9.4, SAS Institute, Cary, NC). The animal variables, which were averaged per cow and period (n=18), were analyzed using the following model:

$$Y_{ijk} = \mu + \text{square}_i + \text{period}_j + \text{treatment}_k + \text{square}_i \times \text{treatment}_k + \text{group}_{l(i)} + e_{\text{ijk}}$$

where Y_{ijk}, μ, square_i, period_j, treatment_k, $\text{square}_i \times \text{treatment}_k$, $\text{group}_{l(i)}$ and e_{ijkl} represent the analyzed variable, the overall mean, the fixed effects of the square, the fixed effects of period, the fixed effects of treatment, the fixed effects of square × treatment interaction, the random effect of group nested in square and the residual error, respectively. The fixed effect of treatment × period interaction was not significant for any animal variable, and was removed from the model.

The herbage variables were averaged per paddock and period (n = 12) and analysed using the following model:

$$Y_{ij} = \mu + \text{period}_i + \text{treatment}_j + e_{\text{ij}}.$$

where Y_{ij}, μ, period_i, treatment_j and e_{ij} represent the analysed variable, the overall mean, the random effect of period, the fixed effect of the treatment and the residual error, respectively.

The variables were tested via orthogonal polynomial contrasts to determine the linear and quadratic effects of the proportion of herbage inclusion in the diet. The least square means were considered as significantly different if $P<0.05$, P values between 0.05 and 0.10 were considered trends and SEM were reported to describe variations.

7.4. RESULTS

Pre- and post-grazing herbage mass of annual ryegrass averaged 1804 and 906 kg DM/ha, respectively (Table 3). Pre- and post-grazing sward height were similar with both treatments and averaged 23.8 cm and 11.4 cm, respectively. Herbage offered with pTMR_{75} and pTMR_{50} was similar in composition, with an average crude protein (CP), NDF and ADF content of 303, 495 and 199 g/kg DM. The morphological composition of ryegrass was similar in paddocks grazed by both pTMR groups, with leaves proportion averaging 770 g/kg DM. The OM digestibility, energetic value and metabolizable protein content of selected pasture were also similar between treatments, averaging 0.82, 7 MJ NE$_L$/kg DM and 120 g/kg DM, respectively.

Table 3. Pre- and post grazing characteristics, grazing management, chemical composition and nutritive value of annual ryegrass ('Maximus') grazed by dairy cows receiving mixed rations.

	Treatment[a]			
	pTMR$_{75}$	pTMR$_{75}$	SEM	*P-value*
Pre-grazing characteristics				
Herbage mass, kg DM/ha	1895	1713	11.2	0.087
Compressed sward height, cm[b]	12.9	11.6	0.72	0.113
Sward height, cm[c]	23.8	23.7	1.13	0.983
Extended tiller height, mm	439	418	5.0	0.247
Extended sheath height, mm	89.1	86.0	2.06	0.295
Post-grazing characteristics				
Herbage mass, kg DM/ha	915	902	7.7	0.712
Compressed sward height, cm[b]	5.8	5.7	0.38	0.743
Sward height, cm[c]	11.3	11.7	0.65	0.206
Extended tiller height,mm	177	184	5.5	0.711
Extended sheath height., mm	79.0	70.8	2.99	0.211
Herbage allowance. kg DM/day				
Above ground level	20.4	30.5	0.50	0.001
Living leaves	15.4	23.9	1.07	0.001
Morphologycal composition, g/kg DM				
Leaves (lamina + sheath)	755	783	16.9	0.194
Stems	208	190	11.4	0.103
Dead material	37.5	27.0	15.0	0.482
Chemical composition, g/kg of DM				
DM	112	111	8.9	0.358
OM	878	866	11.0	0.138
CP	300	306	15.6	0.429
NDF	494	496	12.5	0.619
ADF	198	199	11.4	0.856
Nutritive Value				
OM digestibility	0.83	0.81	0.18	0.419
NE$_L$, MJ/kg DM	7.0	6.9	1.38	0.617
PDIN, g/kg DM[d]	203	201	4.9	0.772
PDIE, g/kg DM[e]	122	119	2.8	0.626

[a] pTMR75 = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d). Net energy for lactation.

[b] Measured with a rising plate meter (F200 model, Farmworks, Feilding, New Zealand).

[c] Measured via 0.5-m sward stick (Barthram, 1986).

[d] PDIN: metabolizable protein when N is limiting for microbial synthesis in the rumen (INRA. 2007).

[e] PDIE: metabolizable protein when energy is limiting for microbial synthesis in the rumen (INRA. 2007).

Throughout the experiment, CP content of ingested annual ryegrass herbage were 318, 308 and 285 g/kg DM in periods 1, 2 and 3, respectively. The annual ryegrass NDF content were 468, 491 and 52.6 g/kg DM and OM digestibility 0.82, 0.81 and 0.79 in periods 1, 2 and 3, respectively.

Total DM intake decreased from TMR_{100} to $pTMR_{50}$ group (Table 4). There was a quadratic reduction in mixed ration intake, and herbage DM intake increased by 1.9 kg/day from TMR_{75} to TMR_{50}. For each kg of herbage DM consumed, intake of TMR with treatments $pTMR_{75}$ and $pTMR_{50}$ decreased by 1.3 and 1.6 kg DM/day, respectively. Thus, total DM intake decreased as the proportion of herbage increased. Diets TMR_{100}, $pTMR_{75}$ and $pTMR_{50}$ supplied 113%, 104% and 92% of energy requirements. The grazing time and DM intake rate increased 20.4 and 15.5%, respectively, in $pTMR_{50}$ compared to the TMR_{75} treatment.

Table 4. Dry matter intake, grazing behavior and chemical composition of the diet of dairy cows receiving mixed rations and with or without grazing access to a annual ryegrass ('Maximus').

	Treatment[a]				*P-value*		
	TMR_{100}	$pTMR_{75}$	$pTMR_{50}$	SEM	ANOVA	Linear	Quadratic
DMI, kg/d							
Total	19.4	18.1	15.8	0.382	0.001	0.001	0.044
Herbage	-	4.8	6.7	0.037	0.001	-	-
TMR	19.4	13.3	9.1	0.324	0.001	0.001	0.001
Grazing time, min/d	-	244	294	4.922	0.004	-	-
Herbage DMI rate, g/min	-	19.9	22.8	1.516	0.014	-	-
Chemical composition of the consumed diet (g/kg DM)							
OM	953	933	916	0.8	0.001	0.001	0.110
CP	149	189	208	1.7	0.001	0.001	0.001
NDF	366	416	427	1.6	0.001	0.001	0.001
ADF	220	218	214	0.9	0.001	0.006	0.286
NE_L supply[b], MJ/d	138	127	111	0.96	0.001	0.001	0.024
NE_L balance[c], MJ/d	15.4	2.50	-10.9	2.96	0.001	0.001	0.879
NE_L balance[d], %	113	104	92.3	2.52	0.001	0.001	0.736

[a] TMR_{100} = Total mixed ration *ad libitum*; $pTMR_{75}$ = 75% of TMR *ad libitum* intake + grazing pasture after morning milking (7 h/day); $pTMR_{50}$ = 50% of TMR *ad libitum* intake + grazing pasture after morning milking (7 h/day). NE_L= Net energy for lactation.

[b] Net energy for lactation supply.

[c] Net energy for lactation balance (NE_L supply - NE_L requirements).

[d] % NE_L requirements.

The milk production and milk fat content did not differ between treatments, averaging 25.6 kg/day and 44.6 g/kg, respectively (Table 5). Milk protein content decreased from 34.2 g/kg on TMR_{100} to 33.8 and 33.1 g/kg in $pTMR_{75}$ and $pTMR_{50}$ treatments, respectively. The MUN concentration increased in pTMR groups compared with cows receiving TMR exclusively.

Table 5. Milk production, milk composition and body weight of dairy cows receiving mixed rations and with or without grazing access to a annual ryegrass (‘Maximus’).

	Treatment[a]				*P-value*		
	TMR$_{100}$	pTMR$_{75}$	pTMR$_{50}$	SEM	ANOVA	Linear	Quadratic
Milk production, kg/day	24.8	26.7	25.2	1.31	0.118	0.413	0.108
4% FCM production, kg/day[b]	26.5	27.9	26.7	0.81	0.695	0.795	0.422
ECM, kg/day[c]	28.1	28.9	28.3	1.32	0.183	0.807	0.071
Milk fat, g/kg	45.5	43.1	45.3	0.87	0.084	0.841	0.028
Milk protein, g/kg	34.2	33.8	33.1	0.73	0.024	0.007	0.704
Milk fat production, g/day	1122	1157	1184	60.3	0.338	0.148	0.910
Milk protein production, g/day	846	898	873	39.2	0.091	0.242	0.059
MUN, mg/L	17.0	19.1	20.8	0.40	0.001	0.001	0.656
Live weight. kg	559	555	556	10.7	0.688	0.483	0.623

[a] TMR$_{100}$ = Total mixed ration *ad libitum*; pTMR$_{75}$ = 75% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d); pTMR$_{50}$ = 50% *ad libitum* TMR intake + grazing herbage after the morning milking (7 h/d).

[b] 4% fat-corrected milk production.

[c] ECM, calculated as kg milk production × (37.6 × fat (g/kg) + 20.9 × protein (g/kg) + 948) / 3,138 (Tyrrell and Reid, 1965).

7.5. DISCUSSION

The main hypothesis of this study was partially confirmed, because the progressive inclusion of herbage in TMR-fed dairy cow diets did not decrease the milk production. However, as cows accessing pasture were not able to avoid reductions in total DM intake, it is logical to assume that in pTMR$_{50}$ milk production has been guaranteed by certain amount of body reserves mobilisation.

7.5.1. *Dry Matter intake and Grazing Behaviour*

The reduction in total DM intake when cows received TMR at 50% of *ad libitum* intake and were given access to grazing is unlikely to be due to the quality of herbage offered, as the relatively low NDF and high CP content of the herbage suggests that quality was good. Indeed, Vibart et al. (2008) found that total DM intake was not reduced when TMR-fed dairy cows had access to high-quality pastures. However, a reduction in pre-grazing herbage mass was shown to reduce herbage intake (Pérez-Prieto et al. 2013), even with good quality pastures. For example, Pérez-Prieto and Delagarde (2012) demonstrated that herbage DM intake decreases linearly when pre-grazing herbage mass (calculated above ground level) decreased from 5500 to 2500 kg DM/ha. In the current study pre-grazing herbage mass averaged only 1800 kg DM ha, even though pregrazing sward heights were over 23 cm, a reflection of the low tiller density of annual ryegrass. Additionally, Ferris (2007) has been reported that once sward height is reduced by grazing, DM intake tended to be smaller on swards of low bulk density compared with swards of high bulk density. Thus, it is likely that the reduction in total DM intake with the $pTMR_{75}$ and $pTMR_{50}$ treatments is, at least in part, due to sward structure restricting intakes.

The reduction in total DM intake as the progressive inclusion of annual ryegrass may be also partially explained by the relatively high moisture content of fresh herbage. The role of herbage moisture content on herbage DM intake has been put in evidence by Cabrera Estrada et al. (2004). Feeding dairy cows indoors with fresh herbage, these authors observed that increasing DM content of fresh herbage from 120 to 300 g/kg allowed to increase herbage DM intake in 130 g per percentage unit increase in herbage DM content. Reductions on total DM intake as the progressive inclusion of fresh hebage for dairy cows receiving both TMR and herbage indoors were already justified by Mendoza et al. (2016) as a consequence of relatively low herbage DM content, which was around 150 g/kg. In the current experiment, herbage DM content was not greater than 112 g/kg.

The greater herbage intake in $pTMR_{50}$ compared to the $pTMR_{75}$ treatment was a consequence of greater grazing time and DM intake rate. This result may be explained because cows receiving the lowest pTMR supply have also consumed the lowest NE_L supply from mixture. Reductions in grazing time because of energy supplied from supplemental intake have been widely reported (Pérez-Prieto et al., 2011; Wright et al., 2016; Ribeiro-Filho et al., *in press*). In the same way, reductions in both daily grazing time and herbage intake rate have been observed when TMR supply was reduced (Pérez-Ramirez et al., 2008; Civiero et al., 2021). However, in this study these grazing behavior adaptations were not sufficient to avoid reductions in total DM intake, which may be explained by relatively several sward structural restrictions and low herbage DM content, as previously explained.

7.5.2. *Milk Production and Milk Composition*

Despite the reduction in total DM intake, the inclusion of herbage in the diet of TMR-fed dairy cow did not decrease milk production, and had no effect on milk composition, thus partially confirming the main hypothesis of this study. However, despite the reduction in intake with $pTMR_{75}$ (compared to TMR_{100}) this diet supplied 104% of energy required for milk production. In contrast, $pTMR_{50}$ provided 92% of energy requirements for milk production, suggesting that milk production was maintained by mobilization of body tissue reserves. Given the short-term nature of the experimental periods, it was not possible to accurately assess changes in body tissue. However, previous studies have demonstrated that cows with similar energy deficiency at mid-lactation can recovery their body condition during final third of lactation and first 4 weeks of the dry period, without any negative effect on performance and reproduction during the next lactation (Roche et al. 2006, 2017). Additionally, when the energy supply is not less than 90% of requirements, there is a reduced likelihood of metabolic disorders (Overton and Waldron 2004).

The lack of diference in milk fat content between treatments may be explained by the narrow range of variation in NDF content of the consumed diet (366 to 427 g/kg DM). According to the values proposed by Stockdale (1997), the milk fat concentration is not affected when the NDF content in the total diet is between 250 and 400 g/kg DM. The relatively high milk fat content observed in this study (44.6 g/kg) can be explained by the breed characteristics, and is in agreement with the milk fat content reported in two previous studies involving cows from the same herd (Dall-orsoletta et al., 2019; Civiero et al., 2021).

As the milk production did not decrease with the progressive inclusion of herbage, the narrow reduction in milk protein content was not sufficient to impose a clear impact in daily milk protein production. It is has been shown that reducing energy supply decreases milk protein production (Nousiainen et al., 2004). Thus, it is logical to assume that the energy supply greater than 90% of energy requirements in $pTMR_{50}$ would be close to the threashold to avoid reductions in daily milk protein reductions. Finally, the higher MUN concentration in treatments with pasture inclusion was clearly a consequence of greater CP content of herbage compared with mixed ration.

7.6. CONCLUSION

Giving cows access to fresh pasture allowed the amount of TMR offered to by reduced by 50% with no loss in milk production. However, pasture access reduced total DMI, forcing cows into negative energy balance. The lower intakes are likely due to the low tiller density, low herbage mass and low herbage DM content of annual ryegrass.

7.7. ACKNOWLEDGEMENTS

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8. RELATIONSHIPS BETWEEN ENERGY BALANCE DURING EARLY LACTATION, AND COW PERFORMANCE, BLOOD METABOLITES, AND FERTILITY[3]

8.1. ABSTRACT

This study was designed to contribute to the understanding of the relationships between energy balance (EB) in early lactation (4 to 21 days in milk (DIM)) and fertility traits (interval to start of luteal activity (SLA), interval to first observed heat (FOH), and conception to first artificial insemination (AI)), and their associated relationships with cow performance and blood metabolites between 4 to 150 DIM. Individual cow data (488 primiparous and 1,020 multiparous lactations) from 27 experiments was analyzed. Data on cow performance, EB (on a metabolizable energy (ME) basis), and fertility traits were available for all cows, while milk progesterone data (to determine SLA) and periodic blood metabolite data were available for 1,042 and 1,055 lactations, respectively. Data from primiparous and multiparous cows were analyzed separately, with the datasets for the two parity groups divided into quartiles (Q1 – Q4) according to the average EB during 4 to 21 DIM (EB range for Q1 to Q4: primiparous, -120 to -49, -49 to -24, -24 to -3 and -3 to 92 MJ/d, respectively: multiparous, -191 to -79, -79 to -48, -48 to -22 and -22 to 93 MJ/d, respectively). Differences between EB quartiles for production and fertility traits were compared. In early lactation (4 to 21 DIM), moving from Q1 to Q4 mean DMI and ME intake increased while mean ECM decreased. During the same period, moving from Q1 to Q4 milk fat content, milk fat-to-protein ratio, and plasma non-esterified fatty acid and β-hydroxybutyrate concentrations decreased, while milk protein content and plasma glucose concentrations increased in both primiparous and multiparous cows. When examined over the entire experimental period (4 to 150 DIM), many of the trends in intakes and milk production remained, although the magnitude of the difference between quartiles was much reduced, while milk fat content did not differ between quartiles in primiparous cows. The percentage of cows with FOH before 42 DIM increased from Q1 to Q4 (from 46 to 72% in primiparous cows, and from 41 to 58% in multiparous cows). Interval from calving to SLA and to FOH decreased with increasing EB during 4 to 21 DIM, with these occurring 9.8 and 10.2 d earlier, respectively, in Q4 compared to Q1 (primiparous cows), and 7.4 and 5.9 d earlier, respectively, in Q4 compared to Q1 (multiparous cows). For each 10 MJ/d decrease in mean EB during 4 to 21 DIM, FOH was delayed by 1.2 and 0.8 d in primiparous and multiparous cows, respectively. However, neither d to first AI nor the

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percentage of cows that conceived to first AI, were affected by daily EB during 4 to 21 DIM in either primiparous or multiparous cows, and this is likely to reflect a return to a less metabolically stressed status at the time of AI. These results demonstrate that interval from calving to SLA and to FOH were reduced with increasing EB in early lactation, while early lactation EB had no effect on conception to the first service.

Key Words: dairy cattle, energy balance, fertility, blood metabolites.

8.2. INTRODUCTION

Over the last few decades the milk production potential of dairy cows in many countries has increased dramatically (Miglior et al., 2005). There is little evidence, however, that cows with greater milk yield have a better metabolic efficiency for milk production (k_l) than cows with lesser milk yield (Agnew et al., 1998). While part of the extra milk produced by greater yielding cows may be supported by their greater intakes (Ingvartsen and Andersen, 2000), intake capacity during early lactation has not kept pace with the increase in milk yields (Veerkamp et al., 2001). Instead, greater milk yields during early lactation have been driven largely by increased levels of body tissue mobilization, with this reflected in increasing extent and duration of negative energy balance (**NEB**; Agnew et al., 1998). During postpartum NEB, glucose is preferentially partitioned to the mammary gland, pancreatic insulin secretion in response to glucose is suppressed, peripheral tissues exhibit insulin resistance, and cows are susceptible to metabolic disorders (Leroy et al., 2008). In addition, energy balance (**EB**) can also influence fertility.

A number of studies have identified relationships between calculated EB and fertility traits. In one of the earliest studies (involving 13 dairy cows), Butler et al. (1981) concluded that EB during the first 20 d of lactation is important in determining the start of luteal activity (**SLA**). Similarly, in a study involving 134 dairy cows, de Vries et al. (1999) identified a relationship between the extent of energy deficit in early lactation and delay in first observed heat (**FOH**), while in a separate study involving 470 first lactation heifers, de Vries and Veerkamp (2000) observed that each 10 MJ (on net energy basis; **NE**) decrease in nadir EB was associated with a delay in ovulation of 1.25 d. Furthermore, cows with a smaller nadir EB and faster recovery to positive EB had fewer d open and a shorter calving interval (Patton et al., 2007; Carvalho et al., 2014). Using a large data set of almost 1,000 cows, Banos and Coffey (2009) observed genetic correlations between d to first estrus and a number of calculated EB traits.

In addition, other studies have demonstrated relationships between indirect indicators of EB and fertility traits. For example, increased BCS loss has been associated with a delay to first ovulation (Butler and Smith, 1989; Gobikrushanth et al., 2019), while Vercouteren et al. (2015) observed a negative relationship between cyclicity by 21 days in milk (**DIM**) and greater body weight (**BW**) loss. Others have examined the relationship between blood metabolites and fertility, with Dubuc et al. (2012) observing that lesser non-esterified fatty acid (**NEFA**) concentrations post calving were associated with earlier ovulation, while Macmillan et al.

(2018) observed a greater incidence of ovulation by 35 d post-partum in cows with lesser NEFA and β-hydroxybutyrate (**BHB**) concentrations.

While relationships between 'energy status' and fertility traits have been established, few studies have been able to examine these in a holistic manner, including the inter-relationships with genetic index, energy intake, milk yield, milk composition, milk progesterone, body tissue and blood metabolites, especially using large datasets. In addition, as primiparous and multiparous cows differ in intakes, performance, endocrine and blood metabolite levels during the transition period (Macmillan et al., 2018), and as primiparous cows continue to grow during their first lactation, it might be expected that primiparous and multiparous dairy cows would exhibit different responses to NEB (Wathes et al., 2007). Thus, the primary objective of this study was to use a large individual cow dataset collected over a 20-yr period to examine the relationships between early lactation EB, and cow performance and blood metabolites, and the impact of early lactation EB on fertility outcomes such as: SLA, FOH, conception to first artificial insemination (**AI**), and time to conception, in both primiparous and multiparous cows.

8.3. MATERIALS AND METHODS

8.3.1. Experiments, treatments and cows

This study involved a meta-analysis of individual dairy cow data obtained from 27 individual studies which were conducted between 1996 to 2016 at the Agri-Food and Biosciences Institute (**AFBI**) in Hillsborough, Northern Ireland. The results of the majority of these studies have been published in peer reviewed scientific papers, conference proceedings, technical reports, and a PhD thesis (Appendix 1). A minimum prerequisite for the inclusion of any experiment in the analysis was that the experiment encompassed the 'early lactation period' (commencing within a few d of calving, and having a mean length of more than 90 d), included data to allow daily EB to be estimated (i.e. daily DMI, daily milk yield, regular milk composition, BW, and detailed information on the ingredients and chemical composition of the diets offered), and had detailed fertility data. The 27 experiments were variable in length, encompassing incomplete lactations, complete lactations, or multiple lactations. In the case of multi-lactation studies, each lactation was designated as a separate experiment within the analysis. While some full lactation and multi-lactation experiments involved periods of grazing in mid/late lactation, data included in the EB calculations were restricted to periods when cows were housed and when individual cow intakes were available. Within all experiments cows were transferred to a free stall cubicle house shortly after calving.

A total of 79 treatments were examined across the 27 studies, with the majority of treatments examining the impact of diet and/or management strategies on cow performance. Although a number of treatments involved ‘alternative’ cow genotypes, only Holstein cows were included within the analysis. Individual cows within experiments were excluded from the analysis if the housed period when individual feed intakes were measured was less than 42 d. Data recorded during the first three d of lactation were excluded from the analysis, while data was included in the analysis up until a maximum of 150 DIM (provided individual feed intake data was recorded during the entire period). In addition, cows with a lactation number > 6 were excluded from the analysis.

The final dataset comprised data from 1,508 individual lactations (derived from 1,009) individual cows, representing 488 and 1,020 lactations for primiparous and multiparous cows, respectively. Genetic indexes were sourced for the majority of the cows (936) from Animal Horticulture Development Board, UK, during December 2018. Cows with pedigree information had a mean Predicted Transmitting Ability (**PTA**) for milk yield of -37 (SD, 212.2) kg, a mean PTA for milk fat plus protein yield of 7.0 (SD, 7.32) kg, and a mean Profitable Lifetime Index (**PLI**) of £60 (SD, 150.5). The £PLI represents the additional profit a sire is expected to return from each of its milking daughters over her lifetime, compared with an average sire of £0 PLI, and comprises the following traits: production (34.4%), survival (15.1%), fertility (15.3%), udder health (13.7%), efficiency (11.8%), leg health (8.1%) and calving ability (1.6%). The fertility component of the index is comprised of 5 traits, as follows: calving interval, non-return rate, body condition score, milk yield around insemination, days from calving to first insemination, and number of inseminations needed to get a cow in calf (AHDB Dairy, 2020).

8.3.2. Diets offered

Diets offered were predominantly based on grass silage and concentrates. However, in a number of studies (n = 16) grass silage was partially replaced with corn silage (usually between 20 - 40% of the forage component of the diet). In addition, in one study, a small quantity of chopped wheat straw (0.3 kg/cow/d) was included in the diet. The mean forage: concentrate DM ratio across the 27 studies was 49: 51. In all studies the forage component of the diet was offered ad libitum (normally between 7 – 10% of the previous day’s intake).

A wide range of concentrate types, feeding levels, feeding strategies and feeding methodologies were adopted within and between studies, according to the objectives of each individual study. The concentrate supplements consisted principally of cereal grains (e.g. barley, wheat, maize), protein supplements (e.g. soybean meal, canola meal), and fibrous by-products from the food industry (e.g. corn gluten meal, sugar-beet pulp, citrus pulp). Additional energy sources (e.g. Megalac® and molasses) were included in some concentrates, while most concentrates contained a mineral/vitamin supplement. The concentrate component of the diets was offered either mixed with the forages (partial mixed ration), separate from the forages (via in-parlor of out-of-parlor feeders), or via a combination of these practices.

8.3.3. Breeding management and fertility records

Heifers entering the AFBI herd have a target age at first calving of 24 months (actual, 24.5 months). Cows in the herd calve from early September through to late December (‘Autumn’ calving), and from early January through to mid-April (‘Spring’ calving). Heat detection commences after calving. Heat detection is based on visual observations, although tail paint was used in a number of studies as an aid to heat detection. There are three defined periods of heat detection during the d, at approximately 10.00 h, 14.00 h and 20.00 h, although all heats observed throughout the d are recorded. Cows with uterine infection (normally based on stock person observations) are normally treated within 3 to 4 wk of calving. During the early years of the dataset, cows failing to show signs of estrus within 8 wk of calving were examined by a veterinarian, and cows with ovarian dysfunction treated as appropriate. During more recent years, veterinary interventions were delayed until approximately wk 10 to 12 of lactation. An exception to the above was cows identified with cystic ovaries, which were treated as soon as the problem was identified.

Throughout the study period all cows in the herd were bred by AI. A minimum 42-d voluntary waiting period was adopted with all cows. Within the autumn and spring calving components of the herd, breeding commenced early in December and early April, respectively. Cows were typically inseminated once per d by trained AFBI staff (assisted on occasions by a local breeding company). Cows observed in estrus after 10.00 h were inseminated the following morning. Pregnancy status was determined by a veterinarian using trans-rectal ultrasonography (scanner) at least 32 d after insemination. Fertility records included cows treated for uterine infection, cows treated with hormones (progesterone, prostaglandin, estradiol benzoate, or gonadotrophin releasing hormone; **GnRH**), observed heats, inseminations, pregnancy diagnosis, and subsequent calving details.

8.3.4. Animal measurements

A number of animal measurement protocols changed over the 20-yr period during which the 27 experiments were undertaken, while others remained largely unchanged. The feed intake of each individual cow was recorded daily using feed-boxes mounted on weigh cells, access to which was controlled by a Calan Gate feeding system (American Calan Inc., Northwood, NH, USA) linked to an electronic cow identification system. All diets were offered ad libitum. In all experiments cows were milked twice daily, with milk yields recorded automatically at each milking, and a total daily milk yield for each cow determined for each 24-h period. In early experiments (n = 7) milk samples were taken in proportion to yield during six consecutive milkings (either weekly or fortnightly), and a single bulked sample analyzed for each wk or fortnight. However, in later experiments (n = 20) samples were taken during two consecutive milkings (normally on a weekly basis) and each individual sample analyzed, and a weighted composition for the 24-h sampling period subsequently determined. Samples in all experiments were analyzed for fat, protein, and lactose concentrations using mid-infrared milk analysis. Fat-to-protein ratio (**FPR**) in milk was calculated as milk fat content (g/kg) divided by milk protein contents (g/kg). The equation (Eq. 1) given by Tyrrell and Reid (1965) was used to calculate the gross energy (**GE**) content of the milk, where fat, protein and lactose content are presented as g/kg:

$$GE, MJ/kg = [0.0384\ x\ fat] + [0.0223\ x\ protein] + [0.0199\ x\ lactose] - 0.108$$

[1]

Energy corrected milk yield (kg/d) was calculated assuming the GE content of 1 kg ‘standard milk’ to be 3.1 MJ/kg (i.e. for milk containing 4.0% fat, 3.2% crude protein, and 4.8% lactose, as described by Muñoz et al. (2015), according to Eq. 2:

$$ECM, kg/d = \frac{milk\ yield\ (kg/d)\ x\ GE\ (MJ/kg)}{3.1}$$

[2]

Milk energy output (MJ/cow per d) was calculated by multiplying the GE content of milk (Eq. 1) by the daily milk yield. Feed efficiency was calculated by dividing ECM yield (kg/d) by the total DMI (kg/d).

In early studies (n = 4) BW was recorded weekly, immediately after pm milking. However, in later studies BW was recorded twice daily (immediately after each milking) using an automated weighbridge, and an average BW calculated for each wk. Body condition scores (BCS) were recorded weekly or fortnightly through each lactation, with BCS assessed on a 1 to 5 scale (Edmonson et al., 1989). Blood samples were collected (from the tail vein) in 26 of the 27 studies, normally between 8.00 – 10.00 h, while the frequency of blood sampling varied according to experiments (normally one sample every 14 – 28 d, until approximately wk 12 of lactation, with less frequent sampling thereafter). Blood serum was subsequently analyzed for BHB and NEFA concentrations, while plasma was analyzed for glucose concentrations.

Milk samples for progesterone determination were taken twice weekly (Monday and Thursday) from each cow in 21 studies for approximately 50 DIM. Milk progesterone concentrations were determined using an enzyme-linked immune-sorbent assay kit (Ridgeway Science Ltd, Gloucestershire, UK), based on the method of Sauer et al., (1986), as described in details by McCoy et al., (2006). Interval to the **SLA** was defined as the interval from calving to the first of at least two consecutive increases in milk progesterone concentrations of >3.0 ng/mL (Darwash et al., 1997). Peak progesterone concentration during first luteal cycle was recorded.

8.3.5. Determination of energy contents of the feedstuffs

In all experiments samples of grass and corn silages offered were collected daily and oven dry matter (**ODM**) determined, with fresh samples normally analyzed weekly for nitrogen (**N**), GE and fermentation products. Silage ODM contents were subsequently corrected for volatile losses during drying, with all intakes presented on a volatile corrected DM basis. Samples of dried silage were composited for each 2 to 4 wk period and subsequently analyzed for fiber and ash concentrations.

In early experiments (n = 8) the digestible OM in total DM content (**DOMD**, %) of silages was determined by offering the silage to sheep confined in 'digestibility crates' at maintenance level (normally 4 sheep per silage). The metabolizable energy (**ME**) content of these silages was then estimated by multiplying the DOMD by 0.16 (assuming that one percentage point of DOMD equates to 0.16 MJ/kg DM of ME (AFRC, 1993). The calculated ME values were then corrected to 'production level of feeding' by multiplying by 0.97 (MAFF, 1975; 1984). In later experiments ($n = 20$), the ME value of the forages offered were derived using NIRS as described by Park et al. (1998). In two experiments where neither sheep digestibility data nor NIRS predictions were available, silage DOMD was initially predicted from nutrient composition (DM, Ash, CP, and NDF) and fermentation characteristics of the silages (lactic acid: total VFA ratio) as described by Yan and Agnew (2004: Eq. 14b), and silage ME content estimated by multiplying the DOMD by 0.16. The mean ME content of the silages offered was 11.3±0.58 for grass silages and 11.2±0.35 for corn silages, while the ME content of wheat straw was assumed to be 6.0 MJ/kg DM (FeedByte®, SRUC, Edinburgh, UK).

Concentrates offered were normally sampled weekly, and composite samples analyzed for each 2 to 4 wk period. The ME content of each concentrate was calculated using the ME content of each individual ingredient, based on values reported in UK feed composition tables (FeedByte®). The mean calculated ME content of the concentrate offered was 12.9 (SD, 0.25) MJ/kg DM. Total ME intakes were determined as the sum of the DM intake of each diet component multiplied by the ME content of that component. Further details of analytical methods used to determine the chemical composition of the feedstuffs and fermentation quality of the silages are presented within the individual studies listed in Appendix 1.

8.3.6. Calculations of estimated energy balances (EB)

Individual cow EB values were initially calculated on a daily basis. Daily EB calculations utilized daily DMI and daily milk yield values. For data that was not available on a daily basis (i.e. BW and milk composition data), measured values were applied to each d during the 3 d period pre and post the d of measurement (in the case of weekly measurements) or to the 7 d period pre, and to the 6 d period post the d of measurement (in the case of fortnightly measurements). The mean ME content of all individual silage samples taken from each silo was applied to all d during which that silage was offered.

The daily EB (MJ of ME/d) of each individual cow was calculated using equations contained within 'Feed into Milk' (**FIM**), the current UK dairy cow rationing system, as the difference between the cow's total ME requirements (maintenance, milk production, and activity) and total ME intake (Agnew and Yan, 2004). The sum of ME requirements for maintenance (including activity: standing, vertical movement and body position changes) and milk production ($ME_{maint+milk}$: MJ/kg of $BW^{0.75}$) was determined using Eq. 4.

$$ME_{maint+milk} = \frac{log_e\left[\left[\frac{[5.06 - Milk\ E.per\ kg\ of\ BW^{0.75}]}{[5.06 + 0.453]}\right]\right]}{-0.1326}$$

[4]

Pregnancy requirements were excluded from the EB calculations in the present study since data used within this analysis was until a maximum of 150 DIM, a 42 d voluntary waiting period is adopted within the AFBI herd, and energy cost of pregnancy is only accounted for from wk 14 of gestation in FIM. Energy requirements for 'walking' were included within the EB calculations as described by Agnew et al. (2004: shown in Eq. 5), using the term (0.0013×BW)/k_m, with the efficiency of utilization of ME for walking assumed to be the same as that for maintenance (**k_m**; AFRC, 1993). This assumes a distance walked of 500 m, which was considered appropriate for housed cows on the AFBI farm.

Finally, daily EB (MJ/d) was calculated by using the following equation:

$$EB, MJ\ of\ ME/d\ = \left([ME_{maint+milk} \times BW^{0.75}] + \left[\frac{[0.0013 \times BW]}{k_m}\right] - 10\right) - \ MEi$$

[5]

The term ME_i is the ME intake (MJ/cow per d). Mean weekly EB values were subsequently calculated for each wk post-calving (up to a maximum of wk 20), with calving date considered as d 1 of wk 1 of lactation. Actual values for BCS were collated on a weekly basis using calving dates as reference points.

8.3.7. Statistical analysis

Within each analyses primiparous and multiparous cows were analyzed separately, while the dataset (or part of the data set) for the two parity groups was divided into quartiles (Q1 – Q4) according to the average EB during 4 to 21 DIM. The primary analysis (on which the EB quartiles described were derived) excluded all cows where FOH followed hormonal intervention (233 cows excluded). This analysis compared cow genetic index, cow performance (intakes, milk production and composition, body tissue and blood metabolites), interval (in d) between calving and FOH, and the percentage of cows with FOH pre-d 42, within each EB quartile. The second analysis excluded cows where first AI followed hormone treatment (279 cows excluded) and compared interval (in d) between calving and first AI, and conception to first AI, within each EB quartile. The third analysis involved cows with milk progesterone data available, but excluded cows where SLA followed hormone treatment (37 cows excluded). This analysis compared interval from calving to SLA, peak progesterone concentration at SLA, and the percentage of cows with SLA pre-d 42, within each EB quartile. The final analysis involved the complete dataset and compared the percentage of cows treated for uterine infection, percentage of cows where the FOH followed hormone intervention, and the percentage of cows where first AI followed hormone intervention, within each EB quartile. Individual cows were not fully nested within a study since individual cows were often used in more than one study. All continuous data were analyzed by REML variance component analysis and differences between treatments tested using Fishers unprotected least significance difference test. The model for continuous data included 'experiment' (1 – 27) and cow as the random effect, and 'EB quartile' (1 – 4) as the fixed effect. For continuous data for the period 4 to 150 DIM, days-on-experiment was also included in the model as a fixed effect, if significant. Binomial data were analyzed via Generalized Linear Model regression analysis using the binomial distribution with a logit link function, and differences between treatments tested using Chi square probability test. The model for binomial data included 'experiment' (1 – 27) and cow as the random effect, and EB quartile (1 – 4) as the fixed effect.

Least mean squares evaluating the interaction between EB quartile (1, 2, 3 and 4) and DIM, were determined for BCS, BW and EB (using weekly data), and general trends produced for the experimental period. The model included experiment and cow within study as random effects.

Data from 401 primiparous cows and 742 multiparous cows were included in a survival analysis to examine the effect of EB quartile (mean of 4 to 21 DIM) on interval from calving to first observed estrus (until 80 DIM). Cows which had a FOH following hormone intervention were excluded from the analysis, as were cows removed from the dataset prior to d 80. Cows which had a FOH after 80 DIM were assumed censored (10, 7, 4 and 4 for primiparous cows in Q1 – Q4; 13, 12, 7 and 11 for multiparous cows in Q1 – Q4). Similarly, data from 462 primiparous cows and 923 multiparous cows were included in a survival analysis to examine the effect of EB quartile (mean of 4 to 21 DIM) on 'non pregnancy' (up to 200 DIM). Cows which had a first AI following hormone intervention were excluded from the analysis, as were cows removed from the dataset prior to d 200. Cows which had a first AI after 200 DIM were assumed censored (29, 17, 17 and 23 for primiparous cows in Q1 – Q4, respectively; 64, 55, 55 and 39 for multiparous cows in Q1 – Q4, respectively). Within each of these survival analyses, the effect of EB quartile on cows without an observed heat and cows not pregnant (survival) was compared using four tests: Log-rank, Wilcoxon (Breslow), Tarone-Ware and Wilcoxon (Peto-Prentice). Kaplan-Meier survival functions were estimated for each EB quartile within each of primiparous and multiparous cows. All statistical analyses were performed using GenStat, Version 20.1 (VSN International Limited, 2019). For all models, statistical significance was declared at $P \leq 0.05$ and trends at $P > 0.05$ to $P < 0.10$.

8.4. RESULTS

With the exception of PTA for fertility, which increased between Q1 and Q4 ($P = 0.025$), none of the genetic values presented for primiparous cows differed ($P > 0.10$) between EB quartiles (Table 1). With multiparous cows, Q1 cows had a lesser PLI than Q4 cows ($P = 0.048$), while none of the other genetic values presented differed between quartiles ($P > 0.10$). With primiparous cows (Table 2) neither concentrate percentage in the diet nor BW differed ($P > 0.05$) between quartiles in either early lactation (4 to 21 DIM), or over the entire experimental period (4 to 150 DIM). During both periods DMI and ME intake increased from Q1 to Q4 ($P < 0.001$), while milk protein content increased in early lactation ($P = 0.008$) and over the entire lactation ($P = 0.002$). In contrast, milk yield, ECM yield and ECM/DMI (Table 2) decreased from Q1 through to Q4 in both periods ($P < 0.001$). Milk fat content decreased from Q1 to Q4 in early lactation ($P < 0.001$), but not over the entire experimental period ($P > 0.05$), while milk fat-to-protein ratio decreased from Q1 to Q4 during both early lactation ($P < 0.001$) and over the entire period ($P = 0.005$). Body condition score did not differ between quartiles during early lactation (4 to 21 DIM), but increased from Q1 to Q4 over the entire experimental period ($P < 0.001$). During early lactation plasma NEFA and BHB concentrations decreased from Q1 to Q4 ($P < 0.001$), while plasma glucose concentrations increased ($P < 0.001$), with similar effects observed over the entire experimental period ($P = 0.008$, $P = 0.004$ and $P < 0.001$, respectively).

Multiparous cows in each of EB quartiles 1 – 4 had a mean lactation number of 3.3, 2.9, 2.9 and 2.8, respectively ($P = 0.021$: Table 3). Concentrate proportion in the diet did not differ ($P > 0.10$) between quartiles in either period. Total DMI and total ME intake increased ($P < 0.001$) from Q1 through to Q4 in early lactation, but not over the entire experimental period ($P > 0.05$). Each of milk yield, ECM, ECM/DMI and BW decreased ($P < 0.001$) from Q1 to Q4 during both periods. Milk fat content and FPR decreased from Q1 to Q4 in early lactation ($P < 0.001$), and over the entire experimental period ($P = 0.002$ and $P < 0.001$, respectively), while milk protein content followed the reverse trend in both periods ($P < 0.001$). While BCS decreased from Q1 to Q4 in early lactation (*P* < 0.001) BCS did not differ between quartiles over the entire experimental period (*P* > 0.05). During both experimental periods plasma NEFA and BHB concentrations decreased from Q1 to Q4 (P < 0.001), while plasma glucose concentrations increased (*P* < 0.001).

Table 1. Predicted transmitting ability (PTA) for primiparous and multiparous cows within each EB quartile, with quartiles based on mean daily energy balance during 4 to 21 DIM.

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
Primiparous cows	Q1 (-120 to -49 MJ/d)	Q2 (-49 to -24 MJ/d)	Q3 (-24 to -3 MJ/d)	Q4 (-3 to 92 MJ/d)	SED	*P*-value
PTA milk (kg)	18.5	-9.2	-15.8	-43.9	26.93	0.208
PTA milk fat (kg)	6.4	5.3	4.3	3.7	0.98	0.065
PTA milk protein (kg)	6.0	5.2	4.7	4.2	0.71	0.117
PTA milk fat (%)	0.07	0.07	0.06	0.06	0.012	0.842
PTA milk protein (%)	0.06	0.07	0.06	0.07	0.006	0.545
PTA fertility	-1.6^{a}	-0.7^{ab}	-0.6^{ab}	0.6^{b}	0.66	0.025
Profitable Lifetime Index (£)	86.6	83.9	70.7	79.9	16.30	0.777
Multiparous cows	Q1 (-191 to -79 MJ/d)	Q2 (-79 to -48 MJ/d)	Q3 (-48 to -22 MJ/d)	Q4 (-22 to 93 MJ/d)	SED	*P*-value
PTA milk (kg)	-64.9	-55.8	-43.4	-39.2	11.23	0.140
PTA milk fat (kg)	2.4	2.5	3.0	3.2	0.32	0.085
PTA milk protein (kg)	3.2	3.4	3.6	3.8	0.27	0.227
PTA milk fat (%)	0.06	0.06	0.06	0.06	0.002	0.973
PTA milk protein (%)	0.07	0.07	0.07	0.07	0.001	0.434
PTA fertility	-0.5	-0.7	-0.5	-0.5	0.17	0.270
Profitable Lifetime Index (£)	40.6^{a}	45.2^{ab}	55.8^{b}	58.7^{b}	6.83	0.048

^{a,b} Values within a row with different superscript lowercase letters differ at *P* < 0.05.

Neither the percentage of primiparous cows treated for uterine infection, nor the percentage of primiparous cows where FOH followed hormone intervention, differed between quartiles (*P* > 0.10) (Table 4). While d to FOH decreased from Q1 to Q4 (*P* = 0.049), the percentage of cows with FOH pre d 42 followed the reverse trend, increasing from Q1 to Q4 (*P* = 0.019). None of the percentage of cows where first AI followed hormone intervention, d to first AI, conception to first AI, or cows pregnant during the first 21 or 42 of the breeding season, differed between quartiles in primiparous cows (*P* > 0.05). However, there was a tendency for an increased percentage of cows in Q3 and Q4 to be pregnancy during the first 84 d of the breeding season (*P* = 0.072). For the sub-set of primiparous cows for which progesterone data was available (Table 4), the interval from calving to SLA decreased from Q1 to Q4 (*P* < 0.001), while peak progesterone concentration at SLA and the percentage of cows with SLA pre d 42 increased from Q1 to Q4 (*P* < 0.001 and *P* = 0.009, respectively).

Within the multiparous cow dataset, the percentage cows treated for uterine infection and the percentage of cows where FOH followed hormone intervention, did not differ between EB quartiles ($P > 0.10$) (Table 5). However, d to FOH was greater with cows in Q1 than cows in Q4 ($P = 0.012$), while the percentage of cows with FOH pre d 42 increased from Q1 to Q4 ($P = 0.038$). None of the percentage of multiparous cows where first AI followed hormone treatment, d to first AI, conception to first AI, and cows pregnant during the first 21 or 42 d of the breeding season differed between quartiles ($P > 0.05$). However, while the percentage of cows pregnant during the first 84 d of the breeding season tended to differ between quartiles ($P = 0.087$), there was no consistent trend between Q1 – Q4. Days from calving to SLA decreased (by 5.8 d) from Q1 to Q4 ($P = 0.003$), while peak progesterone concentration increased by 3.7 ng/mL between Q1 and Q4 ($P = 0.026$). The percentage of multiparous cows with SLA pre d 42 did not differ between quartiles ($P > 0.10$).

Time trends for BCS, BW and EB within each of EB quartiles 1 - 4 are presented in Figure 1 for primiparous and multiparous cows. With the exception of BCS for multiparous cows ($P = 0.101$; Figure 1B) and BW for primiparous cows ($P = 0.398$; Figure 1C) all other parameters differed between quartiles. In addition, there was a significant effect of DIM on all parameters ($P < 0.001$), and a significant interaction between EB quartile and DIM ($P < 0.001$).

Survival curves (FOH before d 80 DIM) for each of the EB quartiles within primiparous and multiparous cows, produced using the Kaplan-Meier survival function, are presented in Figures 2A and 2B, respectively. The estimated time to 25%, 50% and 75% of primiparous cows having a FOH was 33, 50 (95% CI: 44 – 53) and 62 d (Q1), 24, 40 (95% CI: 35 – 45) and 57 d (Q2), 22, 39 (95% CI: 32 – 45) and 56 d (Q3), 21, 34 (95% CI: 30 - 37) and 46 d (Q4). Similarly, the estimated time to 25%, 50% and 75% of multiparous cows having a FOH was 32, 47 (95% CI: 42 – 53) and 61 d (Q1), 26, 43 (95% CI: 38 – 47) and 61 d (Q2), 24, 41 (95% CI: 36 – 45) and 57 d (Q3), 20, 36 (95% CI: 33 - 40) and 54 d (Q4). Differences in survival between EB quartiles, tested using the Log-rank, Wilcoxon (Breslow), Tarone-Ware, Wilcoxon (Peto-Prentice) were found to be significant in both primiparous (All, $P < 0.001$) and multiparous ($P < 0.039$, $P < 0.002$, $P < 0.007$ and $P < 0.002$, respectively) cows.

Table 2. Concentrate proportion in the diet, performance indicators and biological responses of primiparous cows during 4 to 21 DIM, and during 4 to 150 DIM, with quartiles based on mean daily energy balance during 4 to 21 DIM[1]

	Quartiles (mean daily energy balance during 4 to 21 DIM)[1]					
Item[2]	Q1 (-120 to -49 MJ/d)	Q2 (-49 to -24 MJ/d)	Q3 (-24 to -3 MJ/d)	Q4 (-3 to 92 MJ/d)	SED	*P*-value
Days 4 to 21 in milk						
Concentrate proportion in diet	0.50	0.53	0.54	0.52	0.015	0.123
Total DMI (kg/d)	11.5^{a}	12.7^{b}	13.2^{c}	14.3^{d}	0.22	<0.001
Total ME intake (MJ/d)	140^{a}	154^{b}	161^{c}	175^{d}	2.71	<0.001
Milk yield (kg/d)	25.2^{d}	24.0^{c}	22.2^{b}	21.1^{a}	0.44	<0.001
ECM (kg/d)	28.4^{d}	26.0^{c}	23.4^{b}	22.0^{a}	0.48	<0.001
ECM/DMI	2.52^{d}	2.01^{c}	1.79^{b}	1.55^{a}	0.020	<0.001
EB (MJ/d)	-67^{a}	-35^{b}	-15^{c}	14^{d}	0.86	<0.001
Milk fat (g/kg)	49.4^{c}	45.6^{b}	43.4^{a}	42.4^{a}	0.80	<0.001
Milk protein (g/kg)	34.0^{a}	34.8^{b}	35.2^{b}	35.3^{b}	0.37	0.008
FPR	1.46^{c}	1.32^{b}	1.24^{a}	1.21^{a}	0.024	<0.001
BW (kg)	521	514	507	511	5.72	0.123
BCS	2.74	2.69	2.73	2.70	0.036	0.484
Plasma NEFA (mmol/mL)	0.63^{c}	0.53^{b}	0.51^{b}	0.43^{a}	0.041	<0.001
Plasma BHB (mmol/l)	0.75^{b}	0.61^{a}	0.54^{a}	0.52^{a}	0.055	<0.001
Plasma glucose (mmol/l)	3.20^{a}	3.33^{b}	3.43^{c}	3.47^{c}	0.053	<0.001
Days 4 to 150 in milk						
Concentrate proportion in diet	0.50	0.52	0.52	0.52	0.015	0.242
Total DMI (kg/d)	15.4^{a}	16^{b}	16.2^{b}	16.7^{c}	0.24	<0.001
Total ME intake (MJ/d)	187^{a}	195^{b}	197^{b}	204^{c}	3.12	<0.001
Milk yield (kg/d)	28.7^{c}	27.8^{c}	26.2^{b}	24.9^{a}	0.54	<0.001
ECM (kg/d)	29.5^{d}	28.3^{c}	26.6^{b}	25.6^{a}	0.50	<0.001
ECM/DMI	1.95^{d}	1.79^{c}	1.65^{b}	1.53^{a}	0.019	<0.001
EB (MJ/d)	-22^{a}	-7^{b}	3^{c}	17^{d}	2.39	<0.001
Milk fat (g/kg)	41.6	41.0	40.7	41.4	0.59	0.435
Milk protein (g/kg)	32.5^{a}	33.0^{ab}	33.2^{bc}	33.7^{c}	0.29	0.002
FPR	1.28^{b}	1.25^{a}	1.23^{a}	1.23^{a}	0.017	0.005
BW (kg)	512	514	516	520	5.5	0.535
BCS	2.51^{a}	2.52^{a}	2.59^{b}	2.62^{b}	0.027	<0.001
Plasma NEFA (mmol/mL)	0.44^{b}	0.41^{b}	0.37^{a}	0.36^{a}	0.023	0.008
Plasma BHB (mmol/l)	0.66^{b}	0.60^{a}	0.59^{a}	0.58^{a}	0.024	0.004
Plasma glucose (mmol/l)	3.26^{a}	3.37^{b}	3.45^{c}	3.49^{c}	0.026	<0.001

[a,b,c,d] Values within a row with different superscript lowercase letters differ at $P < 0.05$.

[1] Excludes cows where first observed heat followed hormone intervention.

[2] ECM= energy corrected milk; ECM/DMI= gross feed efficiency; EB= energy balance; FPR= milk fat-to-protein-ratio; NEFA= non-esterified fatty acids; BHB= β-hydroxybutyrate.

Table 3. Concentrate proportion in the diet, performance indicators and biological responses of multiparous cows during 4 to 21 DIM, and during 4 to 150 DIM, with quartiles based on mean daily energy balance during 4 to 21 DIM.

	Quartiles (mean daily energy balance during 4 to 21 DIM)[1]					
Item[2]	Q1 (-191 to -79 MJ/d)	Q2 (-79 to -48 MJ/d)	Q3 (-48 to -22 MJ/d)	Q4 (-22 to 93 MJ/d)	SED	*P*-value
Lactation number (upper and lower confidence limit)	3.3 (3.00 – 3.59	2.9 (2.66 – 3.20)	2.9 (2.62 – 3.15)	2.8 (2.50 – 3.03)	-	0.021
Days 4 to 21 in milk						
Concentrate proportion in diet	0.52	0.52	0.50	0.50	0.009	0.785
Total DMI (kg/d)	15.5^{a}	16.8^{b}	16.8^{b}	18.0^{c}	0.24	<0.001
Total ME intake (MJ/d)	188^{a}	204^{b}	205^{b}	219^{c}	3.01	<0.001
Milk yield (kg/d)	37.2^{d}	35.4^{c}	32.2^{b}	29.5^{a}	0.50	<0.001
ECM (kg/d)	41.9^{d}	38.3^{c}	34.7^{b}	30.9^{a}	0.54	<0.001
ECM/DMI	2.73^{d}	2.28^{c}	2.10^{b}	1.75^{a}	0.017	<0.001
EB (MJ/d)	-103^{a}	-61^{b}	-34^{c}	-5^{d}	0.91	<0.001
Milk fat (g/kg)	49.6^{c}	46.0^{b}	45.3^{b}	42.6^{a}	0.58	<0.001
Milk protein (g/kg)	34.8^{a}	35.3^{b}	35.7^{bc}	36.0^{c}	0.26	<0.001
FPR	1.43^{c}	1.30^{b}	1.27^{b}	1.19^{a}	0.017	<0.001
BW (kg)	609^{d}	598^{c}	579^{b}	565^{a}	5.6	<0.001
BCS	2.58^{b}	2.53^{b}	2.45^{a}	2.45^{a}	0.029	<0.001
Plasma NEFA (mmol/mL)	0.70^{d}	0.58^{c}	0.52^{b}	0.42^{a}	0.028	<0.001
Plasma BHB (mmol/l)	0.82^{c}	0.74^{b}	0.69^{b}	0.61^{a}	0.038	<0.001
Plasma glucose (mmol/l)	2.89^{a}	2.97^{b}	3.00^{b}	3.08^{c}	0.036	<0.001
Days 4 to 150 in milk						
Concentrate proportion in diet	0.51	0.51	0.51	0.51	0.009	0.818
Total DMI (kg/d)	19.8	20.1	19.9	20.3	0.21	0.086
Total ME intake (MJ/d)	241	245	242	247	2.70	0.053
Milk yield (kg/d)	38.7^{d}	37.1^{c}	35.1^{b}	29.5^{a}	0.50	<0.001
ECM (kg/d)	39.9^{d}	37.7^{c}	35.7^{b}	33.7^{a}	0.47	<0.001
ECM/DMI	2.03^{d}	1.89^{c}	1.80^{b}	1.68^{a}	0.022	<0.001
EB (MJ/d)	-35^{a}	-17^{b}	-7^{c}	11^{d}	2.23	<0.001
Milk fat (g/kg)	42.6^{b}	41.5^{a}	41.4^{a}	41.2^{a}	0.39	0.002
Milk protein (g/kg)	32.6^{a}	33.0^{b}	33.3^{b}	33.8^{c}	0.17	<0.001
FPR	1.31^{c}	1.25^{b}	1.24^{ab}	1.22^{a}	0.011	<0.001
BW (kg)	602^{b}	595^{b}	585^{a}	578^{a}	4.8	<0.001
BCS	2.37	2.39	2.39	2.40	0.020	0.654
Plasma NEFA (mmol/mL)	0.45^{d}	0.37^{c}	0.34^{b}	0.28^{a}	0.013	<0.001
Plasma BHB (mmol/l)	0.69^{c}	0.64^{b}	0.63^{ab}	0.60^{a}	0.016	<0.001
Plasma glucose (mmol/l)	3.13^{a}	3.18^{b}	3.19^{b}	3.25^{c}	0.017	<0.001

[a,b,c,d] Values within a row with different superscript lowercase letters differ at $P < 0.05$.

[1] Excludes cows where first observed heat followed hormone intervention.

[2] ECM= energy corrected milk; ECM/DMI= gross feed efficiency; EB= energy balance; FPR= milk fat-to-protein-ratio; NEFA= non-esterified fatty acids; BHB= β-hydroxybutyrate.

Survival curves (not-pregnant cows before d 200 DIM) for each of the EB quartiles within primiparous and multiparous cows, produced using the Kaplan-Meier survival function, are presented in Figures 3A and 3B, respectively. The estimated time to 25%, 50% and 75% of primiparous cows becoming pregnant were 81, 112 (95% CI: 98 – 137) and 187 d (Q1), 82, 119 (95% CI: 107 – 137) and 168 d (Q2), 72, 96 (95% CI: 86 – 108) and 139 d (Q3), 78, 111 (95% CI: 96 - 118) and 162 d (Q4). Similarly, the estimated time to 25%, 50% and 75% of multiparous cows becoming pregnant were 82, 117 (95% CI: 109 – 129) and 177 d (Q1), 75, 108 (95% CI: 99 – 118) and 177 d (Q2), 76, 116 (95% CI: 105 – 125) and 185 d (Q3), 76, 104 (95% CI: 98 - 111) and 151 d (Q4).

Differences in survival between EB quartiles, tested using the Log-rank, Wilcoxon (Breslow), Tarone-Ware, Wilcoxon (Peto-Prentice) were as follows in primiparous cows ($P < 0.143$, $P < 0.062$, $P < 0.087$ and $P < 0.064$, respectively) and for multiparous cows ($P = 0.191$, $P = 0.301$, $P = 0.240$, $P = 0.295$, respectively).

8.5. DISCUSSION

Primiparous and multiparous cows were examined separately within this study as the former have significant energy requirements for growth, and are more sensitive to NEB than multiparous cows (Wathes et al., 2007; Macmillan et al., 2018). During the period between 4 to 21 DIM, 78% of primiparous cows and 87% of multiparous cows had a mean EB that was negative. While there is a significant body of research evidence that 'energy status' in early lactation can have adverse effects on fertility traits, many studies have involved relatively small numbers of cows, while other studies have involved indirect measures of EB such as BCS and BW (Buckley et al., 2003; Middleton et al., 2019). In contrast, the current study allows the relationships between calculated EB (determined as the difference between ME intake minus energy requirements, according to equations within Feed into Milk (Agnew et al., 2004)) and fertility parameters to be examined using a large dataset. Furthermore, the availability of information on cow genotype and milk progesterone, together with detailed information on DMI, milk production, milk composition, body weight, BCS, and blood metabolites during both early lactation, and over the first 150 d of lactation, provide a more holistic picture within which to interpret the EB trends and the fertility outcomes observed.

Table 4. Mean fertility performance of primiparous cows within EB quartiles based on mean daily energy balance during 4 to 21 DIM (for binomial data, upper and lower confidence limit in parenthesis).

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
Item[1]	Q1 (-120 to -49 MJ/d)	Q2 (-49 to -24 MJ/d)	Q3 (-24 to -3 MJ/d)	Q4 (-3 to 92 MJ/d)	SED	*P*-value
Percentage of cows treated for uterine infection (entire dataset)[2]	14 (7 - 27)	11 (5 - 23)	18 (9 - 33)	10 (4 -21)	-	0.283
Percentage of cows where FOH followed hormone intervention (entire dataset)[2]	17 (9 - 30)	13 (7 - 24)	9 (4 - 17)	9 (4 - 17)	-	0.273
Days to FOH[3]	47.7^{b}	42.6^{ab}	41.4^{ab}	37.9^{a}	2.38	0.049
Percentage of cows with FOH pre d 42[3]	46 (31 – 62)	58 (43 – 72)	54 (38 – 68)	72 (57 – 84)	-	0.019
Percentage of cows where first AI followed hormone intervention (entire dataset)[2]	20 (10 - 34)	20 (11 - 34)	12 (6 - 23)	14 (7 - 26)	-	0.360
Days to first AI[3]	72.4	71.1	68.9	69.3	3.31	0.735
Conception to first AI (percentage)[4]	32 (24 - 43	30 (21 - 39)	32 (23 - 41)	31 (22 - 40)	-	0.974
Cows pregnant during						
First 21 d of breeding season (percentage)	33 (24 - 43)	24 (16 - 33)	28 (20 - 38)	26 (18 -36)	-	0.507
First 42 d of breeding season (percentage)	48 (39 - 58)	40 (31 - 49)	52 (42 - 60)	46 (37 - 56)	-	0.355
First 84 d of breeding season (percentage)	70 (61 - 78)	66 (56 - 74)	79 (70 - 86)	78 (70 - 86)	-	0.072
For sub-set of cows with progesterone data available[5]						
Interval from calving to SLA	34.6^{c}	28.8^{b}	31.6^{bc}	24.4^{a}	1.59	<0.001
Peak progesterone concentration at SLA (ng/mL)	26.7^{a}	33.6^{bc}	30.5^{ab}	34.2^{c}	1.95	<0.001
Percentage of cows with SLA pre d 42	69 (51 - 83)	83 (69 - 92)	74 (57 - 85)	91 (79 - 96)	-	0.009

^{a,b,c}Values within a row with different superscript differ at $P < 0.05$.

[1] FOH= first observed heat; SLA= start of luteal activity; AI= artificial insemination.

[2] Based on entire data set: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the entire data set were -120 to -50, -50 to -24, -24 to -3 and -3 to 92 MJ/d, respectively

[3] Excludes cows where first observed heat followed hormone intervention.

[4] Excludes cows were first AI followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 were -111 to -50, -50 to -24, -24 to -4 and -4 to 92 MJ/d, respectively.

[5] Excludes cows where SLA followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the data sub-set were -120 to -50, -50 to -25, -25 to -3 and -3 to 69 MJ/d, respectively.

Table 5. Mean fertility performance of multiparous cows within EB quartiles based on mean daily energy balance during 4 to 21 DIM (for binomial data, upper and lower confidence limit in parenthesis).

	Quartiles (mean daily energy balance during 4 to 21 DIM)					
Item[1]	Q1 (-191 to -79 MJ/d)	Q2 (-79 to -48 MJ/d)	Q3 (-48 to -22 MJ/d)	Q4 (-22 to 93 MJ/d)	SED	*P*-value
Percentage of cows treated for uterine infection (entire dataset)[2]	15 (9 - 22)	11 (6 - 18)	14 (9 - 22)	13 (8 -21)	-	0.741
Percentage of cows where FOH followed hormone intervention (entire dataset)[2]	17 (10 - 27)	15 (9 - 24)	14 (8 - 22)	17 (10 - 27)	-	0.766
Days to FOH[3]	49.0^{b}	44.9^{ab}	42.9^{a}	41.6^{a}	2.07	0.012
Percentage of cows with FOH pre d 42[3]	41 (29 - 53)	49 (38 - 61)	55 (43 - 66)	58 (46 - 69)	-	0.038
Percentage of cows where first AI followed hormone intervention (entire dataset)[2]	19 (12 - 29)	21 (14 - 31)	16 (9 - 24)	19 (12 - 29)	-	0.445
Days to first AI[4]	72.7	69.9	68.9	68.6	3.04	0.557
Conception to first AI (percentage)[4]	28 (22 - 35)	29 (24 - 36)	26 (21 - 33)	33 (26 - 40)	-	0.672
Cows pregnant during						
First 21 d of breeding season (percentage)	22 (16 - 29)	20 (15 - 27)	25 (19 - 33)	24 (18 -32)	-	0.629
First 42 d of breeding season (percentage)	43 (35 - 50)	45 (38 - 53)	39 (32 - 47)	45 (37 - 53)	-	0.554
First 84 d of breeding season (percentage)	73 (65 - 80)	75 (68 - 82)	68 (50 - 75)	78 (70 - 84)	-	0.087
For sub-set of cows with progesterone data available[5]						
Interval from calving to SLA	35.7^{b}	29.4^{a}	31.0^{a}	29.8^{a}	1.29	0.003
Peak progesterone concentration at SLA (ng/mL)	24.4^{a}	25.9^{ab}	27.4^{b}	28.1^{b}	2.14	0.026
Percentage of cows with SLA pre d 42	70 (59 - 80)	83 (75 - 90)	78 (68 - 86)	79 (69 - 86)	-	0.103

[a,b] Values within a row with different superscript differ at $P < 0.05$.

[1] FOH= first observed heat; SLA= start of luteal activity; AI= artificial insemination.

[2] Based on entire data set: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the entire data set were -191 – -80, -80 – -47, -47 – -22 and -22 – 93 MJ/d, respectively.

[3] Excludes cows where first observed heat followed hormone intervention.

[4] Excludes cows were first AI followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 were -191 – -79, -79 – -48, -48 – -21 and -21 – 93 MJ/d, respectively.

[5] Excludes cows where SLA followed hormone intervention: actual energy balance range during 4 to 21 DIM for each of Q1 – Q4 within the data sub-set were -185 – -71, -71 – -43, -43 – -16 and -16 – 93 MJ/d, respectively

8.5.1. Relationships between DMI, milk production and energy balance

During the early lactation period (4 to 21 DIM) EB within both parity groups was determined to a large extent by the relationship between DMI and ECM yield, cows with a greater DMI and a lesser ECM having an increased EB. While differences in DMI and ECM continued to drive EB in primiparous cows over the entire experimental period (4 to 150 DIM), differences in EB profiles between quartiles in multiparous cows were driven largely by ECM and not intakes. Broadly similar relationships between ECM and EB have been observed by Patton et al. (2007). The lesser intakes and greater milk yields observed with increasing NEB did not appear to be driven by diet, as concentrate proportion in the diet (an important driver of intake: Lawrence et al., 2015), did not differ between quartiles in either primiparous or multiparous cows. Similarly, these differences are unlikely to have been due to the relatively small difference in lactation number between quartiles with the multiparous cows, while PTA for milk also did not differ between quartiles. While pre-partum management can influence performance post calving, with high BCS cows known to have lesser DMI following calving (Roche et al., 2009; Weber et al., 2013), differences in BCS between EB quartiles were small in both primiparous and multiparous cows. Furthermore, Roche et al. (2009) have suggested that a reduction in intake post-calving only becomes an issue for cows with a BCS greater than 3.5 (5-point scale), considerably greater than mean BCS in the current study.

The increased concentrations of NEFA and BHB in blood in the lower quartile cows may also have inhibited intake during the early lactation. According to the ‘hepatic oxidation theory’ (Allen et al., 2009), for animals in a lipolytic state with shortage of glucose precursors, increasing NEFA concentration, and consequently higher hepatic oxidation, can impact the satiety center decreasing feed intake. Furthermore, greater concentrations of BHB (a ketone body) can also suppress feed intake by hepatic oxidation (Allen and Piantoni, 2013).

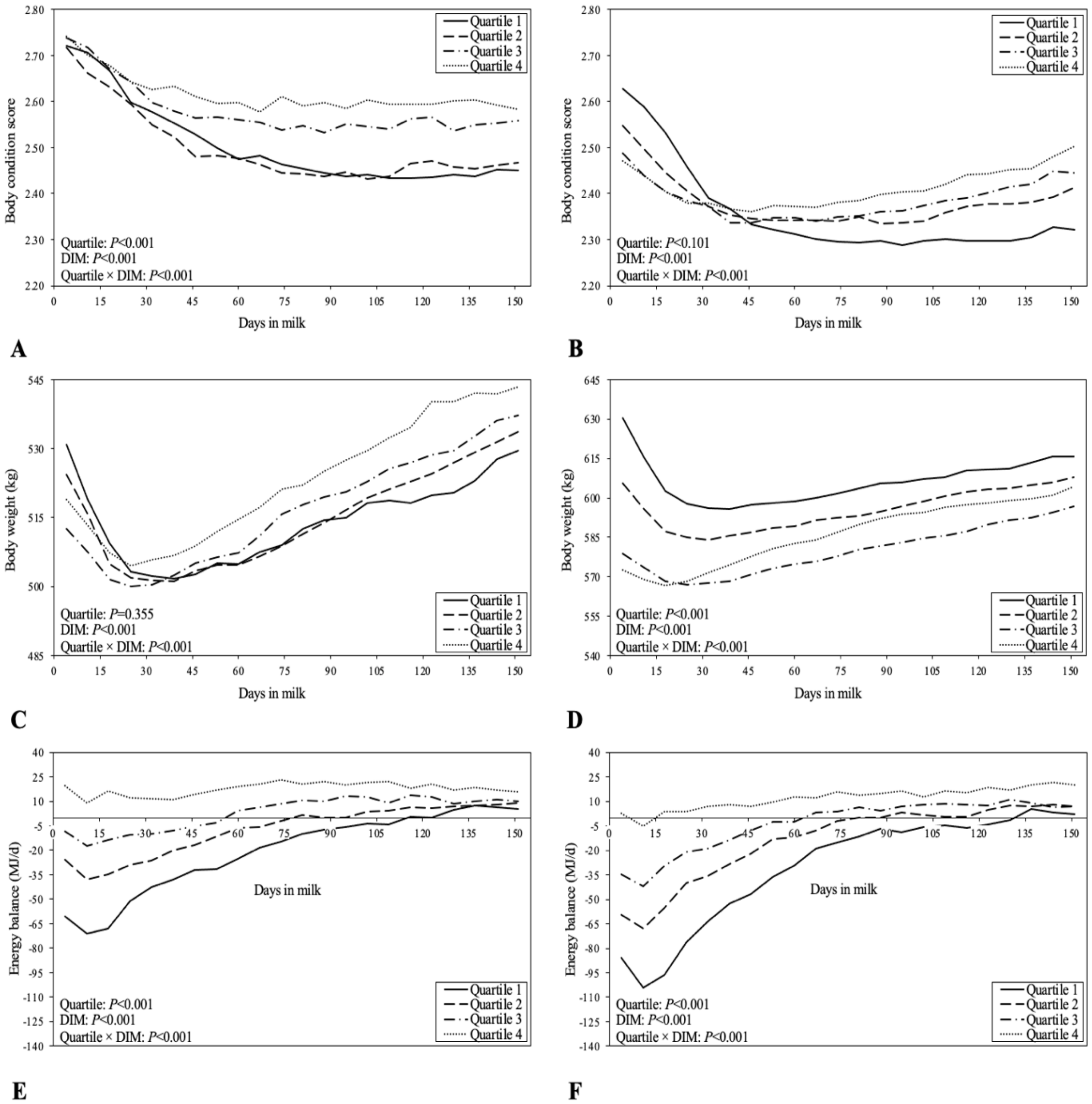

Figure 1. Least squares means (weekly basis, from 4 to 150 DIM), for body condition score (Figures 1A and 1B, primiparous and multiparous cows respectively), body weight (Figures 1C and 1D, primiparous and multiparous cows respectively), and daily energy balance (Figures 1E and 1F, primiparous and multiparous cows respectively) for cows within EB quartiles (Q1, Q2, Q3, and Q4) during early lactation.

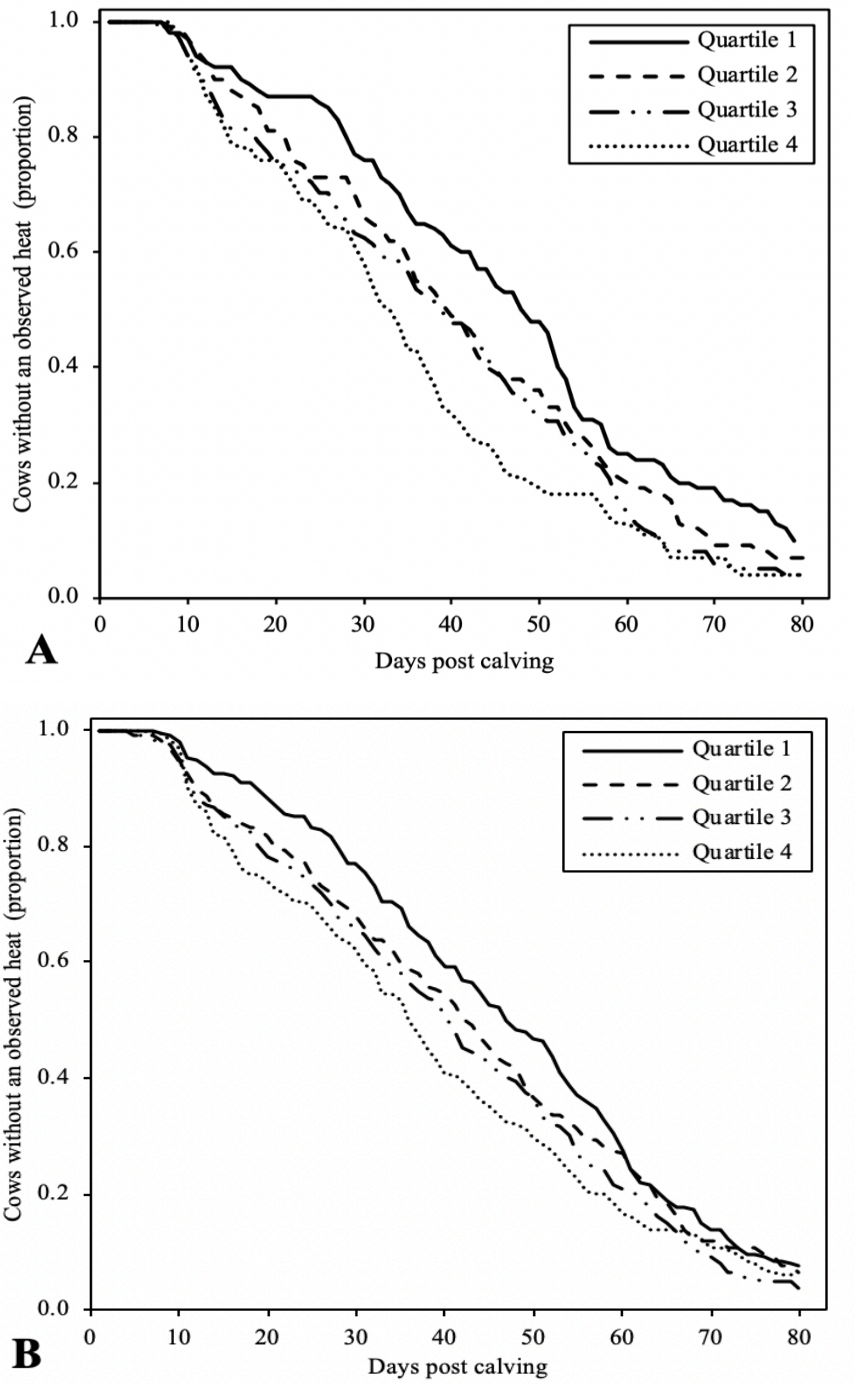

Figure 2. Survival curves showing the effect of EB quartile (based on mean daily EB during 4 - 21 DIM) on the proportion of (A) primiparous ($P < 0.001$: Q1 – 4, -120 to -49, -49 to -24, -24 to -3 and -3 to 92 MJ/d, respectively) and (B) multiparous cows ($P < 0.002$: Q1 – 4, -191 to -79, -79 to -48, -48 to -21 and -21 to 93 MJ/d, respectively) without an observed heat during the first 80 DIM.

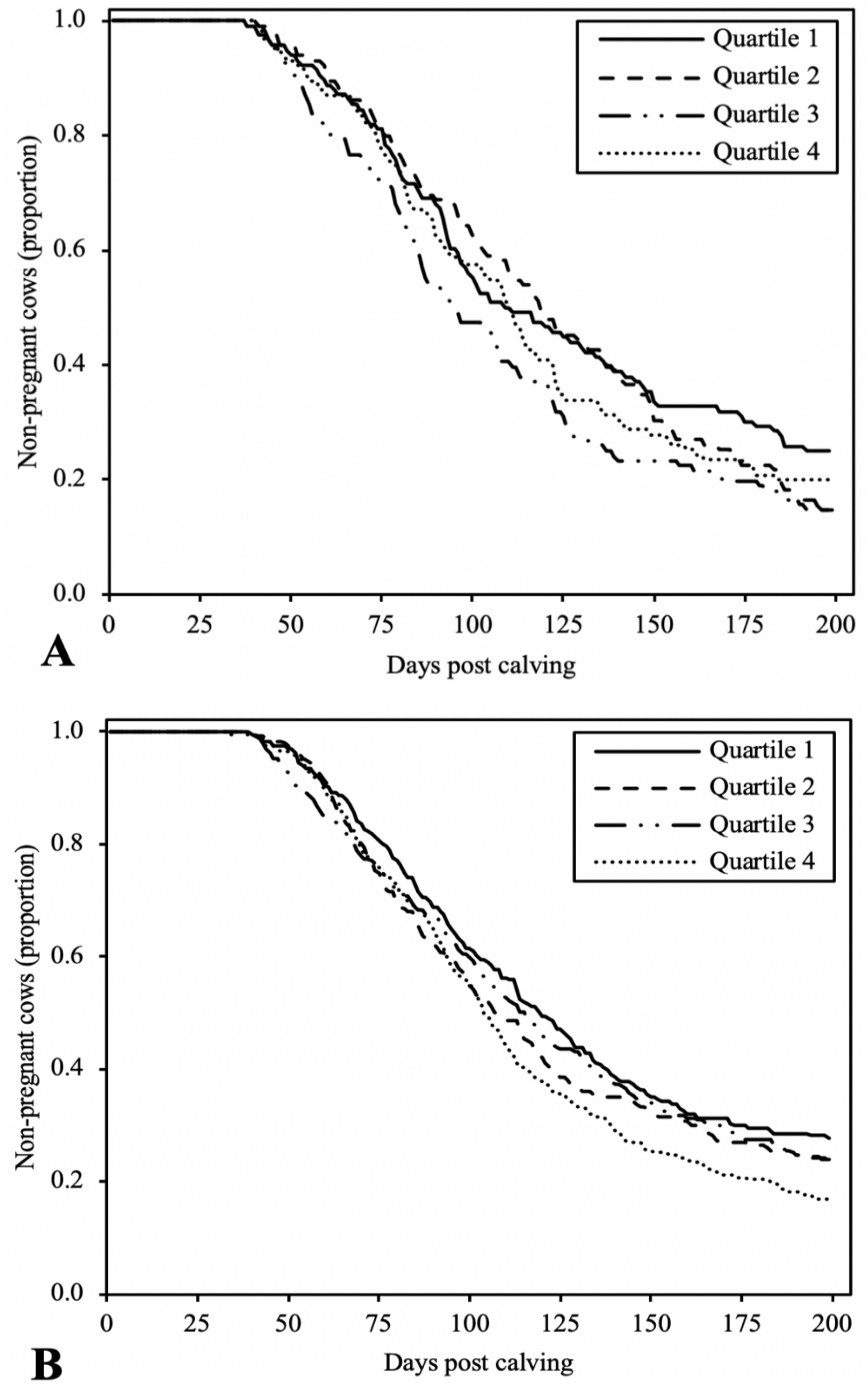

Figure 3. Survival curves showing the effect of EB quartile (based on mean daily EB during days 4 - 21 of lactation) on the proportion of (A) primiparous ($P > 0.05$)]: Q1 – 4, -120 to -50, -50 to -24, -24 to -3 and -3 to 92 MJ/d, respectively) and (B) multiparous cows ($P > 0.05$): Q1 – 4, -191 to -80, -80 to -47, -47 to -22 and -22 to 93 MJ/d, respectively) that were not pregnant during the first 200 days post calving.

8.5.2. Relationships between milk composition, blood metabolites and energy balance

The milk composition and blood metabolite data fully support the EB trends observed. Consistent across both primiparous and multiparous cows, those with a greater NEB during d 4 to 21 DIM had a greater milk fat content. That PTA for milk fat did not differ between quartiles suggests that this was not a 'genetic effect'. Rather, greater milk fat contents in early lactation, as observed previously by Gobikrushanth et al. (2019), is associated with cows mobilizing body tissue reserves, and the incorporation of longer chain length fatty acids into milk fat (Bauman and Griinari, 2001). This is supported by the blood metabolite data for this period, and is reflected in greater NEFA concentrations (arising from the mobilization of body fat reserves to produce glycerol for energy) and greater BHB concentrations (arising from the incomplete oxidation of NEFA to ketones) (Allen and Piantoni, 2013). Blood glucose concentrations followed the reverse trend, increasing with increasing EB, as observed by Gross et al. (2011). The decrease in milk protein content with decreasing EB is also as expected (Beever et al., 2001), and is likely due to a reduced supply of amino acids for milk protein synthesis due to insufficient energy supply for microbial protein synthesis, and for protein synthesis in mammary gland (Nousiainen et al., 2004). The differing trends in milk fat and milk protein contents were reflected in a decreasing FPR ratio with increasing EB. Fat-to-protein ratio is often highlighted as an indicator of energy status (Gross and Bruckmaier, 2019), with a FPR >1.5 claimed to be indicative of cows with sever metabolic stress (Heuer et al., 1999). While differences in mean BCS between quartiles in early lactation (4 – 21 DIM) appear small, the weekly trends presented in Figure 1 highlight distinct differences between primiparous and multiparous cows at this time. For example, while primiparous cows within all EB quartiles had a similar BCS at calving, the range in BCS for multiparous cows was much greater, cows with the highest BCS being in the lowest EB quartile, and vice versa for cows with the lowest BCS. Thus multiparous cows with greater BCS at calving were more prone to body tissue loss.

While the magnitude of many of the differences between EB quartiles observed in early lactation decreased when observe over the entire experimental period, most differences did remain. This is hardly surprising given that differences in weekly EB, BCS and BW profiles between EB quartiles remained throughout the duration of the study. Nevertheless, the large differences in milk fat content observed in early lactation had largely disappeared when examined over the entire experimental period (although still significant in multiparous cows).

8.5.3. Effect of energy balance on incidence of uterine infection and hormone treatments

The link between energy status and uterine infection has also been established, with Vercouteren et al. (2015) observing that cows that lost less BW had a lesser incidence of metritis, while Galvão et al. (2010a) found the incidence of uterine diseases (both clinical and subclinical) to be positively correlated with increasing NEB. Thus the absence of an effect of EB quartile on the percentage of cows treated for uterine infection in the current study was surprising. It is possible that this is due to 'treatment' for infection being on the basis of visual observation followed by clinical examination, rather than a structured check of all cows. In addition, the percentage of cows observed with uterine infection in the current study was relatively low (13%), compared to 25% observed by Galvão et al. (2010a) on commercial farms, perhaps reflecting a higher standard of management within a research environment than on commercial farms.

That the percentage of cows treated with hormones was not affected by EB quartile is perhaps not surprising as interventions did not take place before 70 - 80 DIM in the majority of studies, a time when actual EB differences between quartiles were much smaller. Nevertheless, these cows were not included in the analyses of FOH due to the impact of human intervention, rather than natural onset of estrus (Lucy et al., 2004).

8.5.4. Relationships between energy balance, start of luteal activity and first observed heat

Data on SLA was available from a sub-set of cows, with SLA occurring an average of 12.7 d earlier than FOH. This is not unexpected as progesterone priming influences how estradiol stimulates the hypothalamus, and consequently estrus expression (Sauls et al., 2017). Also, poor energy status during early lactation may decrease estradiol production in the pre-ovulatory follicle, and reduce the sensitivity of the hypothalamus to estradiol resulting in 'silent ovulations' (Ranasinghe et al., 2010). The effects of EB in early lactation on both SLA and FOH were fully aligned, both of these events occurring earlier with improved early lactation energy status. In addition, the percentage of cows showing both SLA and FOH before 42 DIM increased, or tended to increase, with increasing EB in both primiparous cows and multiparous cows, respectively. The overall effect of the latter is clearly highlighted in Figure 2A and 2B. A number of authors (Windig et al., 2008; Patton et al., 2007) have observed that increasing NEB during early lactation is highly correlated with the increase in interval to first ovulation. This effect was quantified by De Vries and Veerkamp (2000), who observed that each 10 MJ decrease in nadir EB (NE_L/d) in primiparous cows corresponded to a delay in ovulation of 1.25 d. Similarly, within the current study each 10 MJ decrease in daily EB (ME basis) (approx. 6.4 MJ/d on a NE_L basis) in early lactation increased the delay to FOH by 1.2 and 0.8 d in primiparous and multiparous cows, respectively, suggesting that the former were more sensitive to NEB. This may reflect the fact that primiparous cows also have a significant competing demand for energy for growth (Wathes et al., 2007; Macmillan et al., 2018).

Cows with improved EB and earlier SLA also had greater milk progesterone concentrations at SLA, supporting the observations of Spicer et al. (1993) of a positive correlation between EB and progesterone concentration during the first estrous cycle. While Windig et al. (2008) observed that cows with a greater milk production had lesser peak progesterone concentrations, as observed in the current study, Moore et al. (2014) noted that greater circulating progesterone concentrations are primarily due to greater corpus luteum synthetic capacity (rather than differences in progesterone clearance rates).

The relationships observed between EB and each of SLA and FOH, are aligned with many of the trends in the production data, and findings of earlier studies. For example, as d to SLA and FOH decreased, total DMI increased while milk yield decreased, in agreement with the findings of Kadokawa et al. (2006), Patton et al. (2007) and Macmillan et al. (2018). Similarly, as in the current study, previous research has identified relationships between the early resumption of reproductive activity and greater milk protein content (Patton et al., 2007), lesser milk fat content (Kadokawa et al., 2006), and consequently a lesser FPR.

A number of studies have observed strong relationships between BCS loss and SLA. For example, Gobikrushanth et al. (2019) found SLA to be delayed in cows that lost more than 0.75 BCS unit (scale 1-5) before 35 DIM, while Barletta et al. (2017) found that greater loss of BCS during the transition period was a key factor in delaying the initiation of ovarian activity after calving. Furthermore, in a study involving 19 Northern Ireland dairy farms (McCoy et al., 2006) SLA was delayed in cows with a lesser BCS during the first 100 d of lactation, while Buckley et al. (2003) observed a reduced likelihood of submission for breeding in cows with greater BW loss in early lactation and a lesser nadir BCS. While BCS profiles in Figure 1 suggest that BCS loss was relatively modest with cows in all EB quartiles, even in Q1, the EB profiles suggest that these cows were mobilizing substantial quantities of body tissue reserves. Thus it is likely that these cows were mobilizing significant amounts of abdominal adipose tissue, something which is more likely to occur with cows in relatively low BCS, as in the current study.

The relationships between blood metabolites and SLA and FOH in the current study agree with earlier findings. For example, Dubuc et al. (2012) and Bossaert et al. (2008) observed a relationship between lesser NEFA concentrations and earlier FOH, while Kawashima et al. (2012) observed a similar effect with greater blood glucose concentration, and lesser blood NEFA concentration. Similarly, Macmillan et al. (2018) observed that cows that had ovulated before 35 DIM had a greater glucose, and lesser NEFA and BHB concentrations compared with cows that ovulated after 35 DIM. It has been suggested that cows with greater serum NEFA, BHB and lesser glucose concentrations have a greater risk of prolonged postpartum anovulation and consequently lesser reproductive efficiency, and as such might benefit from targeted preventive therapy (Wathes, 2012; Vercouteren et al., 2015).

Greater yielding dairy cows make ‘metabolic decisions’ about the utilization of scarce resources such as energy, and in early lactation nutrients are preferentially directed to milk production rather than to initiate pregnancy (Friggens, 2003). The delay in FOH and SLA between Q1 and Q4 in primiparous cows (9.8 and 10.2 d delay, respectively) and multiparous cows (7.4 and 5.9 d delay, respectively) reflect the difference in mean EB profiles (range from -67 to +14 MJ/d in primiparous cows, and -104 to -6 MJ/d in multiparous cows), and is likely due to the impact of energy on activity of the hypothalamic-pituitary-ovarian axis (**HPO**) (Wathes, 2012). The effects of energetic stress on the function of the HPO axis have been examined primarily at the hypothalamus and anterior pituitary, and the loss of pulsatile LH secretion has been shown to result from prolonged inadequate intake of dietary energy (Beam and Butler, 1999; Bisinotto et al., 2012). The underlying mechanism by which NEB reduces LH release is likely to involve the supply of energy to neurons, and hormonal modulation of hypothalamic and pituitary cells (Schneider, 2004). For example, glucose and insulin are the substances that are most likely to exert an impact on HPO, and to influence GnRH secretion, consequently reducing LH pulse and causing a delay in the resumption of reproductive activity (Leroy et al., 2008). In addition, glucose is the preferred energy substrate for neuron metabolism, and lesser concentrations of glucose can inhibit the GnRH pulse generator (Schneider, 2004). In the current study plasma glucose increased as d to FOH and SLA decreased (Q1 to Q4: from 3.20 to 3.47 mmol/l in primiparous cows, and from 2.89 to 3.08 mmol/l in multiparous cows).

8.5.5. Energy balance and fertility outcomes

There were no differences between quartiles in the number of cows treated with hormones prior to first AI, with these treated cows excluded from the subsequent analysis. Nevertheless, for those cows that were cycling normally, interval from calving to first AI was still determined in part by management decisions. For example, a minimum voluntary waiting period of 42 d was adopted for all cows, while for many cows a longer 'delay' occurred to align with the breeding season start dates (namely early December and early April for autumn and spring calving 'herds', respectively). This helps explain why, despite differences between quartiles in interval to FOH, there were no differences in interval to first AI between quartiles (which occurred at a mean of 70.4 and 70.0 d in primiparous and multiparous cows, respectively).

A key finding of this study was that mean conception rate to first AI, the percentage of cows pregnant by 21, 42 and 84 d after start breeding season, and the percentage of non-pregnant cows over the first 150 d of lactation were unaffected by early lactation EB. In contrast, Patton et al. (2007) and Gümen et al. (2005)found that cows with a severe NEB in early lactation had a reduce conception rate at time of breeding. Similarly, a number of studies have established relationships between changes in BCS in early lactation, and pregnancy outcomes. For example, Middleton et al. (2019) found that cows that maintained or gained BCS during the first 30 DIM had increased conception at first AI than those that lost BCS. Similarly, Barletta et al. (2017) observed a greater conception rate (47%) in cows that gained (+0.35 units) BCS in early lactation compared to those that either maintained BCS (33% conception rate) or lost (-0.38 units) BCS (18% conception rate). Carvalho et al. (2014) observed poorer quality embryos in cows that had lost BCS in early lactation.

Nevertheless, in the current study there was a tendency for primiparous cows in Q4 to have an increased pregnancy rate at 84 d after the start of the breeding season, while data in Figure 3B suggests a greater long term pregnancy rate in Q4 multiparous cows. The absence of a clear effect in the current study are likely due to the delay in interval to first AI, and the fact that cows had moved to a less severe metabolic state at the time of AI (on average, 71 DIM). This was highlighted when mean data for the entire experimental period was examined, with all cows having a much-improved EB during this period, with this reflected in the much smaller differences between EB quartiles in milk fat content, milk fat-to-protein ratio and blood metabolites. With regards the latter, in a large scale study Chapinal et al. (2012) observed no relationship between early lactation NEFA and BHB concentration, and subsequent pregnancy rate to first AI. In contrast, Ospina et al. (2010) found a 16% decrease in risk of pregnancy for cows with high (≥ 0.72 mmol/L) NEFA concentrations, with this level only slightly greater than that observed in Q1 cows in early lactation in the current study (0.63 and 0.70 mmol/L for primiparous and multiparous, respectively). The latter is important as it is known that increased NEFA concentrations can adversely affect oocyte quality (Leroy et al. 2005 and 2008). In addition, adequate blood glucose levels are necessary for proper functioning of, and preparation of the ovary, oviducts and uterus (Wathes et al., 2011: Garverick et al., 2013).

Within the current dataset PTA for fertility in primiparous cows increased by 2.2 units between Q1 and Q4, with each 1 unit increase expected to reduce calving interval by approximately 0.6 d and to improve non-return rate by 0.25% (AHDB Dairy, 2020). While this may have made a small contribution to the earlier FOH observed, this was not reflected in a difference in fertility outcomes. Nevertheless, given that PTA for fertility for primiparous cows within the overall dataset ranged from -14 to +11.9, and that there was very considerable overlap in PTA values between quartiles, the relative absence of a genetic-phenotypic relationship is unsurprising. PTA for fertility did not differ between quartiles in multiparous cows, in agreement with the absence of an effect on fertility outcomes observed.

A number of possible reasons why clear relationships between EB and fertility outcomes were not observed in this study have been discussed. However, the potential limitations of numbers of cows involved in the analysis must also be considered. Although numbers were substantially greater than in many other studies, the number of cows within each EB quartile was 122 and 255 cows for primiparous and multiparous cows, respectively.

8.6. CONCLUSION

Dairy cows with more severe NEB during early lactation (4 to 21 DIM) had a lesser DMI and greater ECM yields, while more severe NEB was also reflected in greater milk fat content, and increased concentrations of NEFA and BHB in serum. In addition, increasing NEB in early lactation was associated with a delay in FOH and postpartum SLA. For each 10 MJ/d increase in mean NEB (ME basis) during 4-21 DIM, FOH was delayed in by 1.2 and 0.8 d in primiparous and multiparous cows, respectively. However, early lactation EB had no effect on conception to first service.

8.7. ACKNOWLEDGEMENTS

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8.8. REFERENCES

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8.9. APPENDIX 1

Publications (and experiment ID code, in bold) describing the experiments from which individual animal data was obtained (all experiments undertaken at the Agri-Food and BioSciencs Institute, UK, between 1996 and 2016).

Ferris, C. P., F. J. Gordon, D. C. Patterson, and C. S. Mayne, and M. A. McCoy. 2003. A short-term comparison of the performance of four grassland-based systems of milk production for autumn-calving dairy cows. Grass Forage Sci. 58:192–209. **AFBI experiment codes C18 and C24.**

Ferris, C.P., D. C. Patterson, M. A. McCoy, and D. J. Kilpatrick. 2010. Effect of offering dairy cows diets differing in phosphorus concentration over four successive lactations: 1. Food intake, milk production, tissue changes and blood metabolites. Animal 4:545–559. **AFBI experiment codes C36, C40, C42 and C48.**

Ferris, C.P., Doody, D.G., Laughlin, R., Watson, C.J. and Watson, S. 2013. Cow performance within contrasting milk production systems over three successive lactations. In: Proc. British Grassland Soc. 11th Research Conference: Science and Practice for Grass-Based Systems. 2 - 3 September 2013, Dumfries, Scotland. Paper 6.3. **AFBI experiment codes C54, D91 and D100.**

Ferris, C.P., Gordon, F.J., Patterson, D.C. and Murphy, J. 2002. A three year comparison of four contrasting grassland based systems of milk production. In: Profitable grass and forage: meeting the needs of the farmer and society. Proc. of the British Grassland Soc. Winter Meeting, 27-28 November 2002. Stafford, UK. **AFBI experiment codes C27 and C30.**

Gilmore, H., Young, F.J., Patterson, D.C., Wylie, A., Law, R.A., Elliot, C. and Mayne, C.S. 2011. An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and oestrous behaviour of high yielding Holstein-Friesian dairy cows. J. Dairy Sci, 94: 3510-3526. **AFBI experiment code D80**.

Gilmore, H.S. 2010. An investigation of factors affecting reproductive function in the high genetic merit Holstein-Friesian dairy cow. PhD Thesis, School of Biological Sci., Queen's University Belfast. **AFBI experiment code D84.**

Gordon, F. J., C. P. Ferris, D. C. Patterson, and C. S. Mayne. 2000. A comparison of two grassland-based systems for autumn-calving dairy cows of high genetic merit. Grass Forage Sci. 55:83–96. **AFBI experiment code C8.**

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Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, K. L. Ingvarsten, A. Hameleers, M. A. McCoy, C. S. Mayne, C. P. Ferris. 2011. Effect of pre calving and post calving dietary energy level on performance and blood metabolite concentrations of dairy cows throughout lactation. J. Dairy Sci. 94:808–823. **AFBI experiment code D64.**

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. G. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92: 1001–1012. **AFBI experiment code D71.**

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Purcell, P. J., R. A. Law, A. W. Gordon, S. A. McGettrick, and C. P. Ferris. 2016. Effect of concentrate feeding method on the performance of dairy cows in early to mid-lactation. J. Dairy Sci. 99:2811–2824. **AFBI experiment code D102.**

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Vance, E. R., C. P. Ferris, C. T. Elliot, H. M. Hartley, and D. J. Kilpatrick. 2013. Comparison of the performance of Holstein-Friesian and Jersey x Holstein-Friesian crossbred dairy cows within three contrasting grassland-based systems of milk production. Livest. Sci. 151:66–79. **AFBI experiment code C49 and C51.**

Vance, E. R., C. P. Ferris, C. T. Elliot, S. A. McGettrick, and D. J. Kilpatrick. 2012. Food intake, milk production, and tissue changes of Holstein-Friesian and Jersey × Holstein-Friesian dairy cows within a medium-input grazing system and a high-input total confinement system. J. Dairy Sci. 95: 1527–1544. **AFBI experiment code C54(V).**

Young, F.J., Hameleers, A., Patterson, D.C., and Mayne, C.S., 2006. Effect of additional concentrate supplementation for dairy cows at three time points in lactation. In: Proc. British Soc. Animal Sci. p 123. **AFBI experiment code D56.**

9. CONSIDERACÕES FINAIS

Os resultados desta tese não devem ser interpretados com o objetivo de definir a estratégia ou manejo ideal ou imutável para vacas leiteiras, mas sim como ferramenta de auxílio para tomadas de decisões perante os diferentes objetivos e situações encontradas no campo. Observamos que, com a redução do fornecimento de RTM em até 50% do consumo *ad libitum,* a inclusão progressiva de pasto na dieta de vacas leiteiras no meio de lactação tem pequeno ou nenhum impacto no desempenho produtivo, sem aumentar a intensidade de emissão de CH_4 (g/kg LCE), enquanto as relações observadas no último estudo confirmaram que o BEN é capaz de atrasar o retorno a atividade reprodutiva, mas não altera a taxa de concepção na primeira inseminação.

Dessa forma, pode-se dizer que a hipótese geral do trabalho foi parcialmente rejeitada, uma vez que que a adição de pasto de clima tropical (com maior teor de FDN) não necessariamente aumenta a intensidade de emissão de CH_4 e que mesmo com reduções na IMS o desempenho produtivo se manteve ou apresentou reduções inferiores a 10% em comparação aos animais recebendo exclusivamente RTM. Além disso, diferente do preconizado em estudos prévios, nossos resultados mostram que a fertilidade do rebanho é pouco afetada pela intensidade de BEN no início da lactação.

No experimento 1, a inexistência de diferença na intensidade de emissão de CH_4 (g/kg LCE) com a inclusão de pasto de clima tropical pode ser explicada pelo fato de a diminuição na produção de LCE ter sido acompanhada de uma redução proporcional na emissão diária de CH_4. No experimento 2, os resultados indicam que a manutenção do desempenho produtivo com o maior nível de inclusão de forragem ocorreu às custas de alguma mobilização de reservas corporais, em função da redução na IMS diária e de EL_L. Neste caso, as reduções na IMS podem ser atribuídas à baixa massa de forragem por ha, o que é uma característica frequentemente observada em pastos anuais de inverno, sobretudo nos primeiros ciclos de pastoreio (MIGUEL et al., 2014; MIGUEL; DELAGARDE; RIBEIRO-FILHO, 2019). Contudo, baseado em trabalhos de outros autores (OVERTON; WALDRON, 2004; ROCHE et al., 2017; ROCHE; BERRY; KOLVER, 2006), é possível afirmar que ao ingerirem mais de 90% das exigências de EL_L no terço médio de lactação os animais têm plena capacidade de recuperarem suas reservas durante terço final e no período seco, sem afetar o desempenho reprodutivo e/ou adentrarem em desordens metabólicas.

Os resultados destes dois experimentos permitem a recomendação segura de estratégias de manejo para regiões subtropicais, onde o acesso a pastos de metabolismo C3 (hibernais) e C4 (estivais) na dieta de vacas leiteiras praticamente mantem o desempenho produtivo, agregando bem-estar animal e diminuindo custos da alimentação mecanizada. Além dessas variáveis, a questão ambiental representada no decorrer da tese demonstra que a inclusão do pasto de clima tropical e temperado é capaz de equalizar as emissões de CH_4 quando comparado a sistemas que utilizam exclusivamente RTM. Destaca-se, ainda, que os experimentos aqui mencionados serviram como base para a elaboração de uma análise de ciclo de vida, onde se concluiu que a inclusão de pasto pode manter ou reduzir a pegada de carbono em sistemas de produção de leite em regiões subtropicais (RIBEIRO-FILHO; CIVIERO; KEBREAB, 2020). Isso ocorre devido às menores emissões de GEE advindas das excretas e da produção de alimentos concentrados e silagem de milho quando os animais têm acesso a pasto em comparação a sistemas confinados.

O terceiro estudo permitiu entender melhor as relações que ocorrem em vacas com diferentes intensidades de BE no início da lactação. Animais com maiores severidades de BEN apresentam as maiores produções de leite diária, maiores teores de gordura no leite e maiores relações gordura: proteína no leite, enquanto a IMS e os teores de proteína do leite diminuem. No início da lactação o BEN é resultado das maiores produções de leite e redução do consumo. Porém, durante os primeiros 150 dias de lactação, a produção de leite é o maior contribuinte para o BEN. Quanto aos metabólitos sanguíneos, ocorrem aumentos das concentrações de AGNE e BHB ao passo que as concentrações de glicose diminuem para vacas que adentram em maiores severidades de BEN. Além das relações do BEN com o desempenho e a concentração de metabólitos, foi possível identificar claramente que o BEN atrasou o retorno a atividade reprodutiva de vacas leiteiras, enquanto as taxas de prenhez na primeira inseminação não foram alteradas pela severidade do BEN. Para cada 10 MJ de energia metabolizável houve um atraso de 0,8 e 1,2 dias no primeiro cio observado em vacas multíparas e primíparas, respectivamente. Isso reforça que primíparas são mais sensíveis ao BEN que multíparas, uma vez que ainda necessitam chegar ao tamanho adulto na segunda e terceira lactações.

9.1. REFERÊNCIAS

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