ALINE CRISTINA DALL’ORSOLETTA

EMISSÃO DE METANO ENTÉRICO: UMA ANÁLISE DO ANIMAL AO SISTEMA DE PRODUÇÃO DE LEITE

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

Orientador: Henrique M. N. Ribeiro- Filho

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Banca examinadora:

Orientador:

Professor Dr. Henrique M. N. Ribeiro Filho
Universidade do Estado de Santa Catarina - UDESC

Membros:

Dr. Alexandre Berndt
Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA

Professor Dr. André Sbrissia
Universidade do Estado de Santa Catarina – UDESC

Professor Dr. Gilberto Vilmar Kozloski
Universidade Federal de Santa Maria - UFSM

Dr. Tiago Celso Baldissera
Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina – EPAGRI

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Dedico este trabalho
à minha família.

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“Qualquer caminho é apenas um caminho e não constitui insulto algum – para si mesmo ou para os outros – abandoná-lo quando assim ordena o seu coração. (...) olhe cada caminho com cuidado e atenção. Tente-o tantas vezes quantas julgar necessárias. Então, faça a si mesmo e apenas a si mesmo uma pergunta: possui esse caminho um coração? Em caso afirmativo, o caminho é bom. Caso contrário, esse caminho não possui importância alguma.” Carlos Castañeda

RESUMO

DALL-ORSOLETTA, Aline Cristina. **Emissão de metano entérico: uma análise do animal ao sistema de produção de leite**. 2019. p.120 Tese (Doutorado em Ciência Animal – Área: Produção Animal). Universidade do Estado de Santa Catarina. Programa de Pós-graduação em Ciência Animal, Lages, 2019.

Os principais fatores que afetam a emissão de metano (CH_4) entérico pelos ruminantes são o consumo e a qualidade da dieta. Ao nível de sistema de produção, a emissão CH_4 entérico é influenciada por práticas de manejo ligadas a produtividade e a eficiência alimentar. No entanto, estudos que estimem o efeito de diferentes práticas de manejo sobre a emissão de CH_4 entérico ainda são escassos. Os objetivos deste trabalho foram avaliar o efeito de diferentes estratégias de alimentação sobre a emissão de metano entérico de vacas leiteiras, e estimar o efeito da raça, idade ao primeiro parto e taxa de reposição sobre a emissão total de CH_4 entérico. Para isto, inicialmente estudou-se o efeito de diferentes suplementações energéticas (silagem de milho e milho moído) para vacas leiteiras em pasto de azevém anual sobre a intensidade de emissão de CH_4. Em um segundo momento, a partir de medidas pontuais da emissão de CH_4 entérico, avaliou-se o efeito de duas estratégias de alimentação (suplementação com concentrado ou sem suplementação) e raça (Holandês e Normando). Finalmente, a partir da modelização de dados produtivos coletados durante dez anos na fazenda experimental do INRA (Pin-au-Haras), foi possível estimar a emissão de CH_4 entérico de vacas e novilhas e integrar estes dados ao nível de oito sistemas, combinando duas raças (Holandês e Normanda), duas idades ao primeiro parto (dois ou três anos), duas estratégias de alimentação (alta e baixa entrada de insumos) e taxa de reposição (25, 35,40 e 45%). No primeiro experimento, a suplementação energética com milho moído não reduziu a intensidade de emissão (g CH_4/kg de leite), apenas a emissão de CH_4 por kg de MS consumida. No segundo experimento, animais recebendo a estratégia de alimentação com suplementação apresentaram maior emissão de CH_4 entérico diária, enquanto vacas da raça Holandês mostraram menor intensidade de emissão. No entanto, a medida de CH_4 entérico utilizando medidas pontuais em dois períodos diários pode estar relacionada com o horário de ingestão de alimento, fato que, fragiliza os resultados obtidos, principalmente neste caso, onde medidas durante o período de pastejo não foram realizas. Ao nível de sistema, a emissão total de CH_4 entérico foi sensível ao desempenho individual dos animais, sendo menor em vacas de alto mérito genético quando alimentadas com uma estratégia sem limitações na ingestão de energia. A redução na idade ao primeiro parto teve um efeito expressivo no tamanho do rebanho, e consequentemente, na emissão total de CH_4 entérico. Comparando os dois sistemas com emissão extrema, raça e estratégia de alimentação apresentaram maior potencial de redução na emissão de CH_4 entérico do sistema 17 e 15%, respectivamente, enquanto a taxa de reposição contribuiu em 14% e a idade ao primeiro parto contribuiu em 9 %. Sendo que a combinação destes reduz a emissão de CH_4 entérico em 54 %. Assim, uma maior redução da emissão de CH_4 entérico pelo sistema é esperada quando as práticas adotadas são combinadas; resultando também em maior produção de leite, menor número de animais no rebanho e menor período improdutivo.

Palavras-chave: Eficiência alimentar. Estratégia alimentação. Gás de efeito estufa. Pastejo. Vacas em lactação.

ABSTRACT

DALL-ORSOLETTA, Aline Cristina. **Enteric methane emission: an analysis from cow to the dairy system**. 2019. 120p. Thesis (Doctorate in Animal Science - Area: Animal Production) - Santa Catarina State University. Post Graduate Program in Animal Science, Lages, 2019.

The mains factors affecting enteric methane (CH_4) emission by ruminants are dry matter intake and quality of diet. In the dairy system, the enteric CH_4 emission is affected by management, including productivity and feed efficiency. However, the effect of different management practices on enteric CH_4 emission in the dairy system deserves more studies. The aims of this work were evaluating enteric CH_4 in grazing dairy cows on different feed strategies. And, in a dairy system estimating the effect of breed, first calving age, and replacement rate on enteric CH_4 emission. The first experiment studied the effect of different energy supplementations (corn silage and corn ground) in dairy cows. After that, the second experiment evaluated the effect of feed strategy (with or without supplementation) and breed (Holstein and Normande) on enteric CH_4. For that, measures of enteric CH_4 were performed by spot samples before and after milking in grazing dairy cows. Finally, from performance data collected for ten years at the dairy research farm of INRA (Le Pin-au-Haras) a model was developed. Hence, was possible to estimate the enteric CH_4 emission of dairy cows and heifers. These data were integrated in eight different dairy systems associating two breeds (Holsteins or Normande), two ages of first calving (two or three years), two feed strategies (system with lower or higher inputs) and four replacement rates (25, 35, 40 and 45%). In the first experiment, the energetic supplementation with corn ground was not enough to reduce the methane intensity (g CH_4 /kg of milk), but it was observed a decrease in methane emission *per* kg of DM intake. In the second experiment, the dairy cows in the supplemented feed strategy showed higher daily enteric CH_4 emission. While Holstein dairy cows had a lower methane intensity. However, the enteric CH_4 measures using the spot samples during two periods can be related to the time of feeding. This fact undermines the results described, principally in this case, where the measures during grazing time were not made. At the system level, total enteric CH_4 emission was sensitive to the individual performance of the animals, being lower in dairy cows of high genetic merit when fed with a strategy without energy intake limitations. The reduction in the first calving age had an expressive effect on herd size, and consequently on total enteric CH4 emission. Comparing two dairy system with extreme enteric CH_4 emission, breed and feeding strategies shown a higher potential of enteric CH_4 reduction in the system; 17 and 15%, respectively. While the replacement rate contributed to 14 % and first calving age contributed to 9%. The combination of these factors reduces the enteric CH_4 emission by 54 %. Thus, a comprehensive enteric CH_4 emission reduction in dairy systems is expected when management practices were combined, resulting in higher milk production, fewer animals in the herd and less unproductive period.

Keywords: Feed efficiency. Feed strategy. Grazing dairy cows. Greenhouse gas. Grazing. Dairy cows.

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LISTA DE ABREVIATURAS E SIGLAS

AGV	Ácidos graxo voláteis
ATP	Adenosina trifosfato
CH_4	Metano
CO_2	Dióxido de carbono
CO_2eq	Equivalente de dióxido de carbono
EB	Energia bruta
ECM	Leite corrigido pela energia
EM	Energia metabolizável
FDA	Fibra em detergente ácido
FDN	Fibra em detergente neutro
FPCM	Leite corrigido pela gordura e proteína
GF	GreenFeed
GWP	Potencial de aquecimento global
H_2	Gás hidrogênio
IPCC	Intergovernmental Panel on Climate Change
MO	Matéria orgânica
MS	Matéria seca
N_2O	Óxido Nitroso
NAD	Nicotinamida adenina dinucleotídeo
NADH	Nicotinamida adenina dinucleotídeo reduzida
PB	Proteína bruta
PV	Peso vivo
RPM	Ração parcialmente misturada
RTM	Ração totalmente misturada
SF_6	Hexafluoreto de enxofre
UA	Unidade animal

SUMÁRIO

1 INTRODUÇÃO

A agropecuária é considerada uma das principais fontes antropogênicas de emissão de gases causadores de efeito estufa. Entre mitos e verdades, a produção de bovinos contribuiu com a emissão de uma parcela destes gases, principalmente devido a emissão de metano (CH_4) entérico. O CH_4 entérico é formado naturalmente durante o processo de fermentação ruminal dos alimentos e a partir disto um número expressivo de pesquisas vem sendo realizado com o intuito de avaliar a produção de CH_4 entérico em diferentes categorias de ruminantes, e ao mesmo tempo, identificar possíveis ações que permitam a sua redução. Estas ações são divididas em três diferentes abordagens, sendo relacionadas à nutrição, ao ambiente ruminal e à produtividade animal.

Os resultados obtidos com a avaliação de diferentes estratégias estão frequentemente ligados ao consumo de matéria de seca e a qualidade da dieta consumida pelo ruminante. Neste sentido, dentre as principais ações coerentes ao manejo alimentar podemos citar o manejo da forragem pastejada, o uso de suplementação com alimento concentrado e a utilização de forragem conservada, como a silagem de milho. Já entre as ações relacionadas com a produtividade estas podem atuar principalmente na redução da intensidade de emissão (CH_4/kg de leite produzido). Quando individualmente consideradas, o impacto das ações citadas, sobre a redução da emissão de CH_4, é muitas vezes variável e acaba sendo ligado a outros fatores de influência. Entre eles, fatores ligados ao animal, ao nível de produção, à técnica de avaliação e ao número de animais avaliados. Mesmo com o grande número de pesquisas em relação ao estudo de estratégias pontuais sobre a emissão de metano entérico, visto as fontes de variação, uma descrição mais detalhada do efeito de estratégias de alimentação, por exemplo, sobre a emissão de metano entérico se faz necessária.

Frente a participação considerável do CH_4 entérico na emissão total de gases de efeito estufa em sistemas de produção de ruminantes, atentando à sistemas de produção de leite, aspectos ligados ao aumento da produtividade e da fertilidade, da eficiência de utilização do alimento pelo rebanho e aumento da eficiência do sistema como um todo; indicam permitir o desenvolvimento de estratégias visando o menor impacto ambiental do sistema. No entanto, o assunto ainda requer maiores estudos, principalmente em relação ao efeito da associação de diferentes práticas de manejo que visem a maior produtividade do rebanho sobre a emissão de metano.

Relevando a importância do assunto, este trabalho foi estruturado a fim de avaliar primeiramente a emissão de CH_4 entérico ao nível individual de vacas leiteiras, relevando o efeito de diferentes estratégias alimentação sobre a emissão. Em uma segunda abordagem, o objetivo foi analisar a emissão de CH_4 entérico total do rebanho combinando o efeito de raça, da estratégia de alimentação utilizada, da idade ao primeiro parto e da taxa de reposição. A partir dos resultados gerados espera-se fornecer informações que irão auxiliar no desenvolvimento de estratégias sob uma visão conjunta do sistema de produção de leite.

2 REVISÃO BIBLIOGRÁFICA

2.1 EMISSÃO DE METANO

As emissões do setor agrícola correspondem as emissões oriundas da fermentação entérica de ruminantes, manejo de dejetos animal, solos agrícolas, cultivo de arroz e queima de resíduos agrícolas. Mundialmente a agricultura contribui com cerca de 24 % das emissões totais (FAO, 2014). Segundo, Smith et al. (2014) no ano de 2010 o setor emitiu 5,2 – 5,8 Gt CO_2eq/ano. A partir de estimativas do Intergovernmental Panel on Climate Change (IPCC) a contribuição da produção animal é responsável por 8 – 10,8 % das emissões de gases de efeito estufa, e quando considerado a análise do ciclo de vida completo (incluindo a mudança no uso da terra, produção insumos), a contribuição pode chegar a 18 % (O’MARA, 2011). No Brasil, segundo as recentes estimativas de emissões de gases de efeito estufa (MCTI, 2017), o setor contribui com 31 e 25 % das emissões liquida e bruta totais em CO_2eq (a diferença observada entre os resultados das emissões líquida e bruta corresponde às remoções devido ao crescimento de florestas e campos naturais manejados). Considerando a crescente demanda alimentar mundial e consequentemente exigência em produtos de origem animal, a contribuição do setor tende a aumentar. Diante disto, o último acordo entre países (Acordo de Paris - 2015) visa a aplicação de medidas de redução da emissão de gases de efeito estufa, sendo um dos objetivos manter o aumento da temperatura média global a bem abaixo de 2°C em relação aos níveis pré-industriais e de garantir esforços para limitar o aumento da temperatura a 1,5°C até o final do século. O Brasil se comprometeu a reduzir as emissões de gases de efeito estufa em 37 % até 2025 e apresentou o indicativo de redução de 43 %, até 2030.

Os principais gases emitidos pelo setor são o gás metano (CH_4) e o óxido nitroso (N_2O). A principal fonte de CH_4 é a fermentação entérica pelos ruminantes. O CH_4 possui um potencial de aquecimento superior ao CO_2 e representa, no ruminante, uma forma de perda de energia que varia entre 2 – 12 % da energia bruta (EB) consumida (JOHNSON; JOHNSON, 1995). Entre os anos de 1961 e 2010 a taxa de crescimento anual da emissão de CH_4 entérico foi de 0,95 %, crescendo de 1,3 a 2,0 GtCO_2eq/ano, na qual (SMITH et al., 2014)a Ásia e a América foram os principais contribuidores no últimos cinco anos (TUBIELLO et al., 2013). Nestes casos, a emissão de CH_4 é direcionada pelo tamanho e produtividade do rebanho de ruminantes (O’MARA, 2011). No Brasil, 57 % das emissões totais do setor agrícola é originada da emissão de CH_4 entérico, destes 11 % corresponde a emissão de CH_4 do setor da bovinocultura de leite (MCTI, 2017).

2.1.1 Unidades para expressar a emissão de metano

A expressão da emissão de CH_4 varia segundo ao objetivo final da análise, e devem ser avaliadas de maneira minuciosa para evitar conclusões equivocadas, principalmente, na avaliação de estratégias que visam reduzir a produção de metano CH_4 entérico.

A fim de uma comparação compreensiva de dimensões físicas e econômicas das dimensões das mudanças climáticas e entre emissão de gases causadores do efeito estufa, a emissão de CH_4 pode ser reportada ao potencial de aquecimento global (GWP, sigla em inglês – global warming potential). O potencial de aquecimento compara diferentes gases a partir da sua força radioativa. O gás de referência é o CO_2, a medida em CO_2 equivalente é obtida pela multiplicação das toneladas emitidas do gás considerado pelo seu potencial de aquecimento global, por exemplo CO_2 = 1, CH_4 = 21, N_2O = 310 (IPCC, 2009).

Considerando a emissão de CH_4 entérico ao nível animal, valores absolutos são apresentados como fator de emissão (kg CH_4 produzido *per* animal *per* ano) ou então, produção diária (g CH_4 por dia). Como conhecido, a produção de CH_4 pelo ruminante é acompanhada de perda de EB consumida. Logo, valores absolutos de emissão podem ser reportados como proporção da EB consumida que é perdida. Para uma análise além, os valores de emissão podem ser relacionados com o alimento consumido (g CH_4 *per* ingestão de matéria seca (MS) ou matéria orgânica (MO)), esta permite a comparação da produção de CH_4 evidenciando uma possível comparação entre espécies, e o efeito da composição da dieta consumida.

Do ponto de vista individual e para uma análise de sistema, a intensidade de emissão relaciona-se com a produção animal (g CH_4 *per* produção), indiferente, refere-se à produção de leite e sólidos (gordura + proteína), de ganho de peso e de produção de carne de diferentes espécies e sistemas de produção. Esta, pode ser usada para comparar animais em diferentes estágios de crescimento e níveis de produção, mas também, refletir ao nível de sistema o efeito direto da eficiência de produção e de diferentes práticas de manejo sobre a emissão de CH_4 (BEAUCHEMIN et al., 2008; GERBER et al., 2011).

As unidades utilizadas para expressar a emissão de metano neste trabalho foram: emissão diária (g de CH_4/dia), fator de emissão (kg de CH_4/animal.ano), intensidade (g de CH_4/kg de leite) e produção (g de CH_4/kg matéria seca consumida).

2.2 METANOGÊNESE

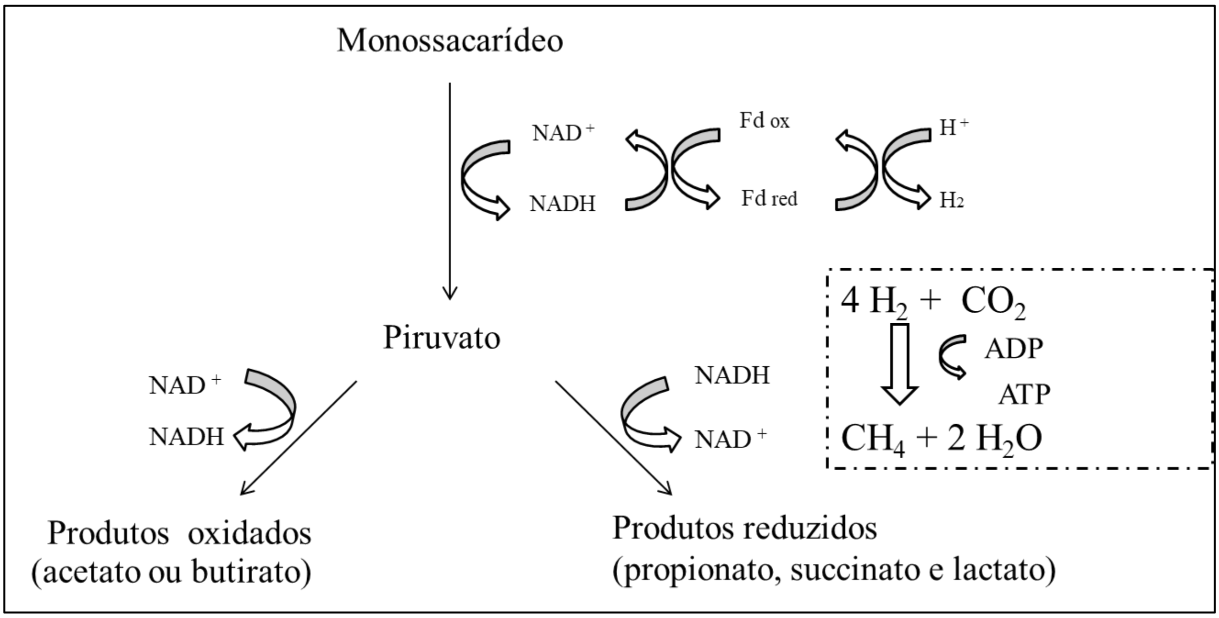
Metanogênese é o processo natural pela qual o CH_4 é formado. A produção de CH_4 ocorre quase que inteiramente a nível ruminal, cerca de 87 – 92 %, o restante é produzido no ceco e intestino grosso (MURRAY; BRYANT; LENG, 1976). No rúmen, a interação entre um ambiente anaeróbico, presença de substrato e a população microbiana, que é composta por bactérias, fungos, protozoários e as metanogênicas *Archaea*, favorece a metanogênese. A microbiota ruminal é responsável pela degradação dos alimentos e a formação de energia a partir de frações do alimento menos aproveitadas por outros animais, em especial carboidratos fibrosos (celulose, hemicelulose) e proteínas ligadas a parede celular vegetal. Os principais produtos da fermentação ruminal são os ácidos graxos voláteis (AGV; acetato, propionato e butirato) e em menor quantidade formato, etanol, lactato, succinato e AGV de cadeia ramificada. Em adição, amônia, dióxido de carbono (CO_2) e gás hidrogênio (H_2) também são produzidos.

As metanogênicas *Archaea* exercem um papel crucial na continuidade da fermentação ruminal. Estas utilizam um número restrito de substrato (receptor de elétrons) para a obtenção de energia para o seu crescimento, a principal via utiliza H_2/CO_2 como substrato, secundariamente, metanol e acetato, cujo produto desta reação é o CH_4. A taxa de formação de CH_4 é determinada pela taxa em que o H_2 é liberado no meio ruminal (JANSSEN, 2010), uma vez que, a taxa liberação de H_2 é dependente das rotas metabólicas utilizadas no processo de fermentação ruminal.

O intermediário comum do catabolismo dos carboidratos pelas bactérias ruminais é o piruvato, o piruvato pode ser metabolizado em produtos mais oxidados como o acetato e butirato, ou para outros mais reduzidos, como o propionato e lactato. A dieta ingerida pelo ruminante irá determinar a proporção em que cada AGV e lactato são produzidos, no entanto, este será depende da concentração de NADH e H_2. O gás H_2 é produzido pela oxidação do NADH em uma reação catalisada por uma desidrogenase e mediada por uma ferredoxina presente na membrana bacteriana (KOZLOSKI, 2011). O aumento na concentração de H_2 na célula desfavorece a desidrogenação do NADH, o qual se acumula e dirige o metabolismo para síntese de produtos mais reduzidos. É neste momento que a atividade das *Archaea* é necessária para a continuidade do catabolismo celular. Estas realizam a retirada do H_2 do meio ruminal que o utilizam para reduzir CO_2 em CH_4. De uma maneira sucinta, quanto mais H_2 é retirado do meio, maior proporção de NADH é convertida a H_2 e maior o rendimento em acetato e de ATP por mol de açúcar fermentado (Figura 1).

De maneira geral, há dois fatores principais que irão determinar o nível de formação de CH_4 a nível ruminal, principalmente, a quantidade de substrato presente e a concentração de H_2 no ambiente ruminal (BENCHAAR et al., 2014; PINARES-PATIÑO et al., 2003). No entanto, a formação de CH_4 entérico é consequência de um complexo processo que envolve condições específicas relacionadas com a formação de AGV, como a população microbiana, as condições físico-química do ambiente ruminal e os substratos disponíveis para a fermentação, além da integração de outros processos, como a biohidrogenação, o metabolismo do nitrogênio e o crescimento microbiano. As diversas interações mostram que a formação de CH_4 não é algo estático, fato que contribui muitas vezes na complexidade de predizer as emissões e definir estratégias de mitigação.

Figura 1 – Vias de fermentação ruminal e formação de metano entérico

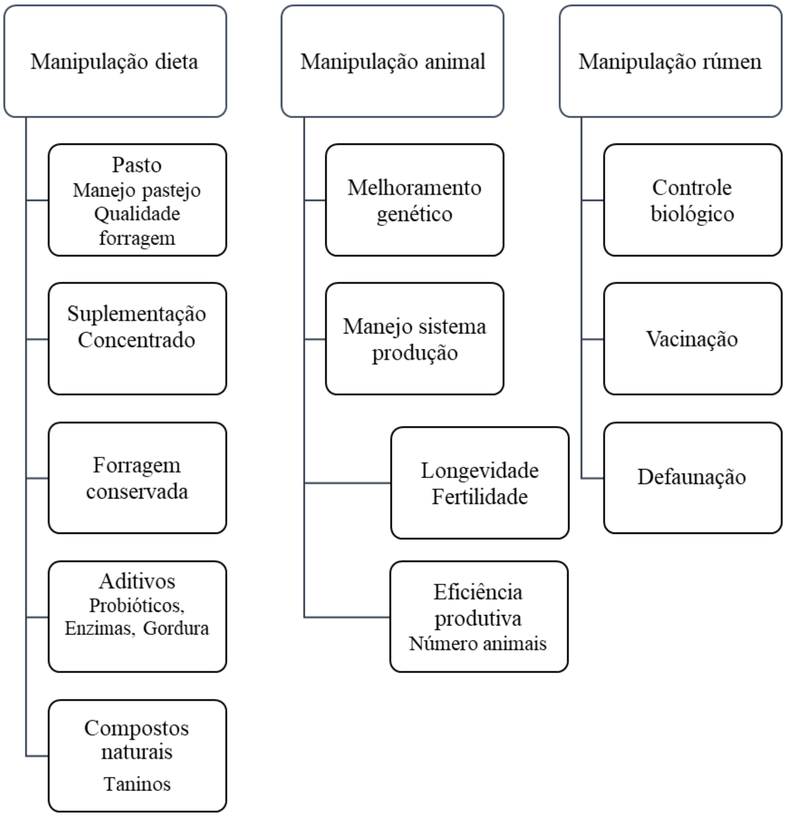


Fd ox, ferredoxinas oxidadas; Fd red, ferredoxinas reduzidas
Fonte: adaptado de Kozloski (2011).

2.3 ESTRATÉGIAS DE MITIGAÇÃO DE METANO ENTÉRICO

Amplas revisões bibliográficas vêm sendo desenvolvidas a fim de compilar diferentes estratégias de mitigação de metano (COTTLE; NOLAN; WIEDEMANN, 2011; HRISTOV et al., 2013a, 2013b; KNAPP et al., 2014). De maneira geral elas são divididas em três categorias dependendo da manipulação realizada (Figura 2). Ações de mitigação a partir do manejo alimentar, diferentes alimentos e estratégias de alimentação podem agir no aumento da produtividade e da eficiência alimentar, e/ou alterando a proporção de AGV produzidos (H_2 disponível). A estratégias em relação ao animal, como melhorias no manejo produtivo que permitam o aumento da produtividade, seja a partir da genética, fertilidade, saúde ou longevidade. E a última categoria refere-se à manipulação ao nível ruminal, ou seja, a partir da utilização de aditivos que atuam direta ou indiretamente na inibição da metanogênese.

Figura 2 – Esquema simplificado das diferentes estratégias de mitigação de metano



Fonte: Adaptado de Eckard; Grainger; De Klein (2010).

2.4 ESTRATÉGIAS DE ALIMENTAÇÃO E O EFEITO SOBRE A EMISSÃO DE METANO ENTÉRICO

2.4.1 Manejo, espécie e qualidade do pasto

Considerando que a forragem pastejada é a base da alimentação em sistemas de produção de ruminantes, discussões do efeito da utilização de diferentes estratégias de alimentação devem partir da sua utilização. Recentemente, Mazzetto et al. (2015) reportam o efeito da intensificação do manejo do pasto baseado em situações de produção de bovinos de corte no Brasil. Estes, avaliam que é possível reduzir a emissão de gases de efeito estufa em 10 kg CO_2eq/kg de carne produzida em um ciclo total entre um manejo extensivo e um manejo melhorado da pastagem, reduzindo a participação do CH_4 entérico (em CO_2eq) de 98% para 57 % em relação a emissão total de gases. Ressaltando que o cenário de manejo extensivo considerado pelos autores, parte de pastos de *Brachiaria spp*. manejados em pastejo continuo, e o manejo melhorado vai até a utilização de irrigação e fertilização nitrogenada visando a redução da sazonalidade da produção de forragem.

Algumas práticas básicas de manejo do pasto podem interferir significativamente na emissão de CH_4 entérico diária, na intensidade de emissão e na emissão por unidade de alimento consumido (Tabela 1). Estas, podem permitir o consumo de uma forragem de melhor qualidade e valor nutritivo. A escolha de uma menor massa pré-pastejo para o manejo de azevém perene (*Lolium perenne*) permite uma oferta maior de lâmina verde e a menor quantidade de colmo e material senescente (ROCA-FERNANDEZ et al., 2011; WIMS et al., 2017), fato que modifica a produção de CH_4 entérico devido ao consumo de uma forragem com maior digestibilidade e teor de proteína bruta (PB) (WIMS et al., 2010; MUÑOZ et al., 2016). No mesmo sentido, Congio et al. (2018) usaram como critério de manejo em pastos de capim elefante (*Pennisetum purpureum* Shum.), para otimizar a produção foliar e o valor nutritivo, a interceptação de luz de 95 %. Com a prática deste manejo foi possível otimizar a eficiência de pastejo e a produção de leite, resultando em reduções de 18 % e 20 % na emissão de CH_4 entérico por kg de MS consumido e por kg de leite, respectivamente.

Como apresentado nos parágrafos acima a produção de CH_4 entérico é afetada pelo consumo e qualidade do alimento. Neste caso, o manejo adequado e a utilização de diferentes espécies de pasto podem determinar esta resposta. Considerando valores médios de 23 g CH_4/kg de MS consumida (DIJKSTRA et al., 2011), a utilização das práticas de manejo citadas podem reduzir a emissão para 20,2 g CH_4/kg de MS consumida (CONGIO et al., 2018). Especificamente, o aumento no consumo reflete no aumento da produção diária de CH_4 entérico (g/dia). No entanto, uma relação inversa com a emissão de CH_4/kg de MS consumida é muitas vezes esperada. Este efeito na redução da emissão por unidade de MS consumida, foi associada, em ovinos, ao enchimento ruminal, ao tempo de retenção do alimento no rúmen, e a digestibilidade da MO da dieta consumida (HAMMOND et al., 2014; PINARES-PATIÑO et al., 2003).

Igualmente, diferenças na emissão de CH_4 entérico ao comparar gramíneas de clima tropical (C4) e temperado (C3) são esperadas. A maior emissão de CH_4 por ruminantes alimentados com gramíneas tropicais é relacionada a natureza das fibras das plantas C4, estas apresentam maior lignificação e resistência física a digestão microbiana do que as fibras das plantas C3. Logo, é também associada ao maior tempo de retenção ruminal (ARCHIMÈDE et al., 2011; ULYATT et al., 2002), e ao padrão de AGV oriundos da digestão da fibra no qual é caracterizado por uma alta relação acetato:propionato, resultando em maior disponibilidade de H_2 no ambiente ruminal.

A inclusão de leguminosas em dietas baseadas em gramíneas traz benefícios nutricionais e ambientais. Incrementos no consumo e na produção de leite são esperados (HARRIS et al., 1997; RIBEIRO-FILHO; DELAGARDE; PEYRAUD, 2003), sendo que esse aumento pode chegar a 30 % e 27 %, respectivamente, com 75 % de inclusão de trevo branco (HARRIS et al., 1997). Adicionalmente, mesmo com o aumento da emissão diária de CH_4 entérico com a utilização de leguminosas em estratégias de alimentação (Tabela 1), uma redução de 16% na emissão de CH_4 em relação a MS ingerida foi observada por Lee et al. (2004) com inclusão de 60 % de trevo branco na dieta total, sendo estimado uma emissão de 15,4 g CH_4/kg de MS consumida com uma dieta composta por 100 % de leguminosa. Estas respostas podem ser explicadas, pela alta lignificação da parede celular e a presença de compostos secundários, (taninos) em algumas espécies de leguminosas (ARCHIMÈDE et al., 2011; LEE et al., 2004). Respectivamente, estas características diminuem a digestão da parede celular das leguminosas no rúmen e inibem a metanogênese, logo, a produção de CH_4 é reduzida.

2.4.2 Utilização de suplementação

A utilização de alimento suplementar em sistemas baseados a pasto é a principal alternativa em situações de baixa disponibilidade de pasto, quando a forragem pastejada não supre as exigências nutricionais do animal, fato observado principalmente em animais de maior produtividade (DELABY; PEYRAUD, 2003). Incrementos no desempenho de vacas leiteiras, consumo total e produção de leite, com a inclusão da suplementação, seja com alimento concentrado ou forragem conservada, são dependentes de uma série de fatores associados entre si, como do valor nutritivo e da quantidade do suplemento e do pasto consumidos, produtividade dos animais e interações digestivas (BARGO et al., 2003). De certa forma, a emissão de CH_4 entérico de vacas leiteiras impostas a alguma estratégia de suplementação está ligada as possíveis interações entre forragem pastejada e o suplemento.

É relativamente conhecido que a utilização de suplementação com concentrado resulta em reduções das emissões de CH_4 em relação a proporção de energia consumida ou quando expressa por unidade de produto (leite ou carne) (BEAUCHEMIN et al., 2008; MARTIN; MORGAVI; DOREAU, 2010). Alguns mecanismos estão envolvidos na menor emissão em relação ao consumo de MS. Inicialmente, o consumo de concentrado pode levar a alteração da estequiometria da fermentação ruminal (SAUVANT et al., 2011), favorecendo a formação de produtos e a utilização de vias metabólicas que produzem menos H_2 (Figura 1). No entanto, nem sempre esta relação é claramente observada (AGUERRE et al., 2011; HART et al., 2009). Ausência desta relação foi relacionada por Aguerre et al. (2011) ao fato de que a proporção de AGV no rúmen não representam necessariamente a proporção que eles são produzidos, uma vez que a quantidade de AGV é resultado do balanço entre a produção e a absorção. Uma segunda causa, seria devido a alteração do substrato disponível, carboidratos estruturais da forragem pastejada (celulose e hemicelulose) por carboidratos não-estruturais (amido e açúcares) do concentrado, resultando em um aumento das taxas de fermentação ruminal que podem alterar o ambiente físico-químico do rúmen (MARTIN; MORGAVI; DOREAU, 2010).

A queda do pH do rúmen inibe a metanogênese e os mecanismos de simbiose entre *Archaea* e protozoários. Por fim, a redução da emissão quando expressa por unidade de produto com a utilização de concentrado seria consequência da diluição das exigências para a mantença em relação ao aumento da produção esperada de leite (MUÑOZ et al., 2015), e adicionalmente, do aumento do consumo total que aumenta a taxa de passagem ruminal, e consequente menor perda de energia na forma de CH_4.

Segundo Hristov et al. (2013a), o aumento da proporção de concentrado na dieta pode diminuir a emissão de CH_4 em relação a quantidade consumida e a produção de leite se a produção for a mesma ou aumentar. Esta relação é evidenciada em situações onde a inclusão de concentrado em dietas baseadas a pasto resultou em um aumento da ingestão de matéria seca, da produção de leite e da emissão diária de CH_4 entérico mas sem afetar a relação entre estes, nos quais valores médios de 19±0,8 g CH_4/kg MS consumida e 15,5±2,6 g CH_4/kg produção de leite foram relatados (LOVETT et al., 2005; MUÑOZ et al., 2015). Como pode ser observado na Tabela 1, a redução linear das emissões em questão, foi observada quando a proporção de concentrado aumentou de 32 para 53 % na dieta total, com diminuições de 31,9 para 25,9 g CH_4/kg MS consumida e de 17,8 para 14,0 g CH_4/kg produção de leite, no entanto, nenhum efeito foi registrado no desempenho produtivo dos animais (AGUERRE et al., 2011). Após uma compilação de trabalhos, Sauvant et al. (2011) reportam níveis de inclusão de concentrado entre 350 – 400 g/kg de MS na dieta total para que reduções ao nível de emissão de CH_4 sejam visualizadas, sendo a resposta proporcional ao nível de ingestão.

Tabela 1 – Síntese do efeito de diferentes estratégias de alimentação sobre a emissão diária, intensidade (*per* unidade de produto) e produção (*per* alimento consumido) de metano entérico.

Estratégia alimentação	**Efeito**	**Emissão CH$_4$ entérico**			**Observações**	**Referência**
		Diária	*Intensidade*	*Produção*		
Manejo, espécie e qualidade do pasto	Massa pré-pastejo	Redução 0-8%	Redução 12%	Redução 8,2%	Efeito de período	Muñoz et al. (2016)
		Redução 0-13%	Redução 0-17,6%	Redução 0-14%		Wims et al. (2010)
	Interceptação luz	s.e.	Redução 20%	Redução 18%	Pasto C4	Congio et al. (2018)
	Leguminosas	Aumento	-	Redução de 21%	Ovinos	Hammond et al. (2013)
		Aumento	s.e.	s.e.	Pastos tropicais	Andrade et al. (2016)
Suplementação	Concentrado	s.e.	Redução 26 – 39%	s.e.		Van Wyngaard; Meeske; Erasmus, (2018)
					Aumento na proporção	Muñoz et al. (2015)
		Aumento 10%	s.e.	s.e.		Aguerre et al.(2011)
		Redução 0-17%	Redução 12-21%	Redução 10-19%		Lovett et al. (2005)
		Aumento 13%	s.e.	s.e.		
	RPM *vs*. Pastejo	Aumento 5,5-14%	s.e.	s.e.		O'Neill et al.(2012)
	RTM vs. Pastejo	Aumento 37%	Aumento 13%	Aumento 11%		O'Neill et al. (2011)
Ração totalmente misturada	Aumento proporção silagem de milho	Aumento	s.e	Redução 2,2-14%	SM:SC[1]	Benchaar et al.(2014)
		Redução	Redução 6%	Redução 14%	SM:SA[2]	Hassanat et al.(2013)
		Redução	Redução 8%	Redução 11%	SM:SP[3]	Van Gastelen et al. (2015)
		s.e.	Redução 8,4%	s.e	SM:SP[4]	Hart et al. (2015)

s.e.; sem efeito; [1]Aumento da proporção de silagem de milho (SM) em RTM em silagem de cevada (SC)
[2]Aumento da proporção de silagem de milho (SM) em RTM em silagem de alfafa (SA); [3]Aumento da proporção de silagem de milho (SM) em dieta baseada em silagem de pasto (SP); [4]Maior proporção de silagem de milho (SM) em relação a silagem de pasto (SP). Fonte: Elaborado pela autora, 2018.

Os efeitos ao nível das emissões de CH_4 entérico com a utilização da suplementação com concentrado também são esperados quando ruminantes são suplementados com forragem conservada, neste caso, principalmente, com silagem de milho. As causas estariam associadas novamente com a modificação do padrão de AGV, uma vez que há a substituição de uma forragem rica em fibras (forragem pastejada) por uma forragem rica em amido (silagem de milho), além do fato de que o amido presente na silagem de milho é mais resistente a fermentação ruminal quando comparado com o amido presente em cereais. Este amido considerado "by-pass" representa cerca de 30% do conteúdo de amido total e pode ser relacionado com a menor produção de CH_4 (DE BOEVER et al., 2016; HART et al., 2015).

Existem poucos trabalhos comparando a emissão de vacas leiteiras em pastejo e recebendo suplementação com silagem de milho. O'Neill et al. (2012) estudaram a suplementação com uma ração parcialmente misturada (RPM) a base de silagem de milho. O aumento da emissão diária com a suplementação com RPM foi proporcional ao aumento no consumo total de MS, no entanto, nenhuma diferença foi observada na emissão de CH_4 quando relacionada com o consumo, em média 25,4 g CH_4/kg MS consumida. Este resultado foi atribuído à qualidade da dieta ingerida, principalmente, ao teor de MO digestível na forragem consumida (844 g MO digestível/kg MS). O consumo de uma forragem pastejada de melhor ou igual valor nutritivo ao suplemento pode assegurar a manutenção da emissão de CH_4/kg MS consumida (MUÑOZ et al., 2015; O'NEILL et al., 2012), ou até mesmo reduzir quando comparado com a utilização de ração totalmente mistura (RTM) (DALL-ORSOLETTA et al., 2016).

Um maior número de trabalhos é encontrado em relação à utilização de diferentes forragens na composição da RTM com o objetivo principal de aumentar o teor de amido na dieta com o aumento na proporção de silagem de milho (Tabela 1). Os resultados encontrados em relação a emissão de CH_4 variam com os níveis de substituição da forragem conservada pela silagem de milho. Substituindo a silagem de cevada por silagem de milho as emissões de CH_4 ajustada pelo consumo de MS ou EB diminuíram com a inclusão de silagem de milho. Esse efeito foi máximo com 54 % de inclusão (19,1 g CH_4/kg de MS consumida e 5,7 % da EB consumida), quando comparado com 27 % de inclusão de silagem de milho na dieta total (21,8 g CH_4/kg de MS consumida e 6,5 % da EB consumida) (BENCHAAR et al., 2014). Similar resposta foi observada com a redução de silagem de alfafa e o aumento da silagem de milho (HASSANAT et al., 2013; JONKER et al., 2016a). A redução da emissão de CH_4 em relação ao consumo de MS foi observada quando a proporção de silagem de milho foi superior a 50 %

da dieta total para vacas em lactação (HASSANAT et al., 2013) ou em ovinos (JONKER et al., 2016a).

No mesmo sentido, em relação à proporção de silagem de milho e silagem de pasto, a emissão de CH_4/kg MS consumida reduziu linearmente com o aumento da inclusão de silagem de milho, sendo máxima com 100% de inclusão (24,6 vs. 22,0 g CH_4/kg MS consumida). No entanto, uma resposta quadrática é observada em relação a emissão diária de CH_4, a intensidade e a porcentagem EB consumida, com valores máximos de emissão com níveis de inclusão de silagem de milho que variam entre 67 e 33 % (VAN GASTELEN et al., 2015). A variação nas respostas em relação a emissão de CH_4 observadas pelos diferentes autores pode ser reflexo da ingestão de MS (HART et al., 2015; VAN GASTELEN et al., 2015). No entanto, em modelo proposto por WARNER et al. (2017) as variações encontradas estariam relacionadas também à composição da silagem. Onde, 82 % da variação da produção de CH_4 entérico passou a ser explicada pelo modelo estruturado quando relacionaram consumo e qualidade da dieta. Estas características do alimento estariam ligadas com a digestibilidade da MO e do FDN (fibra em detergente neutro) bem como ao conteúdo de proteína bruta.

2.5 PRODUTIVIDADE E EMISSÃO DE METANO ENTÉRICO

A variação na emissão de CH_4 entre animais é de 7 a 8 % em ovinos (BLAXTER; CLAPPERTON, 1965), chegando a valores de 18 % em vacas leiteiras quando a emissão é expressa pela quantidade de MS consumida (VLAMING et al., 2008). Como a produção de CH_4 é ligada aos processos microbiológicos durante a fermentação dos alimentos, possivelmente o ambiente ruminal representa uma fonte de variação individual na emissão de CH_4 entérico. Consequentemente, ajustes no manejo alimentar representam a primeira opção de mitigação. Outros fatores de variação individual estão ligados ao animal, na qual estariam sobre controle genético (CLARK, 2013).

De maneira geral, variações na emissão de CH_4 podem ser relacionadas com a produtividade animal. Dados apresentados por Gerber et al. (2011) mostram uma relação positiva entre o aumento da produtividade animal com a emissão de gases de efeito estufa por animal, sendo associado ao fato que, animais mais produtivos são maiores e apresentam maior consumo. No entanto, quando a emissão é associada a produção de leite, a emissão diminui com o aumento da produção (HRISTOV et al., 2013c). A emissão pode variar de 12 kg CO_2eq/kg leite corrigido por gordura e proteína (FPCM) a 3 kg CO2eq/kg FPCM entre animais com produção anual de 2000 kg FPCM a 6000 kg FPCM (GERBER et al., 2011).

A partir de uma ampla revisão bibliográfica sobre os aspectos a serem considerados para a redução da emissão de CH_4 entérico, Knapp et al. (2014) destacam que o desenvolvimento de estratégias para a redução da intensidade de emissão de metano está relacionado com a eficiência de produção, e consequentemente, existem fatores de influência ligados ao animal e ao sistema de produção. Individualmente, estes fatores estão relacionados com a seleção genética de animais mais eficientes, ou seja, que apresentam maior produção de leite com a mesma quantidade consumida. A produção de CH_4 é proporcional ao consumo de energia, reduzindo a proporção de energia utilizada para a mantença enquanto a produção de leite aumenta resulta em diminuição da intensidade de emissão (YAN et al., 2010). Ao nível de sistema, a eficiência de produção liga-se com práticas de manejo que se integram com o manejo nutricional, por exemplo, manejo reprodutivo, mortalidade, taxa de reposição e idade ao primeiro parto.

Particularmente, ao nível de sistema de produção, dentre as fontes de gases de efeito estufa, o CH_4 produzido a partir da fermentação entérica representa em sistemas baseados a pasto e confinamento a principal fonte, 46 e 36 %, respectivamente (O'BRIEN et al., 2012). Neste sentido, diferentes práticas de manejo, as quais se aproximam de situações encontradas em rebanhos comerciais, são utilizadas para descrever um possível efeito sobre a emissão de metano entérico e a emissão total de gases de efeito estufa. Na Tabela 2 é possível observar valores de emissão de CH_4 entérico em diferentes sistemas. De maneira geral, e como já mencionado anteriormente, a maior eficiência no uso do alimento, como por exemplo através da seleção genética de acordo com o sistema de alimentação, pode reduzir as exigências em nutrientes do sistema e consequentemente reduzir a intensidade de emissão (em CO_2eq/ unidade de produto) (BELL et al., 2010, 2011). Vacas leiteiras em confinamento, alimentadas com RTM, apresentam maior fator de emissão (CH_4/animal/ano) e menor intensidade de emissão quando comparada com sistemas a pasto (Tabela 2). Estes resultados são explicados muitas vezes pela maior produção de leite, seleção genética, maior taxa de reposição, e ao maior uso de concentrado em sistemas de confinamento (BELFLOWER et al., 2012; O'BRIEN et al., 2014).

Em relação a seleção genética, Bell et al., (2010) descrevem que é possível reduzir a intensidade de emissão ao longo do tempo a partir da seleção para a produção de sólidos. Sob outra abordagem, mas ainda relacionado com a seleção genética de animais "mais eficientes", o consumo residual individual é usado em sistemas de produção de bovinos de corte como uma forma de selecionar animais com uma maior eficiência alimentar. Bovinos que apresentam menor consumo residual consumem menos alimento do que o esperado para a mantença e produção, enquanto, animais menos eficientes apresentam maior consumo residual e consumo observado maior que o consumo de alimento predito (FORBES, 2005). Considerando o efeito sobre a emissão CH_4 entérico, animais mais eficientes produzem menos CH_4, no entanto, esta associação não é claramente observada em bovinos de corte (MERCADANTE et al., 2015; OLIVEIRA et al., 2016). A ausência de diferença entre a emissão de CH_4 entérico entre bovinos com alto e baixo consumo residual poderia estar relacionada com a maior fermentação ruminal em animais com maior eficiência alimentar, na qual maior quantidade de nutrientes estaria disponível para a produção de CH_4 no rúmen, além de que haveria uma relação positiva entre a eficiência (ganho de peso : consumo de MS) com CH_4 (FREETLY; BROWN-BRANDL, 2013).

Tabela 2 – Intensidade (CH_4/kg de produto) e emissão diária de metano descritos em diferentes sistemas de produção

Descrição sistema	Produção individual	Emissão		Referência
		CH_4	Intensidade	
RTM	26 kg PL4%	392 g/dia	16,0 g CH_4/kg PL4%	Fredeen et al. (2013)
Pastejo+ concentrado	24,6kg PL4%	353 g/dia	15,3 g CH_4/kg PL4%	
Pastejo (2,53 UA[1]/ha) IPP 24 meses TR 18% 92 vacas lactação	6262 kg/ano	-	430,7 kg CO_2-eq/t ECM	O'Brien et al.(2014)
RPM+concentrado IPP 24 meses TR 34% 220 vacas lactação	10892 kg/ano		376,4 kg CO_2-eq/t ECM	
RTM, IPP 26 meses TR 38% 153 vacas lactação	12506 kg/ano		373,6 kg CO_2-eq/t ECM	
Pastejo TR 10% Rebanho 500 animais	5000 kg/ano	119 kg/ano/vaca	582 kg CO_2-eq/t ECM	Belflower et al. (2012)
RTM TR 33% Rebanho 700 animais	10700 kg/ano	202 kg/ano/vaca	448 kg CO_2-eq/t ECM	
Pastejo TR 18% 90 vacas lactação	6639 kg FPCM/ano	124 kg/ano/vaca	666 kg CO_2-eq/t FPCM^5	O'Brien et al. (2012)
RTM TR 18% 90 vacas lactação	8040 kg FPCM/ano	136 kg/ano/vaca	569 kg CO_2-eq/t FPCM^5	

[1] UA; unidade animal, 550 kg

IPP; idade primeiro parto, TR; taxa reposição, PL4%; produção leite corrigido 4% gordura, ECM; produção leite corrigida pela energia

FPCM; produção leite corrigida pela gordura e proteína.

Fonte: Elaborado pela autora, 2018.

2.6 TÉCNICAS DE AVALIAÇÃO DE METANO ENTÉRICO

Recentemente, HRISTOV et al. (2018) abordam as possíveis fontes de erro, e valores discrepantes em inventários nacionais e global de emissão de CH_4 do setor pecuário. Para os autores, possíveis fontes de erros estariam relacionadas com a coleta de dados de emissão que compõem inventários nacionais, estimativas da ingestão de MS, ingredientes e composição química da dieta, além das técnicas de avaliação da emissão de CH_4 entérico. Diferentes técnicas *in vivo* para medir a emissão de CH_4 entérico em ruminantes foram estabelecidas. Dentre estas a utilização de câmaras de respiração, marcador hexafluoreto de enxofre (SF_6) e recentemente, o GreenFeed (C-Lock, Inc., South Dakota, USA). Prós e contras são descritos em cada uma das técnicas, consequentemente, devem ser avaliadas e utilizadas dependendo do objetivo e as condições experimentais de cada trabalho.

Durante o período de medida de CH_4 em câmara de respiração (1 – 7 dias consecutivos) o animal permanece estabulado no interior da câmara sendo possível calcular a emissão de CH_4 total pela multiplicação do fluxo de ar através do sistema pela diferença de concentração entre o fluxo de entrada e saída (HAMMOND et al., 2016a). As câmaras de respiração são caras e apresentam limitação quanto ao número de animais a serem utilizados. A técnica torna-se incompatível para a estimação ao nível de sistema, uma vez que, o comportamento ingestivo do animal pode ser modificado e interações ambientais não são consideradas. Além disso, é impraticável quando se avalia animais em pastejo (STORM et al., 2012).

A técnica do marcador SF_6 foi desenvolvida por Zimmerman, (1993) e seu princípio baseia-se na liberação de uma pequena e conhecida quantidade de SF_6 no rúmen-reticulo. O SF_6 liberado se mistura com o gás de fermentação ruminal e atua como um marcador para o CH_4 produzido no rúmen. Os animais são equipados com um sistema de coleta de ar sendo representativo dos gases produzidos no rúmen e do marcador. As amostras de ar são acumuladas ao longo de 24 horas, e normalmente as coletas são repetidas durante cinco a oito dias, sendo possível realizar amostras acumuladas em medidas durante cinco dias (BERNDT et al., 2014). Essa técnica é principalmente utilizada em animais em condição de pastejo, sendo possível avaliar diferentes estratégias de mitigação em sistemas onde a forragem pastejada faz parte da dieta consumida.

O sistema GF caracteriza-se por medidas pontuais da emissão de CH_4 e CO_2 realizadas a cada visita do animal ao equipamento. Os animais são atraídos ao GF devido ao fornecimento de uma pequena quantidade de concentrado (baixo valor nutritivo) e a emissão estimada a partir do tempo da visita, entre 3 – 5 minutos, no dia e por vários dias (HRISTOV et al., 2015). Uma das dúvidas com o uso do GF está na representatividade das amostras realizadas pontualmente durante o dia para então estimar a emissão diária de CH_4, uma vez que o padrão circadiano de emissão de CH_4 entérico relaciona-se com a ingestão de alimento ao longo do dia (HAMMOND; WAGHORN; HEGARTY, 2016).

Buscando a validação e a comparação entre as técnicas, estudos vem sendo desenvolvidos (ALEMU et al., 2017; HAMMOND et al., 2015, 2016b; HRISTOV et al., 2016). Utilizando novilhas da raça Holandês, HAMMOND et al. (2015) compararam as três técnicas citadas acima em experimentos com dietas baseadas em forragem conservada e forragem pastejada. Os valores de emissão entre as técnicas foram similares, no entanto, quando comparadas a fim de avaliar as diferentes estratégias alimentares em cada um dos experimentos, os efeitos do tratamento na emissão de CH_4 foram significativos somente quando medidos usando câmara de respiração e SF_6, não sendo observada diferença quando as medidas foram realizadas com o uso do GF. Os autores atribuem esta resposta a ausência de visitas ao GF durante o pastejo e no início da manhã. Ao contrário, Hammond et al. (2016b) afirmam que foi possível detectar as mesmas diferenças com a utilização de diferentes fontes de forragem e teor de FDN na dieta independente do uso do GF ou câmaras de respiração. Em média os valores de emissão de CH_4 foram 448 g/dia e 20,9 g/kg de MS consumida usando o sistema GF, e 458 g/dia e 23,8 g/kg de MS consumida utilizando câmara de respiração.

Uma maior variação nas medidas é observada quando compara-se o GF com o SF_6. A baixa relação entre as duas técnicas pode ser influenciada pelas estimativas de concentração de gases no ambiente e a ventilação, principalmente na técnica do marcador SF_6 (HRISTOV et al., 2016). JONKER et al. (2016b) descrevem que a emissão de CH_4 a partir do marcador SF_6 foi 6% menor que a emissão medida em câmara de respiração, possivelmente devido a exclusão da emissão oriunda da fermentação no intestino grosso. Enquanto que em comparação a medida obtida no GF e a câmara, o valor da emissão de CH_4 (g/kg MS consumida) foi similar, mas, 13% superior em comparação a medida obtida no SF_6.

A emissão de CH_4 entérico pode ainda ser estimada de maneira indireta a partir da utilização de modelos empíricos ou mecanicistas. Modelo empíricos são baseados em associações matemáticas ou estatísticas entre o consumo da dieta, a composição dos alimentos consumidos e alguns fatores ligados ao animal, enquanto modelos mecanicistas integram a descrição matemática da bioquímica da fermentação, sendo baseados em princípios bioquímicos, metabólicos e fisiológicos da produção de CH_4 em nível ruminal e algumas vezes no intestino grosso (HRISTOV et al., 2018). A partir do desenvolvimento de modelos é possível estimar a emissão de CH_4 entérico ao nível de sistema (ELLIS et al., 2010).

Modelos matemáticos para a estimativa da emissão de CH_4 de origem entérica são propostos pelo IPCC. Conhecidos como “Tiers”, estes métodos são utilizados dependente do objetivo e do nível de informações necessárias para a estimativa (IPCC, 2006). O Tier 1 baseia-se em fatores de emissão, de forma simples considera a emissão anual de determinada categoria e espécie animal e a população deste no país em questão. Este método, é adequado para a maioria das espécies animais em países onde a fermentação entérica não é a principal fonte ou onde os dados de caracterização da emissão não estão disponíveis. Um pouco mais detalhado o Tier 2 requer dados detalhados sobre a ingestão de energia bruta e fatores de conversão de metano para categorias de animais. Visando uma maior acurácia nas estimativas, o Tier 3 é o método que requer a maior quantidade de informações específicas em relação ao país ou situação em que a estimativa está sendo realizada.

Em relação ao uso destes modelos propostos pelo IPCC, Wall; Coffey; Pollott, (2012) utilizaram Tier 2 e 3 para estimar a emissão de CH_4 entérico e avaliar o efeito do comprimento da lactação na emissão de gases de efeito estufa a partir de informações detalhadas em rebanhos no Reino Unido. A fim de estimar o efeito de práticas de manejo no processo de intensificação da produção de bovinos de corte no Brasil, CARDOSO et al. (2016) utilizaram Tier 2 par estimar a emissão de CH_4 entérico a partir de uma detalhada descrição de das categorias de bovinos utilizadas (peso vivo de machos e fêmeas adultos, peso vivo e ganho de peso de categorias de animais em crescimento), descrição da dieta consumida, e a proporção de energia bruta que é perdida na forma de CH_4, que neste estudo variou de 6,5 % a 3 % EB .

Como mencionado acima modelos empíricos, assim como os modelos propostos pelo IPCC, baseiam-se nos principais fatores de influência na produção de CH_4 entérico. Consumo de MS, consumo de energia metabolizável (EM), FDN, fibra em detergente ácido (FDA), teor de gordura, lignina e proporção de forragem na dieta foram considerados no desenvolvimento de modelos por Ellis et al. (2007). Nas situações avaliadas, em bovinos de leite 69% e 60% da emissão de CH_4 entérico diária foi explicada pela a ingestão de MS e EM, respectivamente, sendo os melhores preditores. Segundo Sauvant et al. (2011), a forma de expressar a emissão de CH_4 estimada pode levar a conclusões equivocadas. Para os autores, estatisticamente o consumo de MO digestível é um melhor preditor que o consumo de MS, sendo possível encontrar uma boa relação entre MO digestível e emissão de CH_4/kg de MS consumida (r^2 = 0,81) e CH_4/kg de peso vivo (PV) (r^2 = 0,90).

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3 QUESTÕES E ESTRATÉGIA DE PESQUISA

A base conceitual e o efeito das principais estratégias de mitigação de metano foram apresentados anteriormente. A partir do exposto, as respostas observadas por diversos trabalhos descrevem o efeito ao nível animal realizadas, normalmente, por curtos períodos. Apesar das recentes pesquisas ao nível de sistema de produção, com a utilização da metodologia de análise de ciclo de vida, por exemplo, discussões sobre a influência de diferentes práticas de manejo ao nível do sistema de produção sobre a emissão de metano entérico ainda merecem atenção.

O desenvolvimento desta tese visa identificar e mesurar inicialmente o efeito de diferentes estratégias de alimentação sobre a emissão de metano entérico a partir da utilização de técnicas *in vivo*, e após, ao nível de sistema de produção de leite, integrar os principais efeitos e identificar as principais práticas de manejo que possibilitem o desenvolvimento de estratégias de mitigação.

Assim, a tese divide-se em três experimentos afim de:

1. Comparar a utilização de diferentes suplementações energéticas para vacas leiteiras em pastos anuais de inverno sob pastejo leniente e identificar seu efeito sobre a intensidade de emissão de metano (g CH_4/ kg de leite produzido). A pergunta central visa responder a magnitude das respostas em produção de leite e ingestão de MS total com a utilização de suplementação energética e o efeito sobre a emissão de CH_4 entérico.

2. Utilizar o momento que os animais estão em estabulação para a ordenha para mesurar a emissão CH_4 entérico a partir de medidas pontuais com o uso do GreenFeed e comparar dois sistemas de produção baseados a pasto (alta e baixa entrada de insumos). Objetivou-se avaliar o efeito de raça e estratégia de alimentação sobre a emissão diária de CH_4 entérico.

3. Estimar a emissão de metano entérico de vacas e novilhas a partir da equação proposta por Sauvant; Nozière (2013). Ao nível do sistema de produção buscou-se identificar práticas de manejo que podem ser consideradas para o desenvolvimento de estratégias de mitigação de metano.

O primeiro experimento foi desenvolvido no Brasil no ano de 2016. Vacas com produção de leite média de 23 kg/dia foram submetidas a duas estratégias de suplementação energética, silagem de milho e grão de milho. A técnica utilizada para mesurar a emissão de

CH_4 entérico foi a do marcador hexafluoreto de enxofre. Além do CH_4 entérico, a produção de leite e consumo de matéria seca (pasto e suplemento) foram medidos. Sabe-se que o uso de suplementação é indicado muitas vezes como uma estratégia de mitigação de CH_4 entérico. No entanto, o efeito do suplemento sobre o consumo de pasto e de MS total pode influenciar a resposta produtiva, e ambos determinar a emissão diária e a intensidade de emissão de CH_4 entérico. Neste sentido, as respostas encontradas na literatura apresentam grande variação, sendo muitas vezes dependentes de fatores ligados ao animal e a dieta consumida.

As outras duas etapas foram desenvolvidas durante o período de doutorado sanduíche no INRA/França durante o ano de 2017 e construídas a partir de um experimento de longo prazo intitulado de “Qual vaca para qual sistema?”. Este experimento está sendo desenvolvido na fazenda experimental do INRA (Institut National de la Recherche Agronomique) no Pin-au-Haras, França. O projeto iniciou em 2005 e tem por objetivo avaliar durante o curso de sucessivas lactações a capacidade de adaptação de diferentes tipos de vaca (raça, idade ao primeiro parto, família genética) diante de duas estratégias alimentares diferentes (alta e baixa entrada de insumos).

No ano de 2017, a medida de metano entérico aconteceu de duas maneiras, inicialmente durante a estação de pastejo foram iniciadas as medidas da emissão de CH_4 entérico. Estas foram realizadas com a utilização do GreenFeed durante o momento que as vacas estavam estabuladas para a ordenha. E uma segunda abordagem aconteceu a partir da base de dados construída depois de 2005, a emissão de CH_4 entérico foi estimada para vacas e novilhas. A partir da integração dos resultados produtivos e dos cálculos de metano foi possível predizer a emissão total de CH_4 entérico de oito sistemas de produção. Estes oito sistemas foram criados combinando os diferentes fatores (duas raças, duas idades ao primeiro parto e duas estratégias de alimentação). Para comparar estes sistemas foi considerado uma produção de leite anual fixa entre eles. Após a compilação das informações necessárias baseadas nos dados reprodutivos coletados durante os dez anos de experimentação, um último fator foi incluído, a taxa de reposição do rebanho. O efeito de diferentes taxas de reposição do rebanho sobre a emissão de CH_4 entérico foi testado em cada um dos sistemas considerando os demais fatores estudados.

4 OBJETIVO GERAL

Avaliar a emissão de CH_4 entérico de vacas leiteiras sobre diferentes estratégias de alimentação e estimar o impacto de diferentes práticas de manejo sobre a emissão de CH_4 entérico ao nível de sistema de produção.

5 HIPÓTESES

5.1 HIPÓTESE GERAL

A estratégia de alimentação utilizada em vacas e em novilhas é o principal fator de influência da emissão diária de CH_4 entérico. No entanto, associado a esta, a escolha da raça; práticas de manejo como idade ao primeiro parto e taxa de reposição irão influenciar a emissão de CH_4 entérico do sistema de produção.

5.2 HIPÓTESES ESPECÍFICAS

a) Em condições de pastejo leniente a utilização de suplementação energética resultará em aumento na produção de leite. Adicionalmente, a redução da intensidade de emissão de CH_4 entérico (g CH_4/kg de leite) será dependente da resposta em produção com a suplementação.

b) Comparando dois sistemas de produção com raças diferentes (Holandês e Normando), vacas em lactação recebendo uma estratégia de alimentação sem restrições em relação as exigências energéticas apresentam maior emissão de CH_4 entérico. Entretanto, a intensidade de emissão (g CH_4/kg de leite) é menor em vacas da raça Holandês devido a maior produção de leite.

c) A adoção de uma maior idade ao primeiro parto, três anos em comparação à dois anos, e a maior taxa de reposição como práticas de manejo resultam em maior emissão de CH_4 entérico do sistema de produção.

6 ARTIGO I – ENTERIC METHANE EMISSION FROM GRAZING DAIRY COW RECEIVING CORN SILAGE OR CORN GROUND SUPPLEMENTATION[1]

6.1 ABSTRACT

This study assessed the effects of corn silage or ground corn supplementation on CH_4 emissions, milk production and total dry matter (DM) intake by dairy cows under lenient grazing conditions. Twelve Holstein × Jersey dairy cows were divided into 6 homogeneous groups and randomly distributed in the experimental treatments, which were compared using a 3 × 3 Latin square design over three periods of 17 days (evaluation period of 5 days). The dairy cows strip-grazed a mixture of annual ryegrass (*Lolium multiflorum*) and oat (*Avena sativa*). The treatments consisted of three feeding strategies being those without supplementation (WS) and supplemented with ground corn (GC) or corn silage (CS; 3.2 vs. 4.2 kg as DM basis). The daily enteric CH_4 emission was measured using the sulphur hexafluoride (SF6) tracer gas technique. The same post-grazing sward height (average = 8.8 cm) between treatments was assured by reducing the herbage allowance (HA) in supplemented treatments. The HA was 41.3, 30.8 and 34.6 kg of DM/cow/day for treatments WS, CS, and CG, respectively. The CH_4 yield (g/kg of DM intake) decreased in the GC treatment group compared to the WS and CS treatments, although the CH_4 emissions (g/day) and CH_4 intensity (g/kg of milk) did not differ between treatments, on average 337 g/day and 15.1 g/kg of milk, respectively. The total DM intake increased by 2.9 kg/day and milk production increased by 1.4 kg/day in the GC treatment group compared to the WS group, whereas the total DM intake increased by 1.5 kg/day and milk production did not differ between the CS and WS treatment groups. In conclusion, it was possible to reduce the HA and remains or increase the total DM intake with corn silage or corn ground supplementation, respectively. In dairy cows grazing temperate grass under lenient grazing condition the energy supplementation was an effective feeding strategy to reduce the CH_4 yield but, it was not effective to reduce the CH_4 intensity.

Keywords: *Avena sativa*, *Lolium multiflorum*, methane intensity, supplementation.

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[1] Este capítulo apresenta o artigo submetido ao periódico Animal Feed Science and Tecnology, seguindo as normas da revista.

6.2 INTRODUCTION

Enteric methane (CH_4) is a normal product of a ruminant's digestive process, and its emission represents approximately 44% of the greenhouse gas emissions (GHG) by the livestock sector (Gerber et al., 2013). The synthesis of enteric CH_4 is directly associated with the hydrogen (H_2) concentration in the ruminal environment, which is a consequence of the proportion of volatile fatty acids (VFA) resulting from microbial metabolism (Janssen, 2010). Hence, the dry matter (DM) intake and diet composition may affect the formation of enteric CH_4 due to differences in the amount and characteristics of the substrate available for ruminal fermentation.

The use of concentrate supplementation has been proposed as a strategy for CH_4 mitigation in grazing dairy cows due to the increase in starch intake and the decline in ruminal pH (Beauchemin et al., 2008; Martin et al., 2010). Reports have shown that increasing the proportion of concentrate in the diet will lower CH_4 yield (*per* unit of feed intake) and CH_4 intensity (*per* unit of milk) if milk production remains the same or is increased (Hristov et al., 2013; Yan et al., 2000). Sauvant and Giger-Reverdin (2009) estimated that the enteric CH_4 emission is reduced when the concentrate supplement ranged from 350 to 400 g/kg of DM in the total diet. However, in grazing dairy cows the concentrate uses as a CH_4 mitigation strategy has displayed conflicting results (Muñoz et al., 2015; van Wyngaard et al., 2018), these ones were attributed to the herbage DM intake.

The nutritive value of conserved forages, main digestibility, can drive the effect on CH_4 yield and CH_4 intensity (De Boever et al., 2016; Hart et al., 2015; O'Neill et al., 2012). Previous studies increased the corn silage proportion on diets based on barley silage (Benchaar et al., 2014), alfalfa silage (Hassanat et al., 2013) and grass silage (Hart et al., 2015; Van Gastelen et al., 2015) showing a decrease in the CH_4 yield. However, the answers had greater variability, which was related to the corn silage proportion and total DM intake. The effect on CH_4 emission has been less reported in the literature with the corn silage inclusion in the grazing-based systems.

The use of supplements is known to have a greater impact on reducing the herbage DM intake and produces a decreased milk production response in dairy cows under non-restrictive grazing conditions compared to dairy cows under restrictive grazing conditions (Bargo et al., 2003; Pérez-Prieto et al., 2011), which may be related to the similarity between the energy supplied by grazed herbage and the energy requirements of non-restrictive grazing conditions. With regards to the effect of different supplements, a theoretical model published by Delagarde et al. (2011) has shown that the impact of forage-conserved supplementation on herbage intake is more pronounced compared to that of concentrate supplementation due to a greater substitution rate (SR, kg herbage DM intake decreased per kg DM supplement intake). In practical conditions, the SR allows for reductions in the herbage allowance (HA) to avoid herbage waste. In this case, the grazing strategy may target similar post-grazing sward heights compared to dairy cows without supplementation. However, studies using grazing management protocols with similar post-grazing sward heights between unsupplemented and supplemented dairy cows are scarce.

In this way, highlighting the high influence of supplementation on the herbage intake, this work aimed to evaluate the effect of corn silage and ground corn supplementations for dairy cows grazing temperate grass on CH_4 yield, CH_4 intensity, milk production, and herbage DM intake. We hypothesized that under lenient grazing conditions, an eventual milk production response to the supplemented treatments may occur. In addition, the enteric CH_4 emission will be dependent on herbage and total DM intake, while the CH_4 intensity with the energy supplementation will be dependent on the milk production response.

6.3 MATERIALS AND METHODS

The experiment was performed from August to November in 2016 in Lages, SC, Brazil (50.18ºW, 27.47ºS; 920 m altitude) in accordance with the regulations of the Santa Catarina State University Ethical Committee (Protocol no. 43.73.09.08.16). During the study, the average maximum and minimum temperatures were 20.8 and 8.6ºC, respectively, and the total rainfall was 161 mm. The average maximum and minimum temperatures and rainfall over 10 years were 20.5ºC, 9.9ºC and 183 mm, respectively.

6.3.1 Animals, experimental design and treatments

Twelve Holstein × Jersey multiparous cows were separated into 6 homogeneous groups according to milk production (23.3 ± 6.9 kg/d), days in milk (101 ± 57.6 d), body weight (492 ± 76.8 kg) and parity (2.3 ± 1.5). Each group was randomly assigned to treatment sequences according to a replicated 3 × 3 Latin square design. The experimental period lasted 17 days and consisted of 12 days of adaptation and 5 days of measurement period.

The treatments consisted of cows with access to annual ryegrass + oat without supplementation (WS) or with different energy supplementation, ground corn (GC) and corn silage supplementation (CS). The quantity of corn silage was calculated to offer the same net energy lactation (NE_L) provided by the ground corn supplementation. The NE_L of supplements was estimated using the equations published by the INRA (2007), where the net energy values are initially calculated from aNDF, crude fiber and crude protein (CP). The quantity offered was 3.2 and 4.2 kg (as DM basis) of ground corn and corn silage, respectively. The supplements were offered individually twice daily after morning and afternoon milking for 60 minutes in the cases of the GC and CS treatments. In the afternoon, any remaining supplement was considered as a refusal. Water and minerals were continually available at grazing and indoors. The chemical composition and nutritive value of the supplements are presented in Table 1.

Table 1 – Chemical composition and nutritive value of the supplements (corn silage and ground corn)

Item	Corn silage	Ground corn
DM, g/kg	281	911
Chemical composition, g/kg DM		
Organic matter	963	984
Crude protein	61	85
aNDF	404	159
ADF	240	31
Nutritive value[1]		
NE_L, Mcal/kg DM	1.53	1.82
PDIN, g/kg DM	37	67
PDIE, g /kg DM	65	87

[1] INRA (2007); NE_L, Net energy for lactation; PDIN, truly digestible protein when degradable nitrogen limits microbial growth; and PDIE, Truly digestible protein when available energy limits microbial growth.

6.3.2 Grazing management and pasture

The ryegrass (Lolium multiflorum cv. Barjumbo) and oat (Avena sativa cv. FUNDACEP - FAPAR 43) pastures were sown in May 2016 on a total area of 6.5 ha. The area was split into six paddocks, two per treatment, and was grazed by the same two cows during each period. The same paddock was grazed three times, once per period. The grazing cycle was on average 32 days (17 days of experimental period + 15 days between experimental periods). During each grazing cycle, each strip had one day of grazing period and 31 days of rest period. After each period, the entire area was mowed to standardize the herbage regrowth between treatments. Between the experimental period intervals, the cows grazed non-experimental mixed perennial pastures.

The grazing method selected for this study was strip grazing, and the area allocated daily to each treatment group was calculated by estimating the pre-grazing herbage mass (HM). In the treatment groups that were not supplemented, the HA above ground level was 35 kg DM/cow. In the supplemented treatment groups (GC and CS) the HA was adjusted to obtain the same post-grazing sward height of the unsupplemented cows.

6.3.3 Animal measurements

The cows were milked twice daily (7 a.m. and 3:30 p.m.). Over the last 5 days of each period, individual milk production was recorded and, milk samples were collected to determine the milk composition. The milk fat and milk protein concentrations were determined by infrared spectrophotometry (Dairy Spect FT, Bentley Instruments Inc, Chaska, Minnesota, USA). The energy-corrected milk (ECM), standardized to 4.0% fat and 3.3% protein, was calculated according to the equation proposed by Tyrrell and Reid (1965):

$$\text{ECM (kg/cow per day)} = \text{milk production kg} \times (376 \times \text{fat\%} + 209 \times \text{protein\%} + 948) / 3{,}138$$

The herbage DM intake was estimated in each group by determining the difference between the pre- and post-grazing HM (Lantinga et al., 2004; for details on the pre- and post-grazing HM estimations, see section 6.3.4 below). The individual supplement intake was quantified daily as the difference between the quantity supplied and the orts.

The NE_L requirements for lactation and maintenance were calculated based on a 4% fat correct milk (FCM) production and body weight as proposed by INRA (2007). The NE_L and metabolizable protein (MP) supply were calculated from the herbage, corn silage and ground corn DM intake and the NE_L and MP concentrations of each feed. The energy balance was calculated as the ratio between the NE_L supply and the NE_L requirements. The energy balance was calculated because the experimental periods were too short to measure any changes in body condition score or live weight. The ruminal degradable protein (RDP) balance was calculated from the difference between the supply of the truly digestible protein when the degradable nitrogen limits microbial growth (PDIN) and the supply of the truly digestible protein when the available energy limits microbial growth (PDIE).

The daily pattern of grazing time was analysed individually through visual observations performed every 5 min (Penning and Rutter, 2004). No behaviour was recorded indoors or at night. The herbage intake rate (g DM/min) was calculated by dividing the daily herbage intake by the mean of daily grazing time.

Daily CH_4 emissions were measured individually using the sulfur hexafluoride (SF_6) tracer technique described by Johnson et al. (1994). Each cow received one SF_6 capsule 15 days before collection of the expired air, average release rate was 2.44 ± 0.11 mg/day. The gas samples were collected on the last 5 days of each period. 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 nitrogen gas (N_2) and pre-evacuated prior to each sample collection. The flow regulators were calibrated to allow a remaining vacuum in the cylinder of about 500 mb (which represents half of the total cylinder volume) at the end of the sample collection period (five consecutive days). In addition to breath samples, an identical apparatus was placed 2m above the soil in the experimental paddocks and indoors to allows the measurement of CH_4 and SF_6 background levels in air. During the last 5 days of each experimental period, three backgrounds samples were taken outdoors, and two samples were taken indoors (local of supplementation was offered).

Concentrations of CH_4 (ppm) and SF_6 (ppt) were determined at the laboratory of Federal University of Rio Grande do Sul, located in Porto Alegre, Southern Brazil, using a gas chromatograph GC-2014 (Shimadzu, Japan), equipped with flame ionization detector (FID) at 250°C and a 1/8” packed column Shimalite Q (0.7 m, 80/100 mesh) for CH_4, and electron capture detector (ECD) at 325ºC and a 1/8” packed column Porapak N (1.5 m, 100/180 mesh) for SF_6. A mixture 5% CH_4+Argon was used as make-up gas in SF_6 analysis (ECD). The gas chromatography column was maintained at 80ºC during the analysis and N_2 was used as gas carrier with a flow of 25 cm^3/min. Calibration curves were established using certified standard gases (White Martins Development Laboratory), with the concentration for CH_4 in ppm (2.41, 5, 10.42 and 203 20) and for SF_6 in ppt (11, 30, 98 and 1000). The minimum detection limits were 0.15 ppm for CH_4 and 5.2 ppt for SF_6.

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

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

where R_{CH4} is the enteric CH_4 (g/cow/day), R_{SF6} is the release rate of SF_6, MW_{CH4} is the molecular mass of CH_4 (16), an MW_{SF6} is the molecular mass of SF_6 (146). The CH_{4BG} and SF_{6BG} concentrations are representative to the CH_4 and SF_6 background concentrations. The CH_4 and SF_6 background concentrations were calculated weighting the predicted indoors and outdoors background concentrations, according to the duration the animals spent in each environment.

6.3.4 Feed and sward measurements

Samples of the silage and ground corn were collected twice daily from day 12 to day 17 in each period. These samples were a composite for each period. Samples of the orts for each cow were collected during the last 5 days of each period and were 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 using a 1-mm screen for subsequent chemical analyses.

The pre- and post-grazing HMs above ground level were estimated using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand), which was calibrated as a function of the DM content in the plate area (0.1 m2; Mannetje and Jones, 2000). For calibration, during each experimental period, samples from 10 points were cut with scissors at ground level before and after grazing and then dried in an oven for 72 h at 60°C. At the end of the experiment, the HM was recalculated by the equations used to estimate the pre- and post-grazing HM.

The pre- and post-grazing herbage heights were measured by averaging 60 readings taken randomly throughout the area allocated for grazing by each group using a rising plate meter (F200 model, Farmworks, Feilding, New Zealand). The pre-grazing extended height of the leaf blade and the highest sheath were measured on 100 tillers at random on day 14 and day 16. The post-grazing leaf and sheath extended heights were measured on day 16 and day 18 on 100 tillers per treatment.

The morphological and chemical compositions of the sward were determined on day 14 and day 16. In each paddock, twenty handfuls of randomly selected herbage (~800 g fresh) were cut at ground level. This herbage sample was separated into two subsamples. One subsample was used to estimate the chemical composition on the herbage selected by grazing. For that, the post-grazing sward height measured with a sward stick was considered to cut the herbage sample. After, 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 separation. The ryegrass and oat were separated into leaf blades, pseudostems + stems, and dead tissue. Each constituent was dried in an oven for 72 h at 60°C to determine the morphological composition of the herbage.

6.3.5 Chemical analysis

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 organic matter (OM) was quantified based on the mass difference. The total N was assayed using the Kjeldahl method (Method 984.13; AOAC, 1997). The neutral detergent fibre (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).

6.3.6 Statistical analysis

The dependent variables were subjected to an analysis of variance using the function PROC MIXED in the software SAS (version 9.3, SAS Institute, Cary, NC, USA). The animal performance (intake, milk production and composition) and methane emissions variables were averaged per animal and period (n = 36) and analysed using the following model:

$$Y_{ijk} = \mu + \text{animal}_i + \text{period}_j + \text{treatment}_k + e_{ijk},$$

where Y_{ijk}, μ, animal_i, period_j, treatment_k and e_{ijk} represent the analysed variable, the overall mean, the random effect of the animal, the random effect of the period, the fixed effect of the treatment and the residual error, respectively.

The herbage variables were averaged per paddock and period (n = 18) and analysed using the following model:

$$Y_{jk} = \mu + \text{paddoc}_{kj} + \text{treatment}_k + e_{jk}$$

where Y_{ijk}, µ, $paddock_i$, $treatment_k$ and e_{ijk} represent the analysed variable, the overall mean, the random effect of paddock, the fixed effect of the treatment and the residual error, respectively.

6.4 RESULTS

The pre-grazing HM and pre-grazing sward heights were similar between treatments and averaged 2534 kg DM/ha and 13.7 cm, respectively (Table 2). The daily offered area per cow decreased in the supplemented treatment groups compared to the unsupplemented group (-17%; $P < 0.01$). The CP, aNDF and ADF contents of the herbage did not differ between the treatments and averaged 212, 447 and 236 g/kg DM, respectively. The OM digestibility, NEL, and truly protein digestible in the small intestine were on average 0.79, 1.67 Mcal/kg DM and 107 g/kg DM, respectively.

The CH_4 emission and CH_4 intensity (per kg milk production and ECM) did not differ between treatments and averaged 337 g/day, 15.1 g/kg milk and, 14.6 g/kg ECM, respectively (Table 3). The CH_4 yield was lower in GC treatment ($P < 0.01$; 20.4 g/kg DM intake) compared to the WS and CS treatments (26.3 g/kg DM intake).

The total DM intake increased ($P < 0.001$) by 2.9 and 1.3 kg/day in the GC and CS treatments compared to the WS (Table 4), respectively. However, the herbage DM intake (-1.8 kg/day) in the CS group decreased when compared with WS but did not differ between the GC and WS treatments ($P < 0.001$). The grazing time was 53 min/day and 16 min/day lower that WS ($P < 0.001$) in CS and CG, respectively. The proportion of time spent grazing (0.66) and the herbage DM intake rate (29 g DM/min) were similar between the treatments.

Milk production (+1.4 kg/day; $P = 0.03$) and milk protein production (+60 g/day; $P < 0.001$) increased in the cows supplemented with ground corn compared to those supplemented with corn silage and those in the unsupplemented group (Table 5). The milk fat concentration was similar between treatments.

Table 2 – Pre- and post-grazing herbage characteristics of annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) grazed by dairy cows with or without corn silage or ground corn supplementation

	Treatments[1]				
Item	WS	CS	GC	SEM	*P*-value
Offered area, m^2/cow per day	172^a	137^b	149^b	9.45	0.007
	Pre-grazing				
Herbage mass, kg DM/ha	2595	2440	2566	106.4	0.564
Rising plate meter, cm	13.8	13.1	14.1	0.64	0.504
Extended tiller, cm	38.9	37.0	38.7	1.39	0.587
Extended sheath, cm	15.8	15.4	16.3	0.71	0.675
Extended lamina, cm	23.1	21.5	22.4	0.81	0.444
	Post-grazing				
Herbage mass, kg DM/ha	1811^a	1572^b	1702^{ab}	58.3	0.041
Rising plate meter, cm	9.3	8.2	9.0	0.37	0.147
Extended tiller, cm	17.3^a	14.7^b	17.7^a	1.16	0.041
Extended sheath, cm	12.3	10.6	11.9	0.71	0.221
Extended lamina, cm	7.23	6.47	7.48	0.330	0.118
	Herbage allowance, kg DM/day				
Above ground level	41.3^a	30.8^c	34.6^b	0.86	<0.001
Green material	39.8^a	30.1^c	32.5^b	0.79	<0.001
Live lamina	23.1^a	18.3^b	18.7^b	0.60	<0.001
	Chemical composition, g/kg DM				
DM, g/kg	170	166	170	4.03	0.509
Organic matter	928	925	928	4.47	0.762
Crude protein	219	204	213	6.25	0.277
aNDF	452	450	439	9.27	0.601
ADF	236	240	231	4.50	0.350
	Nutritive value				
OM digestibility[2]	0.79	0.78	0.79	0.005	0.321
NE_L, Mcal/kg DM[3]	1.67	1.65	1.69	0.019	0.369
PDIN, g/kg DM[4]	141	133	139	3.59	0.305
PDIE, g/kg DM[5]	107	105	108	1.13	0.217

[1] Treatments: WS: cows grazing annual ryegrass + oat without supplementation; GC: 4 kg ground corn supplementation; and CS: 15 kg corn silage supplementation. Means followed by the same letter are not significantly different (*P* > 0.05).

[2] OM digestibility: estimated as a function of the CP and ADF content of selected pasture (INRA, 2007).

[3] Net energy for lactation.

[4] Truly digestible protein when degradable nitrogen limits microbial growth.

[5] Truly digestible protein when available energy limits microbial growth.

Table 3 – Methane emissions of dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) with and without corn silage or ground corn supplementation

	Treatment[1]				
Item	WS	CS	GC	SEM	*P*-value
Methane					
g/day	334	356	321	14.1	0.290
g/kg DM intake	27.7^{a}	25.0^{a}	20.4^{b}	1.32	0.008
g/kg milk	15.3	15.6	14.3	0.59	0.352
g/kg ECM[2]	15.0	15.1	13.8	0.57	0.291
% GE intake	8.0	8.1	7.2	0.53	0.482

[1] Treatments: WS: cows grazing annual ryegrass + oat without supplementation; GC: 4 kg ground corn supplementation; and CS: 15 kg corn silage supplementation.

Means followed by the same letter are not significantly different ($P > 0.05$).

[2] ECM = Energy corrected milk production.

Table 4 – Dry matter intake, energy balance and grazing behaviour of dairy cows grazing annual ryegrass (Lolium multiflorum cv. Barjumbo) and oat (Avena sativa cv. FUNDACEP - FAPAR 43) with and without corn silage or ground corn supplementation.

	Treatment[1]				
Item	WS	CS	GC	SEM	*P*-value
DM intake, kg/d					
Herbage	11.9^{a}	10.1^{b}	11.2^{ab}	0.18	<0.001
Corn silage	-	3.1	-	-	-
Ground corn	-	-	3.6	-	-
Total	11.9^{c}	13.2^{b}	14.8^{a}	0.19	<0.001
aNDF intake, g/kg DM intake	452^{a}	443^{a}	371^{b}	3.78	<0.001
NE$_{L}$ supply, MJ/d[2]	82.8^{b}	80.6^{b}	106.5^{a}	1.80	<0.001
PDIN supply, g/day[3]	1682^{b}	1387^{c}	1800^{a}	37.21	<0.001
PDIE supply, g/day[4]	1278^{b}	1170^{c}	1518^{a}	25.35	<0.001
NE$_{L}$ balance	0.79^{b}	0.75^{b}	0.97^{a}	0.015	<0.001
RDP balance, g/day[5]	404^{a}	217^{c}	282^{b}	14.56	<0.001
Grazing time, min/d	408^{a}	355^{c}	392^{b}	5.3	<0.001
Proportion of time spent grazing	0.67	0.64	0.66	0.010	0.081
Herbage DM intake rate, g/min	29.7	28.6	28.7	0.43	0.160

[1] Treatments: WS: cows grazing annual ryegrass + oat without supplementation; GC: 4 kg ground corn supplementation; and CS: 15 kg corn silage supplementation.

Means followed by the same letter are not significantly different ($P > 0.05$).

[2] Net energy for lactation.

[3] Truly digestible protein when degradable nitrogen limits microbial growth.

[4] Truly digestible protein when available energy limits microbial growth.

[5] Ruminal degradable protein balance.

Table 5 – Milk production and milk composition of dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Barjumbo) and oat (*Avena sativa* cv. FUNDACEP - FAPAR 43) with and without corn silage or ground corn supplementation

	Treatments[1]				
Item	WS	CS	GC	SEM	*P* - value
Milk production, kg/day	21.7^b	21.8^b	23.1^a	0.38	0.030
4% FCM, kg/day[2]	22.7^b	22.8^b	23.8^a	0.31	0.029
ECM, kg/day[3]	22.2^b	22.2^b	23.5^a	0.31	0.011
Milk fat, %	4.34	4.36	4.25	0.053	0.312
Milk protein, %	3.13^b	3.07^c	3.21^a	0.020	<0.001
Milk fat, g/day	931^b	938^{ab}	974^a	12.7	0.056
Milk protein, g/day	673^b	662^b	733^a	11.2	<0.001

[1] Treatments: WS: cows grazing annual ryegrass + oat without supplementation; GC: 4 kg ground corn supplementation; and CS: 15 kg corn silage supplementation.
Means followed by the same letter are not significantly different ($P > 0.05$).
[2] FCM = fat corrected milk production.
[3] ECM = energy corrected milk production.

6.5 DISCUSSION

The current study aimed to evaluate the effects of different energy supplementations (corn silage or ground corn) on dairy cows grazing the upper sward layer of an annual temperate pasture. The goal of grazing management was successfully achieved because the same sward height proportion was obtained between treatments and averaged 40% of the pre-grazing sward height. Additionally, using the same post-grazing sward height, the results showed that by using corn silage and ground corn supplementation, the HA can be reduced by 25 and 16%, respectively.

6.5.1 Methane emission

The average CH_4 emission was consistent with the values reported for dairy cows grazing temperate grasses (O’Neill et al., 2011; Robertson and Waghorn, 2002; Wims et al., 2010) and the CH_4 emission predicted by equations, where the daily emissions ranged from 208 to 461 g CH_4/day (Ellis et al., 2010). It is known that there is a strong relationship between total DM intake and enteric CH_4 emission ($R^2 = 0.86$; Hristov et al., 2013) as reported with concentrate supplementation (Aguerre et al., 2011; Lovett et al., 2005) or corn silage supplementation (O’Neill et al., 2012). However, in agreement with our results, Jiao et al. (2014) and recently, van Wyngaard et al. (2018) did not observe this relationship in supplemented grazing dairy cows. This can be linked with the herbage DM intake and the nutritive value of herbage consumed. Consequently, this modifies the fermentable substrate available in the rumen in association with the supplement.

As the CH_4 yield allows to compare the results regarding the consumed feed composition, the reduction in CH_4 yield in dairy cows in the CS treatment may be explained by the rapidly fermentable carbohydrate (starch) supply combined with the aNDF intake. Hence, the aNDF intake in the GC group was -76 g /kg DM intake (371 vs 447 g aNDF) compared to the WS and GC treatments. This relationship was also described by Aguerre et al. (2011), and it was found to be associated with the differences between the methanogenic potential of the cell wall's components because fermentation of the structural carbohydrates has a higher methanogenic potential compared to non-structural carbohydrate fermentation (Moe and Tyrrell, 1979). Once the CH_4 formation is related to the H_2 available in the rumen, the non-structural carbohydrate fermentation decreases the H_2 availability for CH_4 production by *Archaea*.

The lack of a reduction in CH_4 intensity under the ground corn supplementation compared to that of the unsupplemented cows was a consequence of the milk production response, which did not exceed 7.0% in the dairy cows receiving 3.6 kg of ground corn and, it was not observed milk production increases with corn silage supplementation. Using similar concentrate intake levels, Muñoz et al. (2015) did not find any improvements in CH_4 intensity compared to a 50 g/kg DM concentrate intake. Sauvant and Giger-Reverdin (2009) observed that the relationship between concentrate level in the total diet and CH_4 production is curvilinear. Consequently, the concentrate level for enteric CH_4 emission decreases ranged from 350 to 400 g/kg of DM in the total diet, while it was linked to the level intake. According to O'Neill et al. (2012), for dairy cows grazing pastures with an OM digestibility greater than 0.89, the ability of supplementary feeding to increase milk production may be reduced. In the current study, the average OM digestibility was 0.79. To our knowledge, previous studies have not compared the three feeding strategies used in this work; however, improvements in individual milk production and the absence of an effect on CH_4 *per* milk product were observed when the concentrate inclusion level of the diets was increased (Lovett et al., 2005; Muñoz et al., 2015) or when a partially mixed ration supplementation regimen based on corn silage was used (O'Neill et al., 2012).

6.5.2 Dry matter intake and grazing behaviour

The specific aim in this trial was reduce of the supplementation effect on herbage DM intake. For that the grass management targeted to maintain a high pasture utilization rate and low post-grazing sward height, to compensate the adverse effects on residual pastures, reducing the HA of forage supplemented cows. The herbage DM intake decreased only in the cows supplemented with corn silage, but the total DM intake increased in both supplemented treatments. This answer indicates that the effects of supplementation on the total and herbage intake are linked to the supplement type. It is known that the corn silage supplementation has a higher effect on herbage DM intake that concentrate supplementation (Bargo et al., 2003; Delagarde et al., 2011) being related to the ruminal feedstuff filling capacity (INRA, 2007).

The effect of the supplementation type on the herbage DM intake was a consequence of the reduction in grazing time rather than a reduction in herbage DM intake rate, which averaged 29.1 g/min. However, the grazing time decreased to 16.5 min/kg due to DM corn silage intake. Reductions in grazing time with supplement utilization are usually a consequence of lesser grazing motivation (Bargo et al., 2003). These results are supported at least partially by previous work in which increased corn silage supplementation levels in dairy cows was found to decrease grazing time (Miguel et al., 2014). Additionally, reductions in grazing time to 11.4 and 15.1 min/kg by DM corn silage intake were recorded at low and high herbage allowances, respectively (Pérez-Prieto et al., 2011).

6.5.3 Milk production and milk composition

The higher milk production in dairy cows supplemented with corn ground was a consequence of higher total DM intake and consequently, the higher NEL supply. Dairy cows receiving ground corn supplementation had 0.97 of the energy requirements supplied and presented a positive milk production response of 0.39 kg milk/kg of ground corn intake. This response was lower than that proposed by Delagarde et al. (2011), where the milk production response ranged from 0.5 to 2.2 kg of milk/kg of DM concentrate intake. As discussed by other authors, the milk production response is associated with a concentrate intake level, herbage management, substitution rate, and milk production potential of cows (Bargo et al., 2003; Delaby and Peyraud, 2003; Kennedy et al., 2003). A milk production response from 0.96 to 1.36 kg/kg of concentrate was observed in dairy cows with high genetic merit and milk production (45.8 ± 6.6 kg/day; Bargo et al., 2002). In a similar grazing management condition, Muñoz et al. (2015) reported a milk production response of 0.68 kg/kg of concentrate in dairy cows with average milk production the 27 kg/day.

The absence of milk production response to the silage supplementation is consistent with the energy supply. When using conserved forage supplementation, the milk production is dependent on the nutritional value of the herbage and the supplements (Woodward et al., 2006). The intake of corn silage allowed a little increase on total DM intake but not to the energy concentration, because the nutritive value of the grazed herbage was superior to that of the corn silage (1.7 vs 1.53 Mcal/kg DM). Which was consistent with previous reports where the milk production response can range from -0.4 to 0.8 kg of milk/kg of concentrate (Burke et al., 2008; Delagarde et al., 2011).

The greater energy intake observed in dairy cows receiving ground corn supplementation may also be associated with the slightly increased milk protein production and concentration in this treatment, and similar responses were observed in other works using concentrate (Bargo et al., 2002). Similarly, with the corn silage supplementation, when the energy intake was improved the milk protein concentration increased (Miguel et al., 2014). This study did not report supplementation effect on milk fat concentration. Other studies had shown the lack of corn silage supplementation effect on milk fat concentration (Miguel et al., 2014; Pérez-Prieto et al., 2011). While, it is expected that the concentrate supplementation decreases the milk fat content (-0.6 g/kg per kg DM of concentrate; Peyraud and Delagarde, 2013). In this work, this relationship was not measured probably due to the concentrate feeding levels used.

6.6 CONCLUSIONS

The ground corn and corn silage supplementation strategies implemented here allowed for reductions in the herbage allowance without compromising milk production and total intake. Under these conditions, a moderate level of energetic supplementation for dairy cows grazing herbage with good nutritive value did not seem to be an effective strategy to reduce the enteric CH_4 intensity.

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7 ARTIGO II - BEFORE AND AFTER MILKING ENTERIC METHANE MEASUREMENTS OF GRAZING DAIRY COWS: FEEDING STRATEGY AND BREED EFFECTS[2]

7.1 ABSTRACT

The ruminants take a considerable part in the greenhouse gas emission due to their enteric methane emission (CH_4). The accurate enteric CH_4 measurement is a challenge on grazing cattle farms. The aim of this study was to quantify the enteric CH_4 emission in grazing dairy cows during the indoor period around the two-daily milking by using the GreenFeed (GF, C-Lock Inc., South Dakota – USA). The data collection took place at the INRA's dairy research farm in 2017 during the grazing season. This study was part of an experiment that had started in 2005 on the same farm. The enteric CH_4 emission was measured on Holstein and Normande dairy cows (primiparous and multiparous) that were equally distributed into two grass-based feeding strategies (FS; High and Low FS). The grass consisted of a perennial ryegrass pasture, either pure or associated with white clover. On High FS the dairy cows were supplemented with concentrate and conserved forage when the grass growth couldn't fulfill the animal's demand. On Low FS there was no supplementation. Through 140 days (June 26th to November 12th, 2017) 26 dairy cows on High FS and 29 dairy cows on Low FS had access to the GF unit. The daily visit time on GF unit was from 7 a.m. to 10 a.m. and 3 p.m. to 5 p.m. A minimum of 130 individual recorded visits was considered in the analyses. A total of 4496 recorded visits from 30 dairy cows (12 High FS; 18 Low FS) resulted in 2842 means of daily enteric CH_4 emission which were analyzed by this study. As expected, the High FS increased the enteric CH_4 emission (+ 62 g/day) while the breed affected the intensity of the emission (g CH_4 / kg of milk yield or milk solid yield). The enteric CH_4 emission was driven by the intake, and it was related to the daily period used to measure the enteric CH_4. The GF evaluation of the enteric CH_4 emission must consider the feeding patterns. The indoor results from before and after the milking may not represent the daily enteric CH_4 emission in grazing dairy cows.

Keywords: grazing dairy cattle, GreenFeed, greenhouse gas.

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[2] Trabalho apresentado no “24º Rencontres autour de la Recherche sur les Ruminants”, Paris, 2018.

7.2 INTRODUCTION

The enteric methane (CH_4) is the most important greenhouse gas emitted in ruminant production systems. Naturally produced during rumen digestion process, the enteric CH_4 represents also a significant energy loss to the animal ranging from 2% to 12% of gross energy intake (JOHNSON; JOHNSON, 1995). The two key factors that change the enteric CH_4 production in the rumen are the dry matter (DM) intake and diet composition (BEAUCHEMIN et al., 2008; MARTIN; MORGAVI; DOREAU, 2010). Due to the emission variation between animals, the challenge is measuring the individual enteric CH_4 production at farm level (CABEZAS-GARCIA et al., 2017; STORM et al., 2012).

The respiration chambers (RC), hexafluoride (SF_6) tracer technique, and recently GreeFeed (GF) system are the techniques used in individual enteric CH_4 measurements. Hammond et al. (2015) compared the three techniques. The authors reported a similar enteric CH_4 estimative between them. However, the measures to grazing ruminants have shown greater difficulties. The RC is not advised for studying the grazing systems. When the grass is offered in the RC, the diet selection and the grazing behavior are limited, and this can reflect on enteric CH_4 production. In the SF_6 technique, a breath sample is accumulated over 24 hours, and the collections are repeated over five to eight days. This technique has been chosen to grazing animals (BERNDT et al., 2014). This one is known to consider the variability of animal intake and behavior in grazing animal, but at the same time, there is a frequent cow handling and a relative intensive labor. The GF systems can be used indoors or at grazing animals for estimating the individual CH_4 and carbon dioxide (CO_2) production. The animals entering an automatic feeding are recognized, and concentrations of CH_4 and CO_2 are measured.

The GF system uses spot samples during the day to estimate the gas emissions (CH_4 and CO_2). When used for grazing animals the experimental design has a difficulty to meet the representative samples and records visits per animal per day (WAGHORN; JONKER; MACDONALD, 2016). Recently, Velazco et al. (2016) showed that is possible to estimate the daily CH_4 emission and CH_4 production (g CH_4/kg of DM intake) using short-term measures. The idea of this work was using the indoor period in grazing dairy cows. The period before and after milking were used to estimate the CH_4 emission with the GF system. From an indirect approach, Garnsworthy et al. (2012) proposed the CH_4 emission estimative at farm level from eructation frequency and CH_4 released per eructation using a sampling air released by eructation during milking.

The present work was based on experiment "Which cow for which system?", which started in 2006 at INRA dairy research farm Le Pin-au-Haras. In this one, the Holstein and Normande dairy cows are evaluated in two feeding strategies in order to evaluate their production and reproduction capacity for a long-term. The feeding strategies were representative of two nutritive inputs, High or Low nutritive inputs. During the grazing season of the year 2017, the aim was to integrate individual measurement with the use of GF. This chapter will give a description and discussion of indoors enteric CH_4 estimative in grazing dairy cows before and after milking, and the possible effect of breed (Holstein and Normande), parity (primiparous and multiparous) and two grass-based feeding strategies on enteric CH_4 emission.

7.3 MATERIALS AND METHODS

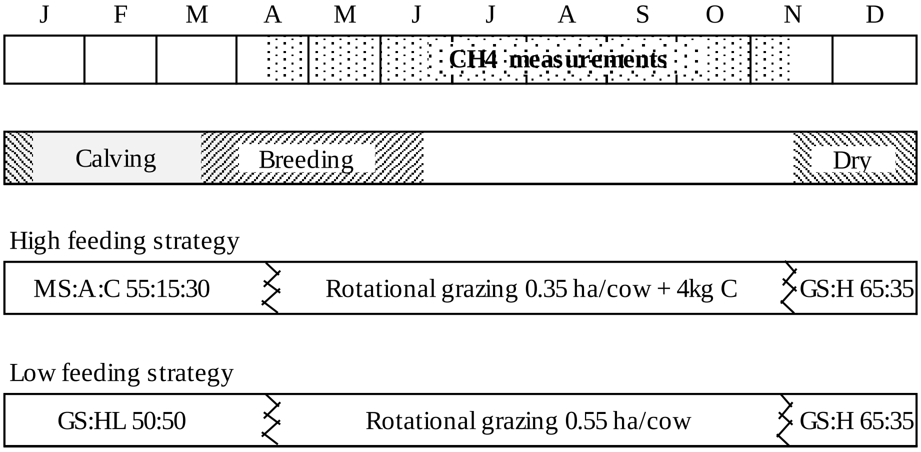
This work was based on an experiment studying the possible breed × feeding strategy (FS) interactions. It was conducted in Normandy, France, at INRA experimental dairy farm in Le-Pin au-Haras (48.448N, 0.098E). The herd was managed under a compact calving system (BEDERE et al., 2017). Briefly, in each year cows calved throughout three months: January, February, and March (Figure 1). All the heifers calved at two or three years old. From November to April, the cows were housed and fed indoors only. After a two-week transition period, the cows were maintained on two grass-based feeding strategies (High and Low FS). In the 2017 experimental year was measured the individual enteric CH_4 emission during the grazing season. The following sections contain the specific descriptions of animals, feeding strategies and enteric CH_4 measurement.

7.3.1 Animals and feeding strategies

The experimental year started with 55 dairy cows. Where there were twenty-eight Holstein and 27 Normande dairy cows (20 primiparous and 35 multiparous). The dairy cows were assigned in two grass-based feeding strategies (High FS or Low FS). Each FS groups were balanced for parity, calving date, a breeding index for milk yield, fat and protein content, body condition score (BCS), body weight (BW) and feeding group of the previous year for multiparous cows. With these conditions, 26 and 29 dairy cows were allocated in High FS and Low FS, respectively. The dairy cows in High FS received 4 kg *per* day of concentrate, 300 g of minerals and vitamins. When the grass offered was insufficient, cows were supplemented with maize silage and haylage (5 – 10 kg of DM *per* day). In Low FS the dairy cows received

500 g of minerals and vitamins and no supplement was provided. The chemical composition and nutritive value of feedstuffs used are presented in Table 1.

Figure 1 – Yearly calendar experimental design of feeding strategies experiment conducted in the INRA dairy research farm Le Pin-au-Haras



░ Grazing season. MS, maize silage; A, dehydrated alfalfa; C, concentrate; GS, grass silage; HL, haylage; H, hay.

Table 1 – Chemical composition and nutritive value of feedstuffs offered during the grazing season for dairy cows on two grass-based feeding strategies

	Grass Grazed		Maize silage	Haylage	Concentrate	GF Concentrate
	High FS	Low FS				
DM, % fresh weight	21.5	21.3	34.5	71.8	93.8	92.6
			Chemical composition, g/kg DM			
OM	888	893	960	924	948	907
CP	182	181	69	145	156	107
NDF	502	506	450	428	211	464
ADF	257	259	222	221	96	269
			Nutrive value[1]			
OMd[2], %	75.5	75.4	72.5	71.5	84.4	74.4
UFL[3], kg DM	0.89	0.89	0.92	0.86	1.11	0.84
PDIN[4], g/kg DM	122	122	42	95	110	71
PDIE[4], g/kg DM	104	105	67	97	126	87

FS, feeding strategy; GF, GreenFeed; DM = dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

[1] INRA, 2018

[2] OMd = organic matter digestibility

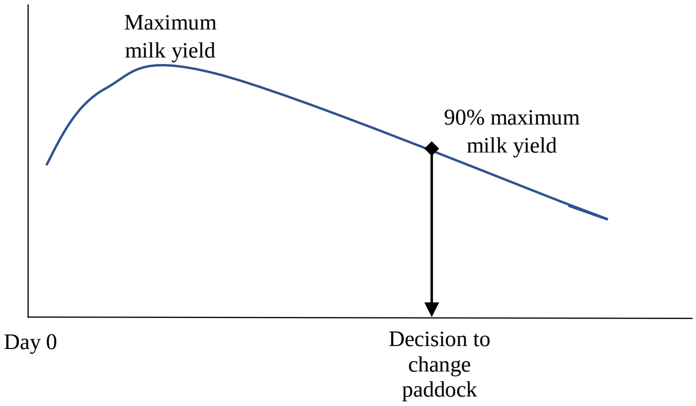
[3] UFL: energy feed unit equivalent to 1700 kcal of net energy for lactation

[4] PDIE and PDIN: protein digestible in the intestine according to energy (E) or nitrogen (N) supply.

7.3.2 Grazing management

An area of 30.9 ha was divided into 11 paddocks, and the pasture was an association of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The FS were managed in separate paddocks, being 11.6 and 19.3 ha for High FS and Low FS, respectively. The grazing management was a simplified rotational grazing system (HODEN et al., 1991). A fixed number of cows were used in each FS (35 dairy cows). Consequently, dairy cows were added in order to obtain one stocking rate of 3.0 cows/ha in High FS and 1.8 cows/ha in Low FS. However, no measurement was performed in these animals. When necessary the forage conserved supplementation was used to benefit the grass growth in following paddocks increasing the total time in the paddock. The paddock was changed as a function of the evolution of the milk yield profile (Figure 2), when milk yield over the previous three days corresponded to 90% of the maximum milk yield value observed on the paddock (HODEN et al., 1991).

Figure 2 – The evolution of dairy cows milk yield during the days in a new paddock and criteria of decision for change of paddock



The grazing season started on March 22nd and finished on December 1st. Both feeding strategies allowed the evaluation of an intensive and a self-sufficient dairy system. The supplementation with conserved-forage under High and Low FS depended on the grass condition throughout the grazing season. The grazing conditions from June 26 to October 8 demanded conserved forage supplementation for the High FS. The mean of supplement intake was 6.9 kg DM (4.2 kg DM of maize silage and 2.7 kg DM of haylage). The Low FS did not demand any supplementation. The grass DM intake was not evaluated.

7.3.3 Sampling and measurements

The cows were milked twice daily at 0630 a.m. and 1600 p.m.. Individual milk yield was recorded by flow meters (Metatron, Westfalia, Germany). Three times a week, both a.m. and p.m. milk samples were collected and analyzed for fat and protein content using an infrared analyzer (MilkoScan^{TM}, Foss Electric, Hillerad, Denmark). The milk solids were considered as milk fat concentration more milk protein concentration. The BW was measured regularly once a week. The dairy cows had access to the automated feeder and to forage conserved supplementation from 7h to 10h and from 15h to 17h. The quantities of concentrate and minerals consumed by each cow were recorded every day using the automated feeder. The quantities of maize silage and haylage offered during the short periods were daily weighed and recorded by treatment. The forage-conserved supplement intake was measured in each FS by the difference between offered and orts. A sample of each intake feed was collected once a week. All samples were dried and conserved for chemical composition analysis.

Pre and post-grazing grass height were measured using an electronic platemeter, on average 150 measures/ha. To estimate the pre-grazing mass density, six to ten strips of 10 m x 55 cm were cut at 5.0 cm above ground level *per* paddock. The grass height on each strip was measured using an electronic platemeter before and after cutting (10 measurements per strip). A sub-sample of fresh pasture per strip was taken to determine the grass DM concentration and the chemical composition of the offered grass (> 5.0 cm).

7.3.4 Enteric methane emission quantification

The enteric CH_4 measure period started on May 19. One GF was provided in each FS. The GF were placed indoor and the access time was from 7h to 10h and from 15h to 17h. The cows had not any previous experience on the GF system, and an adaptation period was necessary. This adaptation allowed the free access to GF units and there was varied interest by the cows. From twice to three times per week the cows were trained to use the GF.

The GF system measures the CH_4 emission using sensors that identified the animal and its head position within a sampling hood, air flow, and CH_4 and CO_2 concentrations in exhaust air. Details of GF operation and analyses can be consulted in HAMMOND et al. (2015) and HRISTOV et al. (2015). Shortly, the GF system allows the punctual measure of individual enteric CH_4 emission. The dairy cows are attracted to visit the GF by concentrate distribution. When an animal visited the GF, it was identified by their radio frequency ear-tag and feed pellets were dropped. Concentrate distribution was programmed so that each cow was able to access to five drops per visit (36 ± 2.1g of concentrate/drop), with the 30s between each drop. During the measuring period (morning or afternoon) the same cow could return a visit to the GF two hours after the last visit. The GF system was refilled with concentrate every week, and this one had a low nutritive value (Table 1).

The regular maintenance of the GF system was accomplished. The air filter was changed once or twice a week as soon as airflow fell below 35 L/s. An aspiration flow-controlled sample enabled CH_4 and CO_2 concentrations measurement continuously (1-s basis) by a non-dispersive infrared sensor. The calibration of the non-dispersive infrared sensor was automatically performed once a day, by injecting a calibration gas mixture with certificated concentrations of CH_4 (1002 ppm) and CO_2 (10 002 ppm) in nitrogen (N_2). In addition, the CO_2 recovery rate of the system was calculated as the ratio between the quantity of CO_2 recovered in the exhaust air and the quantity of CO_2 released into the system during three minutes in triplicate every three weeks.

Data were automatically transferred to the C-Lock server and then handled by C-Lock. Forty-seven dairy cows had regular visits. To assure a satisfactory evaluation, we selected the period with more visit records. Firstly, the evaluation period used to analyze the individual enteric CH_4 emission indoor before and after milking was designed from June 26 to November 12, totalizing 140 days. Secondly, we selected the cows with one minimum of 130 visits records for 140 days. The measurement period (140 days) was divided into 5 sub-periods of 28 days. Daily enteric CH_4 emission was estimated by averaging multiple emissions measurements made overall visits per day at the GF.

7.3.5 Statistical analysis

Before variance analysis the data were processed by calculating the distance (dist) of each point to axis, from the joint distribution of CO_2 versus CH_4 (g/day). Data were eliminated if $\text{dist} > 3.5 \times \sqrt{1-r}$, where r is the correlation coefficient between CH_4 and CO_2.

The data were subjected to variance analysis using PROC MIXED of SAS software (1999, version 9.0, SAS Institute, Cary, NC). The studied variables were analyzed using the following model:

$$Y_{ijklm} = \mu + \text{breed}_i + \text{parity}_j + FS_k + \text{sub-period}_l + \text{first calving age}_m + e_{ijklm}$$

where, $У_{ijkl}$ was enteric CH_4 (g/day, g/kg of milk, g/kg of milk solids, g/kg of BW), and milk yield (kg/day), µ was the mean of the variable of interest, breed (i = Holstein or Normande cows), lactation (j = primiparous or multiparous), feeding strategy (k = high or low), sub-period (l = to 1 from 5), first calving age (m = 2 or 3 years old), and e_{ijklm} the random residual effect. We considered the measures as repeated in time and cows as random effect.

The enteric CH_4 average *per* hour measured on each FS was assessed following the model:

$$Y_{ijklm} = \mu + \text{breed}_i + \text{parity}_j + \text{FS}_k + \text{period}_l + \text{first calving age}_m + \text{hour}_n + \text{hour} \times \text{FS}_{nk} + e_{ijklmn}$$

where, $У_{ijklm}$ was daily enteric CH_4 emission *per* hour, µ was the mean of the variable of interest, the independent variables were as described earlier with addition of hour (m = 07:00 to 10:00h; 15:00 to 17:00h).

7.4 RESULTS AND DISCUSSION

7.4.1 Visits records on GF system

Fifty-five dairy cows had the access to GF system before and after the milking, which 47 had at least one visit record. After the criteria used for CH_4 data analyses (see Enteric methane emission quantification section), data of 30 dairy cows were considered, 12 in High FS and 18 in Low FS. For 140 days used to measure enteric CH_4 the number of visits changed from zero to three visits *per* day. A total of 4496 records (Table 2), amounting 2842 daily enteric CH_4 averages were analyzed. Discussing about reliability of the technique and sampling, Manafiazar et al., 2016 determined that averaging over 7 to 14 d with minimum of 20 spot samples was needed to produce repeatable, while Renand; Maupetit (2016) showed that the test duration for enteric CH_4 emission should be at least two weeks, with a minimum of 50 spot measures. In this work, considering the measuring period (28 days), the individual number of visits records ranged from 27 to 34.

Table 2 – Visits records and total daily methane average data in dairy cows feeding of two grass-based strategies in GF system during indoor period

	Feeding system	
	High	Low
Visits Records		
Morning	1451	1366
Afternoon	831	848
Total	2282	2214
Daily methane data	1441	1401
Nº visit/day/cow	1.43	1.42

Grazing dairy cows with indoor access to the GF unit had 1.42 visits/day, being the same between High and Low feeding strategies. On pasture or in a free-stall facility, successful use of the GF system is reliant on animal visitation to the unit (COTTLE et al., 2015). The daily number of visits is linked to local of access to GF. When the access to GF unit was indoor with an interval between visits of 2 – 4 hours, the number of visits to the GF unit *per* day changed from 2.58 to 3.54 (HAMMOND et al., 2016a) until 5.0 visits (ALEMU et al., 2017). Studies where the GF access happened on the paddock, the same local of graze, the number of visits to the GF unit *per* day changed from 1.1 to 1.6 (HAMMOND et al., 2016b; WAGHORN; JONKER; MACDONALD, 2016).

7.4.2 Daily enteric methane emission and variation over the measure time

Dairy cows receiving Low FS had lesser daily enteric CH_4 emission (- 62 g/day; Table 3). No difference between breed or parity was found in daily enteric CH_4 emission. The daily enteric CH_4 emission ranged from 325 to 387 g/day. These data are close to the described in other studies (BENCHAAR; POMAR; CHIQUETTE, 2001; ELLIS et al., 2007). The principal effect was related to the feeding strategies. In part, this can be explained by the difference between DM intake and to the specific time used to measure the CH_4 enteric.

During the experimental period (140 days), for 97 days the dairy cows in High FS received forage conserved supplementation in addition to the concentrate supplementation due to the limited grass allowance. The mean of supplement intake in High FS was 6.9 kg DM (4.2 kg DM of maize silage and 2.7 kg DM of haylage). However, the grass DM intake and consequently the total DM intake are not known. But, in the similar conditions and feeding strategy, Delaby et al. (2009) described increases on total DM intake with concentrate supplementation. In addition, when the grass characteristics (allowance, pre-grazing mass) are limited, the maize silage supplementation increased the total DM intake (MIGUEL et al., 2014; PÉREZ-PRIETO; PEYRAUD; DELAGARDE, 2011). A strong relationship between DM intake and enteric CH_4 (~0.80) has been supported in the literature (BENCHAAR; POMAR; CHIQUETTE, 2001; ELLIS et al., 2007; HRISTOV et al., 2018; YAN et al., 2000). The least enteric CH_4 emission on Low FS can be due to the lower total DM intake. Increases until 14 % on enteric CH_4 emission with supplementation uses were estimated (LOVETT et al., 2005; MUÑOZ et al., 2015; O'NEILL et al., 2012).

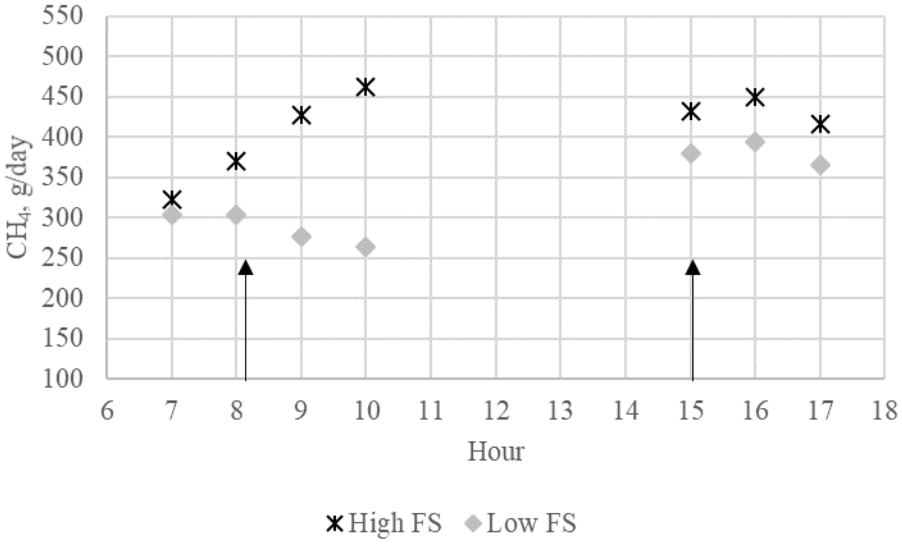
Table 3 – Breed, parity and grass-based feeding strategy effects on milk yield and enteric methane emission in grazing dairy cows

	Breed		Feeding strategy		Parity		
	Ho	No	High	Low	Primiparous	Multiparous	RMSE
MY, kg/day	20.8^{a}	14.8^{b}	19.3^{a}	16.3^{b}	16.3^{a}	19.3^{b}	2.77
Methane,							
g/day	352	360	387^{a}	325^{b}	343	368	34.3
g/kg of MY	17.4^{a}	27.6^{b}	22.1	23.1	22.4	22.9	7.63
g/kg of MS	261.4^{a}	344.1^{b}	293.5	312.0	295.8	309.7	69.9
g/100 kg BW	56.5	53.4	58.6^{a}	51.3^{b}	54.4	55.5	4.41

MY; milk yield, MS; milk solid yield, BW; body weight.

Another reason was linked to the available time to cows visit the GF unit. The same time was used for supplementation intake on High FS. In the Figure 3 is possible to observe the effect of feeding strategy on enteric CH_4 during the daily measure time. The variation in enteric CH_4 production over the day is affected by the feeding strategy where the CH_4 emission rate is highest during and after a meal (WAGHORN; JONKER; MACDONALD, 2016). The daily enteric CH_4 rate is linked to intake (HAMMOND et al., 2015; JONKER et al., 2014). The Figure 4a was showed by Jonker et al. (2014). The authors described the effect of different feeding levels and feeding frequencies on circadian variation in CH_4 emission measured in the respiration chamber. There is a visible feeding time influence on CH_4 emission rate during the day. The lowest rates of CH_4 emission have been seen before the first meal (JONKER et al., 2014; WAGHORN; JONKER; MACDONALD, 2016). The peak emission rates can be 2 to 6 times greater than the basal rate, happening within 140 minutes after eating (CROMPTON et al., 2010). In the High FS, this effect was more visible, which the enteric CH_4 emission increased by 30 % to 7 a.m. until 10 a.m.. The absence of intake on cows in Low FS during the daily measure time resulted in a decrease in enteric CH_4 emission during the same time. Broadly, the emission in the afternoon period was 16 % higher than the emission in the morning, being this difference higher on Low FS. GUNTER; BRADFORD (2015) assessing the difference between enteric CH_4 emission among periods (early morning, late morning, afternoon, and evening periods) showed a maximum variation of 15% between periods.

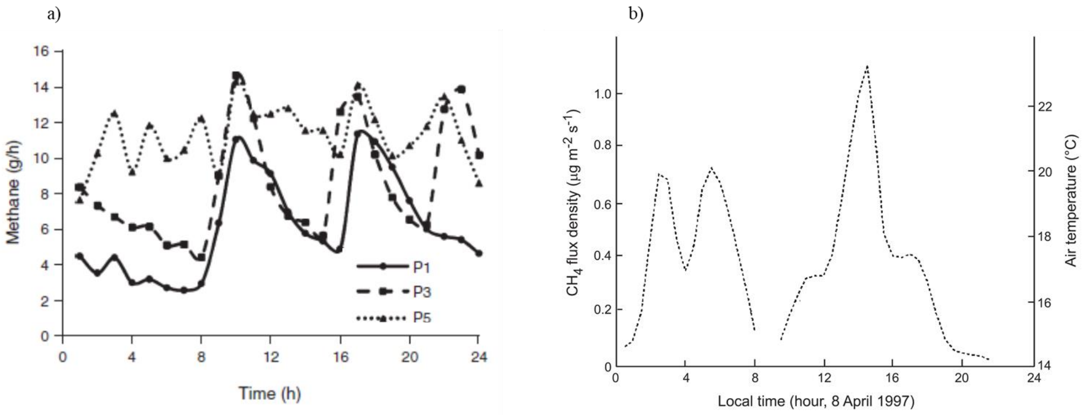
Figure 3 – Evolution methane emission during the daily measure time in dairy cows on two grass-based strategies. Arrow indicate time of indoor feeding in High FS



Considering the factors exposed, the daily enteric CH_4 emission, especially on Low FS, was probably underestimated. The circadian CH_4 patterns in grazing ruminants exhibit two peaks, one in mid-morning and other in the late afternoon (Figure 4b), these coincide with the principal's meals in grazing ruminants (CHILIBROSTE et al., 2015; MATHERS; WALTERS, 1982). With the daily measure time used in this work was not possible to measure the enteric CH_4 emission during the grazing time, consequently, the peaks of enteric CH_4 emission due the more intensive grazing in Low FS were not measured.

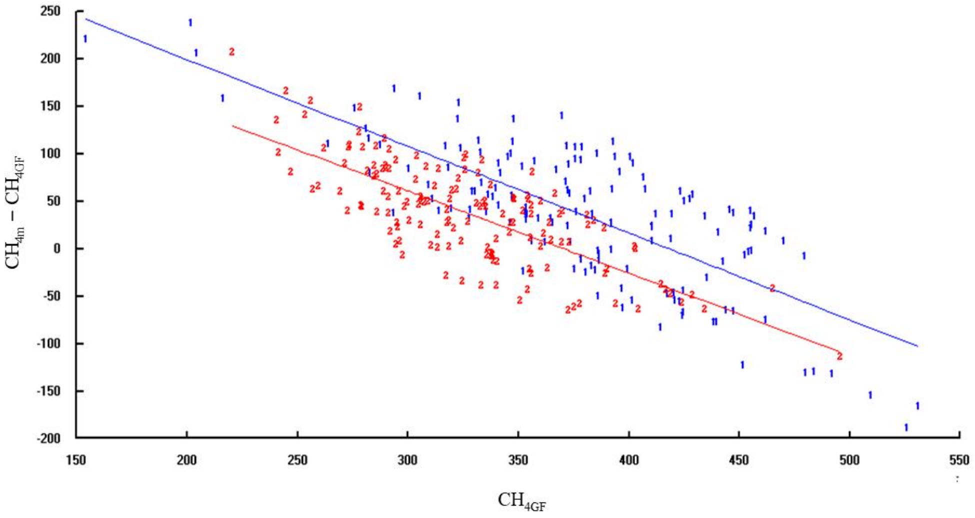
In the Figure 5 is possible to observe the comparison between GF data measured before and after milking (CH_{4GF}) with the values estimated by a model (CH_{4m}; Chapter 3). Analyzing the same herd, the difference taken between them (CH_{4m} – CH_{4GF}) is linked to the GF measurements of CH_4 emission. From all the factors previously discussed, as the measures were not well distributed during the day, a cow with a higher CH_4 rate during the measurement period had the daily enteric CH_4 emission overestimated. And, a dairy cow with a lower CH_4 rate during the measurement period had the daily enteric CH_4 emission underestimated. On average, when compared to the enteric CH_4 emission data estimated by a model, the enteric CH_4 emission in this experiment was underestimated 30 and 50 g/day in High FS and Low FS, respectively.

Figure 4 – Circadian methane patterns in a) supplemented heifers on three feeding strategies (P1; two equal meals daily at 0800 hours and 1530 hours, P3; three equal meals daily at 0800 hours, 1500 hours and 2100 hours, P5; ad libitum feeding), and b) daily methane flux in grazing sheep



Source : a) JONKER et al. (2014); b) JUDD et al. (1999).

Figure 5 – Relationship between before and after milking enteric methane measurements (CH_{4GF}) and methane emission estimated by a model (CH_{4m}) of grazing dairy cows in High (1) or Low (2) feed strategies



7.4.3 Breed and parity effects on enteric methane emission

The Holstein cows had a lesser methane intensity than Normande cows (Table 3). The methane intensity of Holstein cows was 17.4 g/kg of milk and 260.1 g/kg of milk solid, while on Normande cows the methane intensity was 27.6 g/kg of milk and 344.1 g/kg of milk solid. The values agreed with the values reported in grazing dairy cows (FREDEEN et al., 2013; MUÑOZ et al., 2015; O'NEILL et al., 2011, 2012). In Holstein-Friesian cows, the enteric CH_4 intensity (kg of milk yield and kg of milk solids) were 24.2 and 330 g/kg, respectively (O'NEILL et al., 2012). Muñoz et al. (2015) reported a lower methane intensity. The value 13.6 g of CH_4 *per* kg of milk yield was related to the higher daily milk yield, on average 27 kg/day. In this work, the higher milk yield (+6.0 kg/day) and consequently, higher milk solids on Holstein dairy cows, had a dilution effect since there was no difference in daily enteric CH_4 emission between breeds.

The effect of parity on enteric CH_4 was not observed. In the literature the relationship between enteric CH_4 and dairy cow age is controversial (COTTLE; NOLAN; WIEDEMANN, 2011; GRANDL et al., 2016; RAMÍREZ-RESTREPO; CLARK; MUETZEL, 2016). Overall, the differences in enteric CH_4 production between heifers, primiparous and multiparous cows would be expected due to increases in body size, DM intake, and milk yield (GRANDL et al., 2016).

7.5 CONCLUSION

Even though the feeding strategy and breed effects on enteric methane emission in grazing dairy were as expected, the measure of enteric CH_4 with the GF system must consider the management and feeding strategy. Our results from the spot measures arranged indoors before and after milking are questionable, principally in feeding strategy without supplementation.

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8 ARTIGO III - ENTERIC METHANE EMISSION: EFFECT OF BREED, FIRST CALVING AGE AND FEEDING STRATEGY IN A DAIRY SYSTEMS APPROACH[3]

8.1 ABSTRACT

Enteric fermentation by ruminants generates the majority of livestock methane (CH_4). The greenhouse gas (GHG) emission CH_4 is widely reported to adversely affect the climate and needs to be reduced. It is also a waste of animal energy. Enteric CH_4 emission from individual dairy cows have been well reported in the literature, however, the integration of these emissions at the dairy system is less well described. From 2006 to 2015, at INRA dairy research farm, an experiment was conducted to measure, over successive lactations, the effect of breed (Holstein, Ho; Normande, No) and first calving age (two or three years old) on the performance of distinct types of cows in two feeding strategies (FS; High or Low FS). This work aimed to merge the results of this long-term experiment and quantify dairy cows and replacement heifer's enteric CH_4 emissions and integrate this information at the dairy system. The proportion and composition of feedstuffs used on each FS were measured and it was possible to estimate the heifers and dairy cows total dry matter (DM) intake and enteric CH_4. All results obtained were integrated and represent eight dairy systems. Each dairy system sold the same amount of milk sale (400 t). Enteric CH_4 emission factor was related to DM intake. Dairy cows CH_4/head per year varied from 123 to 158 kg. On average, the relationship between enteric CH_4 and DM intake was 21.5 g/kg DM intake. The enteric CH_4 intensity *per* kg of milk yield decreased as dairy cow's productivity improved. The number of cows and heifers required for the same milk sold in different systems varied considerably. The replacement rate and first calving age had a strong effect on the number of dairy cows and replacements heifers, and consequently on enteric CH_4 emission. On average, the Holstein herd emitted 2.5 t/year of enteric CH_4 less than the Normande herd. Reducing first calving age on average decreased dairy systems enteric CH_4 by 2.2 t *per* year. Dairy system annual enteric CH_4 emission increased from 13.1 t to 17.3 t in moving from the Low to High FS. Our results at the dairy system level represent an opportunity to develop mitigation strategies. Each studied factor showed potential to reduce enteric CH_4 reduction mainly by modifying the number of dairy cows and replacements heifers in the herd.

Keywords: dairy cow, greenhouse gas emission, mitigation strategies, replacement rate.

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[3] Este capítulo apresenta o artigo submetido ao periódico Livestook Science na íntegra, seguindo as normas da revista.

8.2 INTRODUCTION

Methane (CH_4) is a potent greenhouse gas (GHG) that is damaging the climate. Its global warming potential is 25 times higher than carbon dioxide (CO_2). The global warming impact of CH_4 is increasing. As a result, there is increasing discussion around developing CH_4 mitigation strategies. Ruminants are an important source of CH_4 emissions in developing and developed countries (FAO, 2010). The majority, about 46% of dairy cattle's total greenhouse gas (GHG) emission results from CH_4 emitted from enteric fermentation (Gerber et al., 2013). In France, CH_4 emissions from the agriculture sector represents around 69% of total CH_4 emissions. The sectors CH_4 emissions are almost exclusively represented by enteric fermentation (CITEPA, 2018).

The CH_4 emitted by e enteric fermentation of feed is caused by ruminal and hindgut microorganisms (Johnson and Johnson, 1995). The volume of CH_4 produced by individual ruminants is dependent on the substrate available for ruminal fermentation, the total dry matter (DM) intake and composition of feed intake (Hristov et al., 2013a; Knapp et al., 2014). Over the years many studies have been conducted to assess the effect diet composition has on enteric CH_4 production. These studies have assessed the effect tropical or temperate grasses and legumes (Archimède et al., 2011; Wims et al., 2010), forage-to-concentrate ratios (Aguerre et al., 2011; Muñoz et al., 2015), forage conserved supplementation (Hassanat et al., 2013; Lettat et al., 2013) and feed additives (Cottle et al., 2011; Alves et al., 2017; Doreau et al, 2018) have on dairy cattle enteric CH_4 emission and proposed feeding management and nutrition mitigation strategies.

In general, the enteric CH_4 intensity is lower in mixed dairy systems than in grass-based systems (FAO, 2010; Gerber et al., 2013). This is partly due to the management approaches used to improve dairy cows productivity (Gerber et al., 2011; Hristov et al., 2013b). However, the understanding of the relationship between feeding and animal management factors at dairy system level and the enteric CH_4 emissions remains limited. Additionally, a lot of experiments have investigated strategies to mitigate CH_4 emitted at the animal level, but few studies have evaluated mitigation strategies at the system level. The biggest difficulties are related to long-term data evaluation and the cost of measuring individual animal's enteric CH_4 emissions. Recent, improvements of the models used to quantify total and enteric CH_4 emissions have shown that it is possible to conduct the evaluations at the dairy system level (Ellis et al., 2010; Appuhamy et al., 2016; Rotz, 2018).

This study aims to develop a model to quantify the impact of dairy cows and replacement heifers on enteric CH_4 emissions. Our goal was to evaluate the effects different grass-based feeding strategies, different breeds (Holstein or Normande), age at first calving (two or three years old) and replacement rate has on enteric CH_4 emissions at the dairy system level. The results are described in detail in order to identify the factors that impact dairy systems enteric CH_4 emission.

8.3 MATERIALS AND METHODS

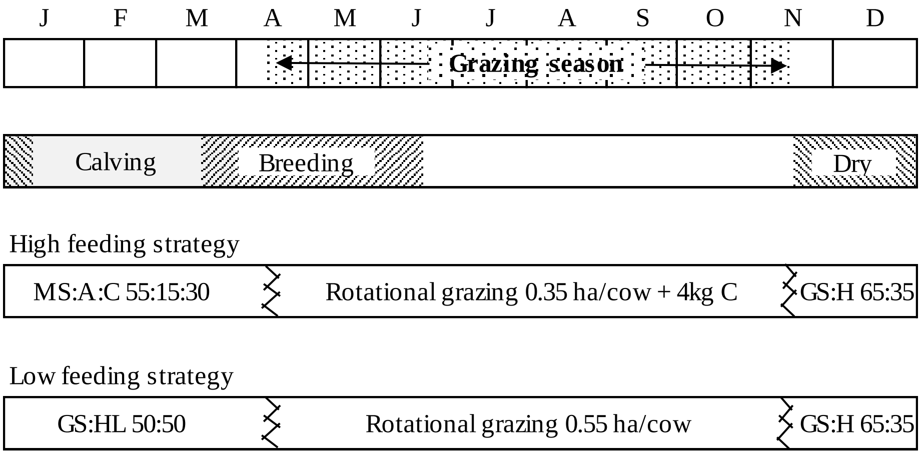
The enteric CH_4 emissions from Holstein and Normande dairy cows under different grass-based feeding strategies, first calving age and replacement rate were quantified using a model and data generated from a ten-year study. This long-term study investigated throughout successive lactations the adaptability of diverse types of cows (breed, age at first calving) on two feeding strategies. The impact of individual management factor on enteric CH_4 emissions was described in eight dairy systems with the same annual milk production.

8.3.1 Experimental design and dataset

From 2006 to 2015, a breed × feeding strategy (FS) experiment was conducted at INRA dairy research farm in Le Pin-au-Haras (latitude 48.72, longitude 0.18; Normandy, France). In short, Holstein and Normande two- or three-year-old dairy cows at first calving, were equally distributed within two FS grass-based every year. At the beginning of the experiment, systems were balanced for parity, calving date, breeding index for milk yield, fat and protein content, body condition score (BCS), body weight (BW) and previous rank of successful insemination. Cows remained in their feeding group until culling (i.e. not pregnant, health problem or death). Replacement primiparous were balanced for age at first calving, breeding index, BCS and BW. The herd was managed under a compact breeding season (13-weeks). This permitted experimental cows to calved over the first three months of the year. The yearly calendar of the experimental design is described in Fig. 1.

The FS were denominated as High FS and Low FS. In the winter, cows in High FS were fed *ad libitum* with a total mixed ration composed of 55% maize silage, 15% dehydrated alfalfa and 30% winter concentrate (% of DM), and during grazing period they were supplemented with 4 kg of grazing concentrate. For the same period, cows in Low FS received an *ad libitum* mixture of 50% grass silage and 50% haylage (% of DM), and during grazing period no concentrate was offered. Concentrates contained soybean, barley, beet pulp, wheat, maize and salt and minerals. From July to November, when the grass growth was insufficient to fulfill the animal demand, cows received maize silage or grass silage supplementation, in High FS and Low FS. During the dry period, cows were fed *ad libitum* with grass silage and hay only.

Figure 1 – Yearly calendar experimental design of the feeding strategies experiment conducted in the INRA dairy research farm Le Pin-au-Haras



Grazing season, breeding season, high feeding strategy and low feeding strategy. MS, maize silage; A, dehydrated alfalfa pellets; C, concentrate; GS, grass silage; HL, haylage; H, hay).

The cows were milked twice daily at 06:30 A.M and 4:00 P.M. Individual milk yields were recorded by flow meters (Metatron, Westfalia, Germany). Milk samples were collected three times a week, both a.m. and p.m. and analyzed for fat and protein content using an infrared analyzer (MilkoScan^{TM}, Foss Electric, Hillerad, Denmark). Bodyweight was measured regularly once a week. During the winter period, the amounts of forage and concentrate offered and refused in each FS were weighed every day to obtain the intake quantities. During the grazing season, the quantities of concentrate and minerals intake by each cow were recorded daily using an automatic feeder in the milking parlor. The grass sward consisted of permanent and perennial ryegrass pastures, either pure or associated with white clover. A sample of each feed offered was collected once a week, and grass sample at each passage of a new paddock. All feed samples were dried and conserved for chemical composition analysis. The typical chemical composition and nutritive value of feedstuffs from experimental years are presented in Table 1.

Nulliparous heifers were separated into two FS depending on calving age, two (Fig. 2.) or three years old (Fig. 3.). The objective of the FS for heifers expected to calve at 2 years was to have higher daily weight gain than the FS for heifers calving at 3 years. Heifers were weighed at birth and then fortnightly. All forages and concentrates offered to heifers were collected regularly to determine their chemical composition and nutritive value. Intake of individual heifers was estimated from BW and intake capacity (INRA, 2007). The BW and DM intake were recorded from birth to calving age and averaged by trimesters.

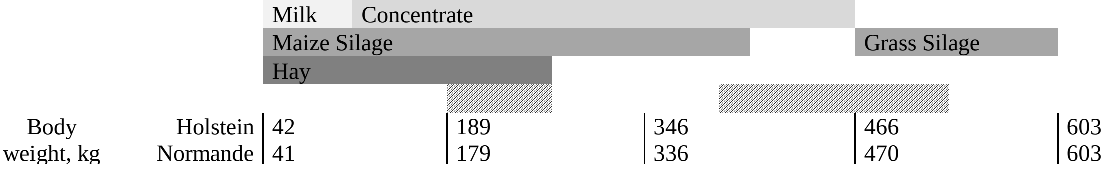
Table 1 – Chemical composition and nutritive value of feedstuffs offered during experimental period (average 2006 – 2015)

	Grass Grazed		Maize silage	Dehydrated alfafa	Grass silage	Haylage	Concentrate
	High FS	Low FS					
DM, % fresh weight	20.7	21.8	32	91	30	67	88
Chemical composition, g/kg DM							
OM	889	889	960	862	901	902	924
CP	178	170	70	214	129	142	217
NDF	505	505	415	348	481	531	174
ADF	249	248	225	233	286	281	80
Nutrive value[1]							
OMd	75	74	73	62	73	71	86
UFL	0.89	0.88	0.91	0.70	0.89	0.83	1.11
PDIN, g/kg DM	112	107	43	139	78	94	154
PDIE, g/kg DM	95	92	68	109	65	94	148

FS, feeding system; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; OMd; organic matter digestibility; UFL, energy feed unit equivalent to 1700 kcal of net energy for lactation; PDIE and PDIN, protein digestible in the intestine according to energy (E) or nitrogen (N) supply.

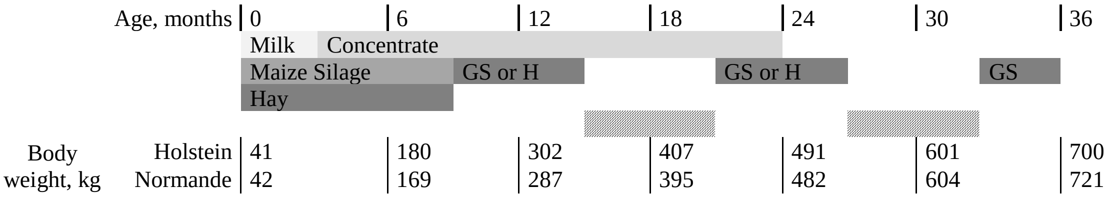
[1] INRA (2007).

Figure 2 – Feeding strategy used in Holstein and Normande heifers with first calving age of two years during experiment conducted in INRA experimental farm Le Pin-au-Haras



Hatched period: grazing period.

Figure 3 – Feeding strategy used in Holstein and Normande heifers with first calving age of three years during experiment conducted in INRA experimental farm Le Pin-au-Haras



GS, grass silage; H, hay. Hatched period: grazing period.

8.3.2 Model description and calculations

A total of 589 lactations from 297 cows (Holstein = 146; Normande = 151) were recorded throughout the experiment, which corresponds to 258 Holsteins lactations and 331 Normande lactations. The lactation rank was divided between primiparous (Lactation 1), and multiparous cows (Lactation 2 and Lactation 3+). Over the experiment, 41% of the 589 lactations were primiparous.

In winter, DM intake was calculated daily based on the difference between what was offered and the orts (refusals). At grazing, the grass disappearance approach was used to quantify grass DM intake. This approach was applied at the paddock level and calculated intake from pre- and post- grass height and grass density measurements as described by Delaby et al. (2001). The total DM intake was measured daily by group during all the experimental period, while the individual total DM intake was estimated considering the individual intake capacity as proposed by INRA (2007):

$$\text{Total DM intake, kg DM/day} = \text{total DM intake by group} \times \text{individual intake capacity} / \text{group intake capacity} \quad \text{(Eq.1)}$$

For each cow, their lactation was composed of twenty-six periods (2-weeks period), being four periods dry and twenty-two periods milking. Individual milk yield, fat and protein milk concentration, BW and total intake averages were grouped by 2-week periods.

The effects of experimental factors (breed, lactation, feeding strategy, and age at first calving) on zootechnical performance (milk production, fat and protein milk concentration, BW and total DM intake) were assessed using the following linear mixed model:

Y_{ijklmn} = μ + $year_i$ + first calving age_j + lactation $rank_k$ + $breed_l$ + FS_m + calving $period_n$ + lactation $rank_k$ × $breed_l$ + lactation $rank_k$ × FS_m + $breed_l$ × FS_m + lactation $rank_k$ × first calving age_j + $breed_l$ × first calving age_j + FS_m × first calving age_j + e_{ijklmn}

where Y_{ijklmn} was milk yield, fat and protein concentration, total DM intake or BW for the ijklmn observation, μ was the mean of the variable of interest, $year_i$ was the fixed effect of experimental year (i = 2006 to 2015), first calving age_j was the fixed effect of calving age (j = two or three years old), lactation $rank_k$ was the fixed effect of lactation (k = 1, 2, or ≥ 3), $breed_l$ was the fixed effect of breed (l = Holstein or Normande cows), FS_m was the fixed effect of feeding system (m = high or low), calving $period_n$ was the fixed effect of possible 2-week calving period (n = 1 to 6), and e_{ijklmn} the random residual effect. All linear mixed model analyses were performed using the *lmer* procedure of the R statistical package (R Core Team, 2016).

8.3.3 Individual enteric methane emissions estimative

Daily enteric CH_4 emission from heifers and dairy cows in different FS was modeled for all experimental years using estimated DM intakes and the measured chemical compositions of feedstuffs. According to the linear coefficients from the model equations described earlier, the DM intake was estimated for 144 distinct types of cows combining breed (2), feeding strategy (2), age at first calving (2), lactation rank (3) and 2-weeks period of possible calving (6). For each type of cow, daily enteric CH_4 was estimated for twenty-six 2-week periods (lactation plus dry period). For heifers, CH_4 estimation was performed during trimesters.

Initially, enteric CH_4 emission was estimated as a function of organic matter (OM) digestibility intake, as proposed by Sauvant and Nozière (2013):

$$CH_4\text{, g/kg DOM} = 45.42 - 6.66\ FL + 0.75\ FL^2 + 19.65\ PCO - 35\ PCO^2 - 2.69\ FL \times PCO \quad \text{(Eq. 2)}$$

where, DOM is the the digestible OM in the total tract; FL is the feeding level (DM intake per % BW); and PCO is the proportion of total intake concentrate.

Once CH_4 emission *per* kg of DOM, OM intake, diet OM digestibility corrected by level of intake, proportion of concentrate in the total diet, and rumen protein balance (INRA, 2018) were quantified for dairy cows and heifers across all experimental years, it was possible to estimate daily enteric CH_4 emissions (g/day):

$$CH_4\text{, g/day} = CH_4\text{, g/kg DOM} \times \text{OM intake} \times \text{OM digestibility} \quad \text{(Eq. 3)}$$

Total enteric CH_4 emission was calculated for fifty-two weeks (twenty-six 2-weeks periods) for dairy cows, and from birth to age at first calving (two or three years old) for heifers. The quantity of CH_4 emitted *per* year was the unit used for heifers and cows.

8.3.4 Dairy systems enteric methane estimative

The factors studied during the experimentation were used to model enteric CH_4 from eight different dairy systems (two breeds, two feeding strategies and two ages at first calving). The model quantified total enteric CH_4 emission *per* system. This was calculated by integrating individual enteric CH_4 emission from dairy cows for the lactation and dry period with enteric CH_4 emission from heifers for the birth to calving period.

The results obtained *per* 2-weeks period were aggregated by year for each type of cow (144). The proportion of cows in each lactation rank (rank 1, 2 and ≥ 3) was estimated according to the herd demography for each system (Table 2). The primiparous proportion was used as the replacement rate and, consequently, estimated the number of replacement heifers. For each system, the total enteric CH_4 emission was estimated considering the same annual herd milk yield. The quantity of milk sold was set at 400 t. This corresponded to an annual milk yield of approximately 434 800 kg milk. Approximately, eight percent of the milk production was considered unsold (mastitis, calf rearing). The herd for this production was presented as cows, heifer and total animals (cows plus replacement heifers). One livestock unit (LU) was equivalent to a dairy cow with milk yield of 6000 kg/year. The corresponding LU values for heifers 0-1, 1-2 and 2-3 years old were 0.3, 0.6 and 0.9 LU, respectively.

The effect of different replacement rates on enteric CH_4 was estimated from the observed reproductive performance and the herd demography (i.e. proportion of cows in each lactation ranks) for each studied system. The total enteric CH_4 emission from dairy cows and heifers for replacement rates 25, 30, 35, 40 and 45% were quantified for dairy systems producing same quantity of milk (400 t sold). The simulations accounted for interactions between management options (replacement rate, FS, first calving age, breed). The potential effect of these options on enteric CH_4 emission was estimated at a dairy system level by the following linear model:

$$Y_{ijkl} = \mu + \text{breed}_i + \text{first calving age}_j + FS_k + \text{replacement rate}_l + e_{ijkl}$$

where Y was the total enteric CH_4 emission for the ijkl observation, µ was the mean of enteric CH_4, breed_i was the fixed effect of breed (i = Holstein or Normande cows), first calving age_j was the fixed effect of calving age (j = two or three years old), FS_k was the fixed effect of feeding system (m = high or low), replacement rate_l was the fixed effect of replacement rate (l = 25 to 45), and e_{ijkl} the random residual effect. The analyses were performed using SAS (1999).

Table 2 – Distribution of lactation rank (%) according to breed, feeding strategy and first calving age (2006 – 2015)

	Holstein				Normande			
	High		Low		High		Low	
First calving age	2	3	2	3	2	3	2	3
Lactation 1	51	46	60	55	37	45	47	46
Lactation 2	27	32	22	30	31	28	32	25
Lactation 3+	22	22	18	15	32	27	21	29

8.4 RESULTS

8.4.1 Feeding strategies description

The different FS used for nulliparous replacement heifers calving for the first time at two or three years had average daily gain (ADG) of 780 and 620 g/day, respectively. The dietary composition (i.e. proportion of each feedstuff) of heifer's total intake was not similar for the different calving ages or feed strategies (Table 3). The grazed grass proportion of the diet was 50% of the total DM for heifers calving at 3 years and 30% for heifers calving a year earlier. The total DM intake from birth to calving was on average 3435 kg greater for heifers calving at three-years-old compared to two-years-old, regardless of breed.

Table 3 – Dry matter intake and proportion of each feedstuff on total intake of Holstein and Normande nulliparous heifers with first calving age of two or three years old

	Holstein		Normande	
First calving age	2	3	2	3
Total DM intake				
0 – 12 months	1480	1321	1379	1257
12 – 24 months	3423	2914	3357	2835
24 – 36 months	-	4072	-	4110
Total[1]	4903	8307	4736	8202
Feedstuff				
Grass grazed	0.30	0.50	0.31	0.50
Maize silage	0.31	0.06	0.30	0.05
Grass silage	0.26	0.39	0.27	0.39
Hay	0.03	0.02	0.03	0.01
Concentrate + minerals and vitamins	0.09	0.04	0.10	0.04

DM, Dry matter.

[1] Total DM intake from birth to calve.

During the years of experimentation two feeding strategies were used to compare different breed across successive lactations. The Low FS represents a system with lower inputs and a strong self-sufficiency (0.55 ha/cow), based almost entirely on grass grazed (66% of total diet, Table 4) and conserved forage (31%). The High FS corresponds to a high producing system *per* animal and *per* hectare (0.35 ha/cow at grazing) due to the concentrate and conserved forage supplementation. Grass grazed represented 44% of total DM diet for HS, while the conserved forage and concentrate supplementation proportion were 34 and 22%, respectively. On average, the dairy cows in High FS had a total DM intake 800 kg/year greater than those on the Low FS.

8.4.2 Intake, milk yield and milk solids

The averages observed across successive lactations for Holstein and Normande performance across two feeding strategies and two ages at first calving is presented in Table 5. The total DM intake was 577 kg DM/year greater in Holstein cows (6748 kg DM/year) when compared to Normande cows (6171 kg DM/year) and was more pronounced for the High FS (+ 676 kg DM/year). The effect of age at first calving on the total DM intake was similar for breed and feeding strategy. Three-year-old cows at first calving intake was 9.5% greater than two-year-old cows at first calving. The total DM intake increased as cows progressed through their initial lactations and then stabilized. The intake difference between two- or three-year-old cows at first calving was only 3% in lactation 3+.

Table 4 – Total intake and proportion each feedstuff on total diet of dairy cows in high and low feeding strategies during lactation and dry period

	High feeding strategy	Low feeding strategy
Total DM intake, kg/year	6860	6060
Lactation		
Grass grazed	0.44	0.66
Maize silage	0.23	-
Dehydrated alfafa pellets	0.03	-
Grass silage	0.08	0.24
Haylage	-	0.07
Concentrates + minerals and vitamins	0.22	0.03
Dry period		
Grass silage	0.65	0.65
Hay	0.35	0.35

DM, Dry matter.

The Holstein dairy cows had a higher milk yield (+ 1852 kg milk/year) and milk solids (+ 94 kg/year) than the Normande cows. Independent of lactation number the milk yield and milk solids were greater in High FS when compared to Low FS, averaging 2109 kg milk/year and 148 kg milk solids/year. As expected, the largest milk yield and milk solids difference between two- or three-year-old heifers at first calving was in lactation 1 (746 kg of milk yield and 58 kg of milk solids). This difference decreased in lactation 2 (436 kg of milk yield and 34 kg of milk solids) and lactation 3+ (126 kg of milk yield and 6 kg of milk solids). The Normande two-year-old cows at first calving were more sensitive to FS than Holstein cows. On Low FS the milk yield was 11% and 8% less in Normande and Holstein cows, respectively, when compared to three-year-old cows at first calving.

Table 5 – Total intake, milk yield, milk solids and feed efficiency of Holstein and Normande dairy cows according to feeding strategies, first calving age and lactation rank (average 2006 - 2015)

	Holstein				Normande			
	High		Low		High		Low	
First calving age	2	3	2	3	2	3	2	3
Total intake, kg/year								
Lactation 1	6108	6765	5425	5941	5466	6114	4981	5487
Lactation 2	7133	7555	6263	6544	6441	6854	5769	6040
Lactation 3+	7658	7956	6738	6894	6978	7266	6256	6402
	6966	**7425**	**6142**	**6460**	**6295**	**6745**	**5669**	**5976**
Milk yield, kg/year								
Lactation 1	7145	7764	4820	5640	4945	5618	3644	4518
Lactation 2	8798	9107	5948	6457	6327	6690	4501	5065
Lactation 3+	9714	9712	6723	6922	7211	7263	5244	5497
	8553	**8861**	**5830**	**6340**	**6161**	**6524**	**4463**	**5027**
Milk solids, kg/year								
Lactation 1	484	536	324	382	364	421	264	328
Lactation 2	595	624	411	445	471	503	339	379
Lactation 3+	664	664	473	481	539	542	399	412
	581	**608**	**403**	**436**	**458**	**489**	**334**	**373**
Feed effiency[1]								
Lactation 1	1.17	1.15	0.89	0.95	0.90	0.92	0.73	0.82
Lactation 2	1.23	1.21	0.95	0.99	0.98	0.98	0.78	0.84
Lactation 3+	1.27	1.22	1.00	1.00	1.03	1.00	0.84	0.86
	1.22	**1.19**	**0.95**	**0.98**	**0.97**	**0.96**	**0.78**	**0.84**
Feed effiency[2]								
Lactation 1	79.2	79.3	59.8	64.3	66.7	68.8	53.0	59.8
Lactation 2	83.5	82.6	65.6	68.0	73.2	73.4	58.7	62.7
Lactation 3+	86.7	83.4	70.3	69.8	77.2	74.6	63.7	64.4
	83.1	**81.8**	**65.2**	**67.4**	**72.4**	**72.3**	**58.5**	**62.3**
Body weight, kg								
Lactation 1	573	654	532	610	614	686	558	628
Lactation 2	634	686	586	635	688	732	626	667
Lactation 3+	695	727	632	661	758	781	680	700
	634	**689**	**583**	**635**	**687**	**733**	**621**	**665**

[1] Feed efficiency = kg milk yield/kg DM intake.
[2] Feed efficiency = kg milk solids/kg DM intake.

8.4.3 Individual enteric methane emission

There was an important effect of age at first calving on the total enteric CH_4 emission in heifers (Table 6). When considering the period from birth to calving, independently of breed, three-year-old heifers at first calving enteric CH_4 emission was 84 kg greater than the two-year-old heifers at first calving. For dairy cows, the average daily CH_4 emission of the Normande and Holstein breeds during the experimentation were 389 and 374 g/day, respectively.

Table 6 – Accumulated enteric methane emission of Holstein and Normande nulliparous heifers with first calving age of two or three years old (average 2006 – 2015)

	Holstein		Normande	
First calving age	2	3	2	3
Accumulated CH_4 emission, kg				
0 – 12 months	34	30	31	29
12 – 24 months	83	70	81	68
24 – 36 months	-	100	-	101
Total[1]	117	200	112	198
CH_4, g/kg of DM intake				
0 – 12 months	22.9	22.9	22.6	22.8
12 – 24 months	24.2	23.9	24.1	23.8
24 – 36 months	-	24.7	-	24.7
Total	23.5	23.8	23.4	23.8
CH_4, g/kg of ADG[2]				
0 – 12 months	110	114	104	115
12 – 24 months	317	364	299	341
24 – 36 months	-	474	-	418
Total	214	317	202	292

[1] Accumulated methane emission from birth to calve.
DM, dry matter; ADG, average daily gain.

Dairy cows on the High FS emitted 410 g CH_4/day and those receiving Low FS emitted 353 g CH_4/day, which resulted in a 21 kg increase in enteric CH_4 per year for the High FS when compared to the Low FS (Table 7). Three-year-old dairy cows at first calving emitted 11.0 and 7.5 kg of CH_4 *per* year more in High and Low FS, respectively, when compared to the two-year-old heifers at first calving. The lactation rank influenced the relationship between CH_4 and milk yield or milk solids yield. Dairy cows in lactation 3+ emitted less enteric CH_4 per unit of output (g/kg of milk yield and milk solids) regardless of the breed, feeding strategy and age at first calving.

8.4.4 Dairy system enteric methane emission

As a consequence of the short breeding period, the replacement rate for all of the systems studied was high (Table 8). The replacement rate ranged from 37% for High FS with Normande cows calving for the first time at two years to 60% for Low FS with Holsteins calving for the first time at three years. Generally, the replacement rate was greater for systems with Holstein than Normande dairy cows (55% *vs.* 44%, respectively), and in Low FS than High FS (52% *vs.* 45%, respectively). For an expected milk sale of 400 000 kg/year the dairy systems with Holstein three-year-old dairy cows at first calving receiving High FS required the least cows and the systems with the Normande two-year-old dairy cows at first calving receiving Low FS required the most (50 and 102 cows, respectively). The number of heifers and total herd was greater for systems with three-year-old cows at first calving and Low FS, independently of breed.

The dairy system annual enteric CH_4 emission was mostly influenced by FS and first calving age. The system with two-year-old dairy cows at first calving on High FS had less methane emission (11.8 ton of CH_4 *per* year) than the system with three-year-old cows at first calving on Low FS (18.2 ton of CH_4 *per* year). The relationship between CH_4 and milk yield (CH_4 intensity) was expressed *per* cow and total herd. When expressed *per* cow, the CH_4 intensity ranged from 17.3 to 27.9 g CH_4/kg of milk and from 24.6 to 44.2 g CH_4/kg of milk at herd level. There was a large effect of breed and FS on CH_4 intensity in the studied systems. Nonetheless, the enteric CH_4 emission *per* kg DM intake was similar between different cows (21.5 g/kg DM intake) and herds (22.3 g/kg DM intake).

The system with Normande three-year-old dairy cows at first calving receiving Low FS and with a 45% replacement rate showed the greatest enteric CH_4 emission (19.0 t/year), while Holstein two-year-old dairy cows at first calving receiving High FS, and with a 25% replacement rate showed the lowest enteric CH_4 emission (8.8 t/year) when compared to other systems (Table 9). The difference between the two extremes systems total enteric CH_4 emission shows the max reduction potential for systems emissions was 54% (Fig.4). The segmentation between factors indicates that breed was the most impacting factor, being capable of reducing enteric CH_4 emission by 17.1%, followed by the feeding strategy effect (-14.8%). The first calving age effect is nearly half of the two previous (- 8.7% for two-year-old cows at first calving). Decreasing the replacement rate 10% induced 1290 kg less of CH_4 emission (13.6% difference between the two extreme systems).

Table 7 – Enteric methane emission of Holstein and Normande dairy cows according to feeding strategies, first calving age and lactation parity (average 2006 - 2015)

	Holstein				Normande			
	High		Low		High		Low	
First calving age	2	3	2	3	2	3	2	3
CH_4, g/d								
Lactation 1	357	401	310	345	339	381	297	329
Lactation 2	409	438	354	374	394	420	341	359
Lactation 3+	443	462	381	393	430	447	370	380
	403	**434**	**348**	**371**	**388**	**416**	**336**	**356**
CH_4, kg/year								
Lactation 1	130	146	113	126	124	139	108	120
Lactation 2	149	160	129	137	144	153	124	131
Lactation 3+	162	169	139	143	157	163	135	139
	147	**158**	**127**	**135**	**141**	**152**	**123**	**130**
CH_4, g/kg of DM intake								
Lactation 1	21.3	21.6	20.9	21.2	22.6	22.7	21.7	21.9
Lactation 2	20.9	21.1	20.6	20.9	22.3	22.4	21.6	21.7
Lactation 3+	21.1	21.2	20.6	20.8	22.5	22.4	21.6	21.7
	21.1	**21.3**	**20.7**	**20.9**	**22.5**	**22.5**	**21.6**	**21.8**
CH_4, g/kg of OM digestible								
Lactation 1	32.7	33.0	33.0	33.2	34.1	34.2	33.9	34.0
Lactation 2	32.3	32.5	32.6	32.8	33.8	33.8	33.6	33.7
Lactation 3+	32.5	32.6	32.6	32.7	33.9	33.9	33.6	33.6
	32.5	**32.7**	**32.7**	**32.9**	**33.9**	**34.0**	**33.7**	**33.8**
CH_4, g/kg of milk								
Lactation 1	18.2	18.8	23.5	22.3	25.0	24.7	29.7	26.6
Lactation 2	17.0	17.5	21.7	21.1	22.7	22.9	27.7	25.9
Lactation 3+	16.7	17.4	20.7	20.7	21.8	22.4	25.8	25.2
	17.2	**17.9**	**21.8**	**21.3**	**23.0**	**23.3**	**27.5**	**25.9**
CH_4, g/kg of milk solids								
Lactation 1	269	273	349	330	339	330	410	366
Lactation 2	251	256	314	307	305	305	368	346
Lactation 3+	244	254	294	298	291	301	339	337
	253	**260**	**315**	**310**	**309**	**311**	**367**	**349**

OM, organic matter.

Table 8 – Total intake, milk yield, milk solid, and total enteric methane emission in Holstein and Normande herd with a milk sold of 400 ton *per* year on different dairy systems

	Holstein				Normande			
	High		Low		High		Low	
First calving age	2	3	2	3	2	3	2	3
Replacement rate	51	46	60	55	37	45	47	46
Number of								
Dairy cows	53	50	80	71	71	68	102	88
Heifers, LU[1]	24	42	43	71	24	55	43	73
Total, LU	88	104	119	143	96	126	129	153
Milk yield, kg/cow/year	8168	8632	5430	6098	6067	6364	4266	4951
Milk solids, kg/cow/year	604	647	405	455	494	519	345	399
Total DM intake, kg								
Dairy cows	6723	7277	5842	6261	6244	6625	5499	5889
Total herd	5580	5393	5910	5422	5940	5613	6104	5567
CH_4, ton/year								
Dairy cows	7.53	7.79	9.69	9.37	9.98	10.16	12.11	11.24
Total herd	10.71	12.42	15.31	17.21	12.93	16.22	17.48	19.20
Per LU, kg/year	122	119	129	120	135	129	136	126
CH_4, g/kg DM intake								
Dairy cows	21.1	21.3	20.7	21.0	22.4	22.5	21.6	21.7
Total herd	21.8	22.2	21.8	22.2	22.7	22.9	22.2	22.5
CH_4, g/kg MY								
Dairy cows	17.3	17.9	22.3	21.6	23.0	23.4	27.9	25.9
Total herd	24.6	28.6	35.2	39.6	29.8	37.3	40.2	44.2
CH_4, g/kg milk solids								
Dairy cows	234	239	299	289	283	286	344	321
Total herd	333	382	472	531	367	457	496	548

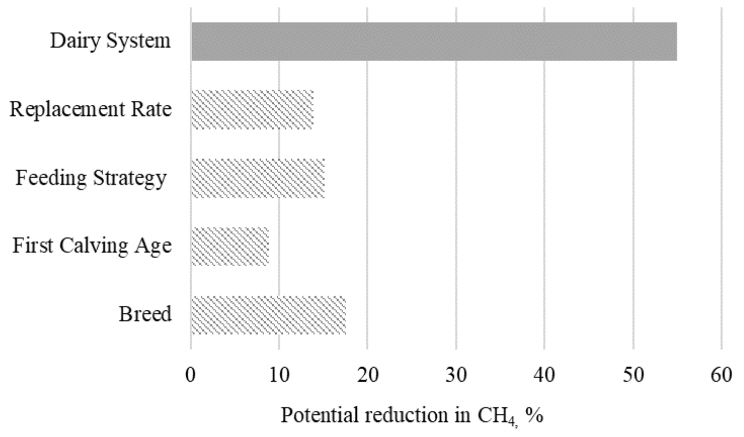
LU, Livestock Unit; DM, dry matter; MY, milk yield.

Table 9 – Necessary number of cows, replacements heifers and total enteric methane emission for a different herd with a milk sold of 400 t/year in different replacement rate, and the reduction expected with the improve on replacement rate

	Holstein				Normande			
	High		Low		High		Low	
First calving age	2	3	2	3	2	3	2	3
Replacement rate	*Number of cows*							
25	49	48	72	67	68	65	93	84
30	50	48	73	68	69	66	95	85
35	51	49	74	68	70	66	96	86
40	52	50	76	69	72	67	99	87
45	52	50	77	70	73	68	101	88
	Heifers replacement, LU							
25	11	22	16	30	15	29	21	38
30	13	26	20	37	19	35	26	46
35	15	30	23	42	21	41	30	53
40	19	36	27	50	26	49	36	63
45	21	41	31	57	30	55	41	71
	Total CH_4 emission, t							
25	8.82	10.07	11.40	12.52	11.69	13.17	14.30	15.29
30	9.16	10.61	11.93	13.26	12.19	13.91	14.97	16.20
35	9.45	11.05	12.36	13.87	12.60	14.51	15.54	16.95
40	9.88	11.72	13.02	14.80	13.22	15.44	16.39	18.08
45	10.25	12.29	13.59	15.58	13.75	16.22	17.11	19.04
	CH_4 reduction from 25 %							
30	3.8	5.1	4.4	5.6	4.0	5.3	4.5	5.6
35	6.7	8.9	7.8	9.8	7.2	9.2	8.0	9.8
40	10.8	14.1	12.4	15.4	11.5	14.7	12.7	15.4
45	14.0	18.1	16.1	19.7	15.0	18.8	16.4	19.7

LU, Livestock Unit

Figure 4 – The potential reduction of replacement rate, feeding strategy, first calving age and breed on enteric methane emission (t/year) comparing two dairy system emissions



8.5 DISCUSSION

The CH_4 emissions quantified during this work was based firstly on the extensive description of diet and DM consumed by heifers and dairy cows and secondly using a modeling approach that integrated performance data collected during ten years of experimentation. The equation proposed by Sauvant and Nozière (2013) allowed the calculation of the individual enteric CH_4 emission on dairy cows during unproductive and productive periods from the diet description.

Measurements of enteric CH_4 emission from growing heifers are scarce and very variable (Cottle et al., 2011; Morrison et al., 2017). According to IPCC (2006), the emission factor for growing heifers in Western Europe is 57 kg. Recently, Jiao et al. (2014) reported that grazing Holstein heifers have an emission factor of 36.2 kg from birth to 12 months of age, and 64.3 kg from 13 to 24 months. In the present study, two-year-old heifers calving emitted on average 57 kg of CH_4 per year, whereas from birth to 12 months of age the emission factor was 31 kg and, from 12 to 24 months of age the factor was 75.5 kg. This variation may be due to the feeding system used and the age of growing heifers.

The daily CH_4 emission observed from cows was similar to other European studies (Appuhamy et al., 2016) with grass-based FS (Muñoz et al., 2015; O'Neill et al., 2012; Robertson and Waghorn, 2002). The emission factor (kg CH_4/head/year) was greater than that reported by IPCC (2006) and Vermorel et al. (2008). This result may be associated with the total period used to estimate the enteric CH_4 emission. In French dairy systems, the observed emission factor was 118 kg in dairy cows with milk yield of 6300 kg/year during a period of 47 weeks (Vermorel et al., 2008). In our study, the annual milk yield was 6470 kg producing an emission factor of 139 kg for 52 weeks (lactation and dry period).

8.5.1 Effect of feeding strategy on enteric methane emission

As expected, the individual enteric CH_4 emission was driven by DM intake. This relationship has been well discussed in the literature (Beauchemin et al., 2008; Benchaar et al., 2001; Martin et al., 2010; Knapp et al., 2014). In growing heifers, our data allow a description of enteric CH_4 emission from birth to calve and remark the breed and first calving age effects on CH_4 emission. Excluding the effect of breed three-year-old heifers at first calving emitted more enteric CH_4 than heifers calving at two years old. From birth to calving, 50% of enteric CH_4 emission was emitted between 24 and 36 months for 3 year old heifers. This result was directly influenced by the higher duration of unproductive period, greater DM intake and growth during the last year (Jiao et al., 2014; Morrison et al., 2017). In this view, the enteric CH_4 emissions *per* ADG in two and three-year-old heifers at first calving were 208 and 304 g CH_4/ADG, respectively. Morrison et al. (2017) showed that heifers up to 23 months of age emitted 252 g CH_4 per kg of heifer live weight. However, the CH_4 emission *per* ADG in the last year of heifers with expected first calving at three years old was on average 446 g CH_4/ADG. This result may be, at least partially, explained by the higher DM intake and slower growth of this animal over this period.

The FS had an evident effect on an annual enteric CH_4 emission, with dairy cows in High FS showed greater DM intake and enteric CH_4 production than Low FS. The association between enteric CH_4 emission and DM intake means it possible to discuss the diet composition effect (O'Neill et al., 2011). The CH_4 emission *per* unit of DM intake ranged from 20.6 to 22.7 and averaged 21.5. These values agree with the variation proposed by Berndt et al. (2014). Normally, the use of concentrate and maize silage supplementation can reduce the enteric CH_4 emission relative to DM intake due to changes in fermented substrate (Beauchemin et al., 2008). Replacing structural carbohydrates from forages by non-structural carbohydrates, results in large modifications on rumen physic-chemical conditions and microbial populations, and thus alters CH_4 production (Lettat et al., 2013; Martin et al., 2010). Moreover, it is known that the grazing grass of greater OM digestibility can result in less CH_4 emitted *per* kg of DM intake when compared to pastures with less OM digestibility (O'Neill et al., 2011; Wims et al., 2010). In our work, the close values of this relationship between FS can be associated to three factors. First, the low concentrate proportion, second maize silage was used in winter only for High FS and third the high nutritive value of grass grazed.

8.5.2 Effect of managements factors on dairy system enteric methane emission

Once the enteric CH_4 estimated for each type of cows and heifers studied at INRA dairy research farm was analyzed the objective was to integrate the enteric CH_4 emission at the dairy system level. It was possible to simulate the total enteric CH_4 emission (productive and unproductive periods) from eight different dairy systems aiming to sell the same amount of milk. The different systems were used to evaluate the effect breed, first calving age, FS and replacement rate combinations have on total enteric CH_4 emission. As discussed previously, there was a direct effect of FS on enteric CH_4 emission. The dairy system simulations indicated three factors were associated with CH_4, namely the milk yield potential, first calving age and the replacement rate. These factors directly influenced the number of cows and replacements heifers required for a fixed amount of milk sales and thus affected enteric CH_4.

Improving animal productivity is widely used as a greenhouse gas mitigation strategy. In principle the strategy, reduces the number animals required for the same product output and thus reduces GHG emissions and the environmental footprint (Hristov et al., 2013a). Our research showed that a dairy system with Holstein cows receiving High FS had an annual milk yield of 8400 kg per cow and required 52 cows to sell 400 t of milk, while in the same FS 70 Normande cows were necessary, because this breed annual milk yield was 6215 kg per cow. Consequently, the Normande emitted 25% more annual enteric CH_4. This breed also emitted more enteric CH_4 in the Low FS, but the difference reduced to 18%. Thus, these findings clearly imply that the selection of more productive animals and the use of a FS that allows the expression of the merit genetic of cows reduces emissions. This result is consistent with that showed by O'Brien et al. (2014), where the enteric CH_4 emission (kg of CO_2-eq/t of energy corrected by milk) was 13% less in a high-performing confinement than in grass-based system. These differences in emissions were largely due to variation in feed efficiency i.e. milk yield per unit of intake. Improving feed efficiency mitigates enteric CH_4 emission (Gerber et al., 2011; Yan et al., 2010) and tends to improve financial returns thus.

The compact calving season and the different ability of the cows to be in calf (Bedere et al., 2017) resulted in high replacement rates for the different systems. The effect of fertility on CH_4 emission was previously studied by Garnsworthy (2004). Improving herd fertility influences the heifer replacement number for a fixed herd and reduced CH_4 emission by up to 24%. According to our observations, decreasing the first calving age to two years old was possible and reduced 2.2 t of CH_4/year. This was largely due to the replacement rate (Table 9). It is important to highlight that in each dairy system studied we simulated the replacement rate improvements. Considering the extremes situations, improving the replacement rate from 45 to 25% reduced enteric CH_4 emission from dairy systems with first calving age of two and three years old by 16.4 and 20%, respectively. The additivity of replacement heifers numbers and unproductive period were the key factors influencing total enteric CH_4 emission for each dairy system. Similar to previous reports, fewer replacement animals are needed in herds with better reproduction rates, resulting in a direct effect on CH_4 emission (Crosson et al., 2011; Knapp et al., 2014).

The difference in enteric CH_4 emission between the two extreme systems was more than double (Table 9; Fig. 4.). These results clearly show the strong mitigation potential when the animal and feed factors in the current study are considered simultaneously. Globally, the individual mitigation strategies are applied in an integrated way to a system level (Bell et al., 2011). Strategies that aim to improve animal productivity have the potential to reducer CH_4 by 20 to 30 % (Boadi et al., 2004; Knapp et al., 2014) with feeding, nutrition, and genetic selection strategies representing 15% and 19%, respectively, of the reduction of CH_4 emission *per* energy-corrected milk (Knapp et al., 2014). The management factors in the dairy system studied, breed, FS, replacement rate and first calving age act directly on dairy cows productivity, feed efficiency and unproductive period. When all these factors were analyzed, breed had the greatest potential to reduce enteric CH_4 (17.1%) followed by FS (14.8%).

8.6 CONCLUSION

The initial description of system studied for ten-years confirmed the major individual influences DM intake and feed efficiency has on enteric CH_4 emission. At the dairy system level, breed, feeding strategy, replacement rate and first calving age represent an opportunity to reduce the enteric CH_4 emission. However, the highest reduction was possible when all of them were combined. These results can serve as the basis for quantitative enteric CH_4 emission discussions and as reference to a general approach for quantifying total greenhouse gas emission from dairy systems.

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9 CONSIDERAÇÕES FINAIS

Os resultados observados mostram que não somente a utilização de diferentes estratégias de alimentação, mas também, a raça e práticas de manejo do rebanho devem ser consideradas no estabelecimento de estratégias para a mitigação da emissão de metano entérico. Observamos que atrelados às respostas observadas em relação a emissão de metano *per si* estão o aumento na produtividade, na eficiência de produção e a redução no número de animais improdutivos no rebanho. Estes fatos confirmam a hipótese geral proposta, em que a estratégia de alimentação utilizada é o principal fator de influência na emissão diária de metano entérico. Contudo, a escolha da raça e práticas de manejo, como, a idade ao primeiro parto e a taxa de reposição influenciam a emissão de metano entérico pelo sistema de produção.

Conceitualmente, a construção dos projetos apresentados partiu do pressuposto de que a produção de metano entérico pelo ruminante é ligada ao consumo e a composição da dieta consumida. Na situação prática apresentada no primeiro experimento, a oferta de pasto pôde ser reduzida em proporções diferentes entre a forma de suplementação energética, alimento concentrado ou foragem conservada, sem afetar negativamente o consumo e a produção de leite. O manejo adotado permitiu o consumo de uma forragem de boa qualidade e mesmo com o uso da suplementação energética, a redução na intensidade da emissão de metano entérico não foi evidenciada. Sendo assim, a escolha da estratégia de alimentação deve considerar que a suplementação com concentrado pode não ser eficaz na redução da intensidade de emissão dependendo da qualidade da forragem e do manejo do pasto adotados.

De uma maneira ou outra, os resultados obtidos com o segundo experimento concordam com o reportado na literatura a respeito do efeito da estratégia de alimentação sobre a emissão de metano diária devido ao consumo, e do efeito sobre a intensidade de emissão devido a maior produtividade, neste caso ligado a raça. No entanto, as medidas pontuais utilizadas não foram distribuídas ao longo do dia, afetando a veracidade das informações reunidas nestas circunstâncias. A concomitância entre o momento das medidas e o momento da alimentação, principalmente nos animais que recebiam suplementação, somado ao fato de que não ocorreu a estimativa da emissão de metano entérico durante o período de pastejo, fragilizaram os dados obtidos.

Finalmente, ao nível do sistema de produção buscou-se identificar práticas de manejo que poderiam ser consideradas no desenvolvimento de estratégias de mitigação de metano. Para tal, a descrição dos dados produtivos na utilização de diferentes estratégias de alimentação, o efeito de raça, da idade ao primeiro parto e a criação de novilhas permitiu, a partir da utilização de um modelo, integrar as informações já conhecidas e estimar o efeito sobre a emissão total de metano entérico. A emissão de metano entérico total considerando a estratégia de alimentação com maior entrada de insumos, raça Holandês, 25% de taxa de reposição e idade ao primeiro parto de dois anos foi 10,2 t/ano menor quando comparada ao sistema que contava com uma estratégia de alimentação com baixa entrada de insumos, raça Normando, idade ao primeiro parto de 3 anos e taxa de reposição de 45%. Esta diferença na emissão se deve principalmente ao efeito dos fatores estudados sobre o número de animais no rebanho e sobre a resposta produtiva. Desta forma, raça e estratégia de alimentação apresentaram maior potencial de redução, 17 e 15% respectivamente, seguidos da taxa de reposição (13,6%) e da idade ao primeiro parto (8,7%).

Claramente, os resultados desta tese retratam apenas uma parcela das emissões de gases de efeito estufa. Contudo, considerando a ênfase dada à redução do impacto ambiental na produção de alimentos, a estimativa da contribuição de diferentes práticas de manejo sobre a emissão de metano entérico, um dos principais gases de efeito estufa, em sistemas de produção de ruminantes possui suma importância e abre frentes para outras discussões. Buscando um aperfeiçoamento do modelo apresentado, este poderia ser estendido à análise da emissão de outros gases, como o óxido nitroso, e para sua validação em nível de sistema, pode existir a necessidade de se comparar os dados da emissão estimados com medidas efetivas da emissão de metano. Questões futuras a serem discutidas relacionam-se à eventual aplicabilidade do modelo desenvolvido em outras situações práticas na produção de ruminantes, como em propriedades. Em casos reais como este, seriam possíveis estimativas da emissão de metano entérico e a estruturação de estratégias de mitigação singulares.