Universidade Federal de São João del Rei – UFSJ

Programa de Pós-Graduação em Bioengenharia de Sistemas Ecológicos

JOÃO PAULO SACRAMENTO

EXIGÊNCIAS NUTRICIONAIS DE ENERGIA E ESTRATÉGIAS ALIMENTARES PARA VACAS DE LEITE EM DIFERENTES SISTEMAS DE PRODUÇÃO E CONDIÇÕES CLIMÁTICAS

SÃO JOÃO DEL REI
MINAS GERAIS – BRASIL
2021

JOÃO PAULO SACRAMENTO

EXIGÊNCIAS NUTRICIONAIS DE ENERGIA E ESTRATÉGIAS ALIMENTARES PARA VACAS DE LEITE EM DIFERENTES SISTEMAS DE PRODUÇÃO E CONDIÇÕES CLIMÁTICAS

Tese submetida ao Programa de Pós-Graduação em Bioengenharia da Universidade Federal de São João Del - Rei como parte dos requisitos necessários para a obtenção do título de "Doctor Scientiae" (DS).

SÃO JOÃO DEL REI
MINAS GERAIS – BRASIL
2021

JOÃO PAULO SACRAMENTO

EXIGÊNCIAS NUTRICIONAIS DE ENERGIA E ESTRATÉGIAS ALIMENTARES PARA VACAS DE LEITE EM DIFERENTES SISTEMAS DE PRODUÇÃO E CONDIÇÕES CLIMÁTICAS

Tese submetida ao Programa de Pós-Graduação em Bioengenharia da Universidade Federal de São João Del - Rei como parte dos requisitos necessários para a obtenção do título de “Doctor Scientiae” (MS).

Aprovado: 10 de junho de 2021

Dra. Fernanda Samarini Machado
(Membro externo)

Dra. Camila Flavia de Assis Lage
(Membro externo)

Dr. João Paulo Pacheco Rodrigues
(Membro externo)

Dr. Thierry Ribeiro Tomich
(Membro externo)

Dr. Luiz Gustavo Ribeiro Pereira
(Orientador)

Dedico este trabalho a minha mãe, Sueli, por toda afeição, esforço e amor.

AGRADECIMENTOS

A Deus e aos amigos espirituais;

A Universidade Federal de São João Del Rei, ao Departamento de Engenharia de Biossistemas (DEPEB) e ao Programa de Pós-graduação em Bioengenharia (PPBE);

A Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Leite;

A University of New Hampshire, em especial ao professor Dr. André Brito, por me acolher e orientar durante o período sanduiche;

Ao meu orientador, Luiz Gustavo Riberio Pereira e os pesquisadores da Embrapa Gado de leite, Fernanda Machado, Thierry Tomich e Mariana Campos por toda atenção e contribuição disponibilizada;

Aos amigos e colegas de trabalho que me acompanharam e apoiaram ao longo dos anos;

A todos os estagiários e alunos da Residência Zootécnica, que colaboraram com a execução do trabalho e aos funcionários do campo experimental José Henrique Bruschi – Embrapa, pela amizade e por toda a ajuda;

Aos companheiros de pós-graduação pelas horas vividas e pela troca de experiência ao longo do período experimental;

Ao meu pai Alexandre e ao meu irmão Victor, pelo amor, educação, dedicação e confiança;

Agradeço ao meu alicerce, minha mãe Sueli, que sempre vibra a cada vitória minha. Sem ela para me manter de cabeça erguida a conclusão deste trabalho não teria sido a mesma;

À Coordenação de aperfeiçoamento de pessoal de nível superior (CAPES), pela concessão da bolsa de estudos e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Embrapa pelo financiamento do projeto;

A todos que contribuíram de forma direta e indireta para realização deste trabalho;

Muito Obrigado!

Contents

REVISÃO DE LITERATURA

1. Eficiência dos sistemas de produção de leite

1.1 Cenário atual da produção de leite

Atualmente, a pecuária é desafiada pelo aumento da demanda de produtos de origem animal frente ao crescimento da população global, acompanhado pela pressão de redução do impacto ao meio ambiente (RANGANATHAN et al., 2016). Além disso, os produtores estão mais atentos aos processos relacionados à cadeia do leite, precisando lidar com fatores que reduzem as margens de lucro como a escassez de mão de obra, alta de salários e dos custos dos insumos e queda no preço do produto (LLANOS; ASTIGARRAGA; PICASSO, 2018).

As fazendas leiteiras passaram por mudanças e processo de modernização em período curto, incluindo as mudanças em layouts dos galpões, surgimento de novas tecnologias de ordenha, novas práticas de formulação de dieta e mistura da ração, além da mudança do perfil do tamanho das propriedades (LUIK-LINDSAAR; PÕLDARU; ROOTS, 2019). Nesse cenário, existe uma tendência global de intensificação técnica dos sistemas de produção de leite, principalmente no aumento da produção do leite por hectare, número de vacas leiteiras por hectare, ração mais concentrada na dieta e melhoria do mérito genético das raças (BAVA et al., 2014). A intensificação dos sistemas leiteiros pode ter efeitos sobre a eficiência e resultados econômicos de fazendas e tem sido proposta como estratégia para alimentar a população mundial (LLANOS; ASTIGARRAGA; PICASSO, 2018).

Nos Estados Unidos, as primeiras mudanças que contribuíram para a melhora em índices de eficiência em fazendas leiteiras foram observadas a partir de 1940. Desde então, embora tenha sido observada queda no número total de vacas e de fazendas leiteiras, houve crescimento no tamanho médio do rebanho das propriedades que acompanharam as mudanças do setor (VON KEYSERLINGK et al., 2013).

Capper et al. (2009), relataram que de 1944 até 2007, os EUA produziram 59% mais leite, com 64% menos vacas, que consumiam 77% menos ração e 65% menos água por litro de leite produzido, graças ao melhoramento genético dos animais, nutrição e manejo do rebanho.

A intensificação convencional da pecuária leiteira geralmente é baseada em uso de insumos como fertilizantes e agrotóxicos e energia fóssil em grande escala (ALEXANDRATOS E BRUINSMA, 2012), que pode resultar em impactos ambientais como erosão do solo, lixiviação de nutrientes, contaminação da água, além da emissão de gases do efeito estufa (MEUL et al., 2017). Uma resposta aos problemas ambientais gerados por este modelo foi o surgimento de sistemas alternativos, dentre eles o orgânicos, que fundamentam a produtividade de forma sustentável, integrando indicadores ambientais e agregando valor aos produtos, por meio da exploração de mecanismos ecológicos (LLANOS; ASTIGARRAGA; PICASSO, 2018).

Para Von Keyserlingk et al. (2013), a globalização desempenha papel importante na formação do mercado leiteiro atual. No entanto, embora a globalização da indústria de laticínios tenha tornado os custos totais de produção nos países exportadores mais semelhantes, a estrutura dos custos é variável. Sistemas baseados em pastagem, comuns a regiões tropicais, têm custos fixos altos associados ao preço de compra da terra, enquanto custos variáveis de produção como suplementação alimentar, mão de obra, equipamentos e estrutura são relativamente baixos. Em contrapartida, sistemas onde as vacas são confinadas, têm maiores custos variáveis devido a maior demanda de insumos, mesmo considerando os maiores níveis de produtividade dos animais.

1.2 Fatores relacionados à eficiência de fazendas leiteiras

Tradicionalmente, a eficiência para produção de leite e lucratividade é expressa por hectare de área da propriedade utilizada para a atividade (BASSET-MENS; LEDGARD; BOYES, 2009). Diversos trabalhos tem defendido a necessidade de adotar práticas de gestão e tecnologias, com intuito de reduzir custos de produção (LUIK-LINDSAAR; PÕLDARU; ROOTS, 2019; MA; BICKNELL; RENWICK, 2019; MARETH et al ., 2019). Segundo os autores supracitados, tais estratégias seriam ponto chave para aumento da eficiência e sobrevivência de propriedades, além de minimizar o impacto ambiental causado pela atividade (BELL et al., 2011).

Luik-lindsaar et al. (2019) afirmaram que a eficiência dos processos de produção de leite depende diretamente da relação insumo-produto e indiretamente das decisões tomadas na fazenda e no animal, como decisões tomadas sobre a higiene da fazenda, produção de leite das vacas leiteiras, idade das vacas ao primeiro parto, entre outros. O nível de produção e eficiência dos sistemas de produção de leite podem variar em decorrência à quantidade de concentrado oferecido aos animais, produção total de leite da fazenda, fertilidade e saúde das vacas, fatores também relacionados à sobrevivência da propriedade, emissões de gases de efeito estufa e a necessidade por terra (BELL et al., 2011).

Cabrera et al. (2010) analisaram a relação entre a intensificação e eficiência em fazendas de leite no estado de Wisconsin (EUA). Observaram que sistemas intensivos eram mais eficientes do que as fazendas extensivas. Esses resultados corroboram com Alvarez et al. (2008), que usaram análise de cluster para classificar fazendas do Norte da Espanha por nível de intensificação, de 1999 a 2007. Os autores mostraram que fazendas intensivas produziam com custo total médio inferior e apresentavam níveis de eficiência maiores do que fazendas extensivas. Os autores ainda sugeriram que sistemas de produção intensivos seriam mais fáceis de ser gerenciados, com base na maior variância do índice de eficiência do grupo de fazendas extensivas.

O tamanho da fazenda (CABRERA; SOLÍS; DEL CORRAL, 2010; MA; BICKNELL; RENWICK, 2019; MARETH et al., 2019); e a frequência de ordenha (CABRERA; SOLÍS; DEL CORRAL, 2010; MA; BICKNELL; RENWICK, 2019) também têm efeitos positivo sobre a eficiência de fazendas leiteiras. Outros determinantes como o tipo de galpão, a intensidade da irrigação (JIANG; SHARP, 2015); custos com alimentação, mão-de-obra e assistência técnica (MARETH et al., 2019) foram relacionados positivamente à eficiência técnica em pesquisas realizadas na Europa e no Brasil.

Luik-lindsaar et al. (2019), observaram que fazendas mais eficientes usam menos mão-de-obra e área agrícola por vaca. Em pesquisa realizada com 253 fazendas leiteiras no Sul do Brasil, Mareth et al. (2019) observaram que fazendas ineficientes têm custos de mão de obra mais elevados que fazendas eficientes. Cabrera et al (2010) mostraram que quanto maior a proporção de mão de obra familiar no quadro de funcionários, maior o efeito sobre o valor de eficiência técnica da propriedade. Conforme Carter (1984), em geral, membros de uma família trabalham pelo bem estar coletivo ao invés de pensar de forma individual e, consequentemente, proporcionam maior esforço para a produção.

É importante ressaltar que, embora maiores níveis de eficiência técnica sejam associados ao aumento da produtividade de leite, não está claro se a eficiência do sistema tem efeito significativo sobre a lucratividade. O impacto na lucratividade dependerá dos preços relativos dos insumos usados e do preço do leite pago ao produtor. A intensificação também pode estar associada a efeitos ambientais negativos (MA; BICKNELL; RENWICK, 2019).

A eficiência do uso de nutrientes (MU et al., 2017) e o mérito genético (BAUMAN et al., 1985), são fortemente associados ao desempenho de fazendas leiteiras. O manejo nutricional é importante e afeta a lucratividade do sistema, já que é responsável por metade dos custos totais da produção de leite (VANDEHAAR, 1998). Por sua vez, técnicas associadas a melhoramento genético e a previsão inicial de mérito genético de vacas leiteiras tem potencial para melhorar a eficiência do sistema de produção de leite, pela manipulação destes processos (BAUMAN et al., 1985).

1.2.1 Manejo Nutricional

O fornecimento da dieta para vacas leiteiras é um componente importante das despesas agrícolas, onde a eficiência é expressa como o retorno de produto vendável por unidade de insumo alimentar (BAUMAN et al., 1985). Apesar de fazendas leiteiras mais eficientes apresentarem maiores custos de alimentação, o custo da dieta por kg de leite, é menor nessas propriedades (LUIK-LINDSAAR; PÕLDARU; ROOTS, 2019). Ondersteijn et al. (2003) observaram efeito positivo nos resultados financeiros com o aumento da eficiência nutricional por meio de manejo mais preciso ou adoção de tecnologias.

Bauman et al. (1985) sugeriram que eficiência digestiva, aumento da ingestão, variação da taxa metabólica, utilização de energia metabolizável para produção e a partição de nutrientes são os principais fatores responsáveis pelo aumento da eficiência em fazendas leiteiras. Há consenso na literatura que a intensificação da produção de leite por meio da suplementação com concentrado, melhora a eficiência técnica de fazendas leiteiras. Esses resultados foram apresentados por Kompas e Che (2006) para fazendas australianas, Alvarez et al. (2008) para compilado de pesquisas realizadas na Espanha entre 1999 e 2006, Cabrera et al. (2010) para os Estados Unidos, Ma et al. (2019) para sistemas a pasto da Nova Zelândia e Mareth et al., (2019) para fazendas localizadas no sul do Brasil.

Cabrera et al. (2010) avaliaram o impacto da inclusão de duas estratégias de manejo alimentar como variáveis (*i:* proporção de ração comprada para o tamanho do rebanho, e *ii)* uso de sistema de alimentação de ração total misturada *("total mixed ration"* - TMR). Concluíram que a alimentação comprada tem um impacto pequeno, mas significativo na eficiência. Já o impacto do uso da TMR era muito maior em magnitude, embora fosse menos significativo estatisticamente.

A TMR limita a capacidade das vacas selecionar os ingredientes individuais da dieta, aumentando as chances de os animais consumirem as quantidades corretas dos ingredientes. Como resultado, têm-se melhoras na fermentação e digestibilidade pelas bactérias do rúmen, o que pode ser traduzido em melhor ingestão e, consequentemente, maior eficiência para produção de leite (SORIANO; POLAN; MILLER, 2001).

2.2.1 Seleção de rebanho

O principal objetivo dos programas de melhoramento é aumentar a produção, e os níveis de gordura e proteína do leite, melhorar a saúde e fertilidade do rebanho, a fim de alcançar uma boa curva de lactação. Todos esses parâmetros afetam os resultados econômicos de uma fazenda leiteira (LUIK-LINDSAAR et al., 2018).

Luik-lindsaar et al. (2018) analisaram o efeito total que o valor genético relativo, englobado por potencial de produtividade do rebanho e habilidades de seleção de reprodutores pelo produtor, sobre a eficiência da produção de leite. Os autores presumiram que quanto maior o mérito genético, maior é a capacidade de conversão alimentar das vacas e a qualidade do leite, e esses fatores podem afetar positivamente a eficiência de fazendas leiteiras.

Roibas e Alvarez (2010, 2012), observaram que o melhoramento genético de rebanhos teve impacto significativo nos teores de proteína e gordura do leite e aumentou a lucratividade de fazendas leiteiras. Os autores concluíram que embora o melhoramento genético das vacas tenha aumentado os gastos com ração, no futuro será preciso que os produtores invistam em melhoramento genético, para manter a eficiência do sistema.

Ramsbottom et al. (2012) observaram que o índice de reprodução econômica foi positivamente relacionado à margem líquida e receita bruta por vaca e por litro de leite. Portanto, pode-se presumir que o mérito genético dos animais, combinado a outros fatores de produção (taxa de lotação, tamanho do rebanho e quantidade de ração) afeta a eficiência das fazendas leiteiras.

É importante observar que para estimar o potencial genético de um rebanho, é preciso considerar as influências externas na variação da produção (RENNÓ et al., 2002). Na escolha de uma raça ou cruzamento, devem ser considerados aspectos como o sistema de produção a ser adotado na propriedade e o clima (MIRANDA; FREITAS, 2009). Assim, as características genéticas de uma raça ou cruzamento, devem estar alinhados as condições climáticas de uma região a fim de assegurar a máxima produção de leite (PERISSINOTTO et al., 2018), principalmente em países com diversidade de sistemas de produção como o Brasil. Portanto, conhecendo as zonas de conforto térmico das diferentes raças de bovinos leiteiros e os recursos de manejo alimentar, reprodutivo e das condições de instalações, o produtor de leite será capaz de realizar a escolha mais adequada para as condições climáticas de sua propriedade (MIRANDA; FREITAS, 2009).

2. Exigências Nutricionais de Vacas Leiteiras

As exigências nutricionais de bovinos leiteiros vem sendo estudados ao longo dos anos (AFRC, 1993; ARC, 1980; CSIRO, 2007; NRC, 2001). Embora qualquer nutriente possa ser considerado limitante no desempenho produtivo dos animais, os níveis de energia e proteína da dieta, são os primeiros a serem estimados (FAO, 2015).

Diferentes metodologias podem ser utilizadas para estimar as exigências energéticas para mantença e produção de vacas leiteiras. A calorimetria permite mensurar a produção de calor pelo animal por métodos físicos (calorimetria direta), ou por medições das trocas gasosas com interações com a excreção de nitrogênio urinário (calorimetria indireta), como nos ensaios respirométricos (DIJKSTRA et al., 2020). Nessas condições, a energia produzida pelo animal é mensurada por meio das trocas gasosas e produção total de calor (RODRIGUES; VIEIRA, 2011). Tabelas de exigências de sistemas como ARC (1980), AFRC (1993) e CSIRO (2007), foram baseados em respirometria calorimétrica.

O abate comparativo é outro método frequentemente adotado no Brasil e foi também o procedimento utilizado pelo Sistema Californiano de Energia Líquida (CNES), no qual o NRC (1996) tomou como base para desenvolver suas tabelas de exigências (FONTES et al., 2005). Nesse caso, a produção de calor é determinada pela diferença entre a energia retida (ER) e o consumo de energia metabolizável (CEM) (GARRETT; MEYER; LOFGREEN, 1959), o que permite que as exigências de energia sejam determinadas em condições mais proximais da produção real das vacas (FONTES et al., 2005).

O ponto de partida de sistemas de alimentação baseados em energia é a energia bruta (EB), que foi definida por Armsby (1912) como o máximo de energia quantificada durante a combustão completa dos alimentos (MEIGS, 1925). Essa fração é medida em bomba calorimétrica e uma parte dela, não digerida, é perdida nas fezes (VANDEHAAR; ST-PIERRE, 2006).

A diferença da EB ingerida e da eliminada nas fezes (EF) é chamada de energia digestível (ED). A proporção de ED em relação à EB do alimento pode variar de 30% (para forragens) a 90% (grãos). A ED é estimada facilmente em ensaios que refletem a digestibilidade da dieta, porém há falha em não considerar as perdas ocorridas em função de processos digestivos, assim, nem toda energia perdida está diretamente relacionada a fração não absorvida da dieta. Dessa forma, há uma grande chance da ED atribuir valores acima dos reais para alimentos de menor digestibilidade (volumosos) em relação aos alimentos com maior digestibilidade (grãos), de forma que é mais correto considera-la como energia digestível aparente (EDA) (NRC, 1981). O NRC (2001), aceita que um quilo de NDT equivale a 4,4 Mcal de energia digestível, no entanto a ED sofre forte influência do consumo alimentar do animal, principalmente em dietas de animais de alta produção (MEIGS, 1925).

Parte da energia digestível é perdida através da urina e durante a fermentação de gases (principalmente metano - CH_4), restando à energia metabolizável (EM) (VANDEHAAR E ST-PIERRE, 2006), que representa a fração da energia da dieta que fica disponível para o animal, e será aproveita nas funções fisiológicas e produtivas do organismo. A EM pode ser convertida em outras formas de energia, e é a base para avaliação da mantença dos animais e formulação de rações (NRC, 2000).

A digestibilidade do alimento, a quantidade e tipo de alimento consumido interferem na disponibilidade de EM (NRC, 1981). A avaliação precisa da EM das dietas de bovinos, é feita por meio de ensaios de digestibilidade *in vivo*, medindo-se a EB dos alimentos e das perdas energéticas pelas fezes, urina e CH_4 (AFRC, 1993).

Baseando-se em uma série de trabalhos de Armsby e Fries publicados entre 1903 e 1907, Meigs (1925) discutiu a aplicação prática do uso de EM para medir os níveis de energia dos alimentos. O autor concluiu que os valores dos nutrientes digestíveis totais são a abordagem existente mais próxima da medida da EM contida nos alimentos, e por isso pode-se supor que os valores da energia metabolizável podem ser utilizados para expressar o valor da energia nos alimentos.

Como parte da EM é perdida na forma de produção calor (PC) gerado por processos fisiológicos de mantença da vaca e pela digestão do alimento, quando obtidos os valores de EM e PC, pode-se calcular a energia líquida (EL) (FERRELL; OLTJEN, 2008), a fração de energia utilizada para produção do leite e ganho (VALADARES FILHO et al., 2016).

A energia líquida de mantença (EL_M) é igual ao metabolismo do animal em jejum, e a energia líquida para a produção de leite (EL_P) é igual ao valor calórico do produto formado (MOE; FLATT; TYRELL, 1972). Flatt et al. (1965), encontraram valor de 73 kcal/kg $PV^{0,75}$ para a PC, em vacas leiteiras secas e vazias. O NRC (2001) preconiza correção de 10% para atividade em relação ao valor descrito pelo autor, que define a EL_M de vacas em lactação em 80 kcal/kg $PV^{0,75}$. O conselho ainda assume uma equivalência de 85% do peso de corpo vazio metabólico adotado pelo NRC (2000) de 77 Mcal/kg $PCVZ^{0,75}$, em relação ao peso vivo metabólico, para adotar a exigência de 65 kcal/kg $PV^{0,75}$.

Já o AFRC (1993) considera os requisitos para EL_M como 69,76 kcal/$(PV/1,08)^{0,67}$ para machos castrados e novilhas. O multiplicador 1,08 faz referência à relação existente entre o peso vivo e o peso vivo em jejum. Para a produção de leite é acrescida uma margem de segurança de 10% ao valor base, assumindo o valor de 70 kcal/$(PV/1,08)^{0,67}$. No Brasil, Silva (2011) utilizou câmara respirométrica para a determinação das exigências nutricionais de fêmeas bovinas em crescimento. O autor comparou animais da raça Gir leiteiro, Holandês e F1 Holandês x Gir. Os valores de EL_M encontrados foram 85,2; 96,4 e 102,3 Kcal/$PV^{0,75}$, respectivamente.

As exigências de mantença sofrem influência do peso do animal, o nível de produção, a atividade e o ambiente em que os animais são mantidos. Assim, as necessidades dos animais devem ser determinadas com base em valores representativos das condições ambientais do animal (temperatura, velocidade do vento, superfície específica e isolamento térmico), tipo (*Bos taurus*, *Bos indicus* e cruzamentos) e histórico nutricional anterior, estimado a partir do escore de condição corporal (ECC) (FOX et al., 1995).

A EL para a lactação (EL_L) é definida como a energia contida no leite produzido, e equivale à soma dos calores de combustão da gordura, proteína e lactose do leite (NRC, 2001). Os valores do calor de combustão determinado por análise de regressão da energia da gordura, proteína e lactose do leite, comumente utilizados são 9,29, 5,71 e 3,95 Mcal/kg, respectivamente (TYRRELL; REID, 1965). Em geral, apenas gordura e proteína do leite são medidas, visto que o teor de lactose do leite é o componente que menos varia, assim é comum a adoção do 4,85 % de lactose do leite (NRC, 2001).

No NRC (2001), a energia líquida para lactação (EL_L) é usada para expressar o valor da energia dos alimentos, a partir da correção das exigências líquidas por um fator de eficiência de utilização.

Vandehaar e St-pierre (2006), afirmaram que a eficiência de utilização da EM para produção de leite (k_L) e semelhante à eficiência de utilização da EM para mantença (k_M). Desse modo, pensando na eficiência da conversão de EM em EL_L, se a exigência de mantença é de 20 Mcal de EL_L ao dia, ela permanecerá constante mesmo que o consumo do animal aumente. De acordo com os autores, o calor extra que é produzido, será considerado como incremento calórico e a maior porção energética será aproveitada para a produção de leite.

Dois aspectos importantes da inter-relação entre nutrientes geradores de energia e proteínas devem ser considerados. Primeiro, mudanças na quantidade de proteína pode alterar os ingredientes da ração, comprometer a digestibilidade dos nutrientes e consequentemente influenciar o desempenho dos animais. Nessa perspectiva, o primeiro objetivo da dieta, seria atender as demandas de nitrogênio, principalmente quando a demanda por N de matéria orgânica fermentável é alta. Em segundo lugar, alterações do suprimento de proteína nos tecidos podem alterar o padrão e a eficiência do uso de nutrientes absorvidos. Por exemplo, no início da lactação, a suplementação de proteína favorece a partição dos nutrientes disponíveis para a secreção mamária. A resposta ao aumento da entrada de AA depende do estado fisiológico da vaca e do equilíbrio de todos os nutrientes absorvidos pelo trato gastrointestinal (OLDHAM, 1984).

A fração proteica de rações para gado leiteiro representa um dos ingredientes mais dispendiosos das dietas (HOF et al., 1997). E para minimizar custos, há cada vez mais interesse em reduzir perdas de compostos nitrogenados e encontrar um ponto de melhor aproveitamento metabólico pelos animais.

A proteína da dieta ou proteína bruta (PB) é definida pelo o teor de nitrogênio (N x 6,25) nos alimentos. A definição parte do pressuposto de que o teor médio de N dos alimentos é de 16 g por 100 g de proteína. Para otimizar a eficiência do uso de PB da ração, é preciso fornecer quantidades adequadas de proteína degradável e não degradável no rúmen (PDR e PNDR respectivamente) a fim de potencializar a atividade microbiana que permite a redução da oferta de PB na dieta, que irão fornecer as quantidades de aminoácidos (AA) absorvidos. Nas necessidades nutricionais do gado leiteiro apresentadas no relatório do NRC (1989) foi assumido que 15% do N ingerido é aproveitado. A proteína necessária para a lactação é baseada na quantidade de proteína secretada no leite. A eficiência do uso de proteína metabolizável (MP) para a lactação é assumida como 0,67 (NRC 2001).

Em uma análise de regressão, Spanghero e Kowalski (1997) avaliaram a ingestão de dietas com diferentes níveis de N digestível. A pesquisa apresentou correlação positiva ($r = 0,444$, $P < 0,01$) entre o balanço de nitrogênio (BN) e a disponibilidade de N da dieta, resultado que pode ser interpretado como superestimação do N retido, ou subestimação de mobilização, à medida que a disponibilidade de N na dieta aumenta.

Wright et al. (1998) avaliaram o efeito da suplementação de PNDR com um padrão fixo de aminoácidos (AA) sobre a produção de proteína do leite e utilização de N em vacas leiteiras e a resposta à quantidade de ração ingerida. Foi observado que o aumento da produção de proteína do leite foi diretamente proporcional à concentração de PNDR na dieta. A eficiência de utilização de N para produção de leite ultrapassou 30% nas vacas que foram suplementadas com os menores níveis de PNDR. Os autores conluiaram que há uma oportunidade de aumentar a produção de proteína do leite usando formulações de PNDR que são balanceadas para AA, minimizando a excreção de N residual.

Os interesses práticos do aumento da eficiência da proteína da dieta incluem redução dos custos da ração, aumento do ganho de proteína do leite produzida, aumento dos rendimentos com a produção do leite, ganho de espaço na dieta para outros nutrientes que irão aumentar a produção e redução do descarte de nitrogênio (NRC, 2001).

É importante lembrar que as exigências nutricionais mudam de acordo com uma série de fatores como alterações do ambiente e raça. Mesmo em casos em que as vacas são semelhantes quanto o tamanho ou a raça, as exigências de energia metabolizável para mantença podem variar entre 8 a 10% (Van Es, 1961).

2.1 Raça Girolando

A raça girolando, é uma raça sintética, bimestiça, desenvolvida no Brasil a partir de 1940 como resultado do cruzamento entre as raças Gir e Holandês. O cruzamento tinha como objetivo gerar animais que aliassem a alta capacidade de produção de leite, a precocidade e a mansidão do gado *Bos taurus taurus* e a rusticidade da raça *Bos taurus indicus*, a fim de conferir a produtores brasileiros condição de produzir leite em qualquer região do país (CANAZA-CAYO et al., 2014).

Com o início do teste de progênie de touros em 1997, realizado em parceria com a Embrapa Gado de Leite, a raça ganhou novos rumos, a intensidade de seleção dos animais aumentou e os criadores passaram a investir mais em melhoramento genético, em novas técnicas de reprodução e de manejo (VINICIUS et al., 2010).

Estima-se que 80% do leite produzido no Brasil provêm de vacas que tenham em sua composição genética as raças Gir e Holandês (VINICIUS et al., 2010). Entre as características inerentes a raça destacam-se a boa produtividade, alta fertilidade e vigor, constituindo-se em uma composição racial importante para a pecuária nacional (ANDRÉS et al., 2012).

Avaliando 289 registros de produção de leite de vacas da raça Holandês e diferentes graus de composições raciais de vacas Girolando, Mellado et al. (2011) compararam características de produção de leite em fazendas de produção intensiva da região subtropical do México. Os autores relataram que embora as vacas da raça Holandês fossem superiores em desempenho produtivo, em ambiente subtropical, o desempenho reprodutivo das vacas puras é inferior, enfatizando a importância do cruzamento de bovinos Holandês x Gir para a produção de leite em regiões tropicais.

Os testes de progênie de touros, iniciados em 1997, impulsionou a intensidade de seleção dos animais, criadores passaram a investir em melhoramento genético, novas técnicas de reprodução e de manejo. Como resultado, a raça ganhou reconhecimento internacional, principalmente por atender às demandas de regiões tropicais do planeta e atender tanto grandes como pequenos produtores (VINICIUS et al., 2010).

A Portaria 79 de 7 de fevereiro de 1996 do Ministério da Agricultura, Pecuária e Abastecimento (Mapa), oficializou a criação da raça, com intuito de obter e fixar uma raça leiteira tropical nacional. A Associação Brasileira de Criadores de Girolando, criada com a finalidade de aprimorar a criação da raça, é a entidade oficial responsável pelo registro genealógico dos animais. No Brasil, a produtividade da raça vem aumentando, graças aos avanços na qualidade genética dos animais, associado a um conjunto de melhorias no manejo nutricional e sanitário dos rebanhos, além da adoção de tecnologias de precisão, na assistência técnica e na estrutura das propriedades (MIRANDA; FREITAS, 2009).

2.1.1 Exigência nutricional da raça Girolando

Sistemas para estimar as exigências de EM para mantença (EM_M) e a eficiência de utilização da ingestão de EM para a produção de leite (k_L) são frequentemente utilizados como base para formulação de rações (NRC, 2001; CSIRO, 2007). No entanto, esses dados são obtidos a partir de pesquisas que utilizam principalmente raças europeias puras em condições de clima temperado, cujas exigências nutricionais podem ser diferentes de animais da raça Girolando, comum nas regiões tropicais (MADALENA et al., 1990). Adicionalmente, a base alimentar das dietas de sistemas de produção dos trópicos é de forrageiras de baixa qualidade, frequentemente deficientes em nitrogênio e energia digestível. Sob condições adversas, pouca chuva e altas temperaturas e umidade relativa do ar, as forragens tropicais passam por um processo de espessamento da parede celular, fator limitante do consumo de matéria seca e da digestibilidade das forragens, que em poucos casos é superior a 55% (MONTOYA; CHARÁ; BARAHONA-ROSALES, 2017).

Dessa maneira, sem o conhecimento adequado do metabolismo de vacas leiteiras cruzadas, os sistemas de alimentação podem ser pouco eficientes para atender às demandas energéticas e de proteína desses animais (CHAOKAUR et al., 2015).

Na Tabela Brasileira de Exigências Nutricionais de Zebuínos Puros e Cruzados (BR-CORTE) a EM representa mais de 82% da ED em dietas balanceadas (VALADARES FILHO et al., 2016) para bovinos de corte. Valor próximo do encontrado no NRC (2000), ARC (1980) e CSIRO (1990), que estabelecem em 80% a fração digestível disponibilizada na EM na maioria das forragens e dietas que associam forragens e cereais. No entanto para gado de leite em condições tropicais esses valores ainda não estão completamente estabelecidos.

Oliveira (2015) e Carvalho et al. (2019), discutiram a importância da realização de ensaios de alimentação, para ajustes nas recomendações de energia do sistema de alimentação de vacas leiteiras cruzadas em condições tropicais. Para os autores a escassez de dados a respeito do metabolismo e partição de energia desses animais, pode contribuir para a formulação de planos de nutrição inadequados, que comprometem a produtividade dos animais.

Baseado em pesquisas realizadas entre 1960 e 2015, Oliveira (2015) observou que vacas leiteiras cruzadas apresentaram valores de EM_M 26% menor e (k_L) 19% menor do que vacas pura *Bos taurus*. Por sua vez, Carvalho et al. (2019) não observaram diferença na eficiência de uso de energia expressa pelos coeficientes "q" e "k", ou na EM_M, entre vacas cruzadas (Holandês x Gir) e vacas da raça Gir, embora os animais F1 tenham apresentado maior ingestão de EB, ED, EM; maior exigência de EL_L e consequentemente maior produção de leite em relação ao Gir.

Os resultados de EM_M observados no primeiro caso, podem estar relacionados a fatores como menores tamanho e atividade das vísceras/fígado, turnover de proteína corporal, gordura visceral e perda de calor corporal, e maior capacidade de resfriamento evaporativo conferida ao cruzamento pelo *Bos indicus*. Já o k_L mais baixo das vacas leiteiras cruzadas pode estar relacionado ao efeito da dieta ou diferenças homeostática e da regulação homeorética. É muito comum que seja fornecida maior proporção de forragem nas dietas de vacas cruzadas reduzindo o k_L devido à maior proporção de acetato em relação ao propionato (Armstrong e Blaxter, 1965). No entanto, k_L foi mais influenciado pelo mérito genético das vacas do que pela dieta (Oliveira, 2015).

O número de trabalhos envolvendo ensaios de metabolismo energético com vacas leiteiras nos trópicos, ainda são insuficientes para estabelecer de forma precisa as exigências nutricionais da raça Girolando. No futuro, será preciso elaborar um banco de dados com as exigências nutricionais de vacas leiteiras cruzadas, com objetivo de fornecer a produtores uma alternativa mais eficaz para produção sustentável em sistemas de produção de leite de países como o Brasil.

3. Sistemas sustentáveis de produção de leite

O comportamento do consumidor de leite e derivados segue uma tendência global baseada em fatores como a expansão comercial mundial, aumento da população urbana, movimentos de migração, aumento da renda e maior acesso a informação impulsionada por novas tecnologias (JAVALGI; GROSSMAN, 2016; ZHLLIMA; IMAMI; CANAVARI, 2015).

As questões ambientais e o bem-estar animal, relacionados à pecuária, são pautas fortemente debatidas tanto em agendas sociais e acadêmicas, como em questões de cunho político e comercial (MULDER; ZOMER, 2017). Este debate parte de dois princípios. O primeiro é que toda produção de alimentos tem um impacto no meio ambiente, e à medida que a população mundial aumenta é fundamental produzir alimentos de alta qualidade em quantidade suficiente a partir de recursos finitos (CAPPER; CADY; BAUMAN, 2009). A segunda é que este debate, oriundo da grande industrialização na produção animal, trouce à tona questões de segurança alimentar, considerações sociais e éticas, situações de abuso aos animais, e conhecimento sobre os estados fisiológicos e emocionais dos animais (ROBBINS et al., 2016).

Pensando no crescimento do mercado de alimentos de origem animal, e baseado na complexidade dos fatores que apoiam o consumo sustentável, será necessário implementar estratégias de marketing responsável (MIRANDA-DE LA LAMA et al., 2019). Para produzir de forma intensificada e ao mesmo tempo conservar recursos naturais, os produtores precisam identificar sistemas e práticas que façam o melhor uso dos recursos disponíveis e minimizem o potencial impacto ambiental (WHITE et al., 2019). Nesse contexto a adoção de sistemas orgânicos é uma opção que pode garantir preço diferenciado e manter margens maiores de lucro na fazenda e indústrias de leite e derivados (ROSATI; AUMAITRE, 2004).

3.1 Sistema Orgânico

A produção de leite orgânico é definida como a criação das vacas com acesso ao pasto, alimentadas com ração orgânica, aliados ao uso restrito de antibióticos e hormônios (Oruganti, 2011). Os sistemas orgânicos são considerados menos prejudiciais ao meio ambiente quando comparados a sistemas convencionais, principalmente pelo fato dos rebanhos orgânicos produzirem menos resíduos. No entanto, a mesma quantidade de leite é obtida explorando-se uma área maior (ROSATI; AUMAITRE, 2004).

Comparada a outras culturas orgânicas, as fazendas leiteiras orgânicas são relativamente novas, com os primeiros produtos lançados no mercado só nos anos 90.

Com as mudanças do perfil do consumidor, que passou a se preocupar com questões relacionadas à saúde, houve crescimento global no consumo de leite orgânico. Diversos acontecimentos críticos podem ser atribuídos ao sucesso e crescimento de fazendas certificadas como orgânicas. Destacam-se a conscientização dos consumidores quanto ao uso de transgênicos e pesticidas em culturas para a alimentação do gado e o uso indiscriminado da somatotropina bovina e medicamentos sintéticos, o que encorajou muitos consumidores a buscar por alimentos considerados mais saudáveis, incentivando a busca por produtos lácteos orgânicos (HAMADANI; KHAN, 2015).

Considerando os sistemas orgânicos, realizar a transição de um sistema convencional não é opção fácil pensando principalmente nas questões econômicas. Porém, fazendas que utilizam pasto como fonte de alimentação podem ter a transição de sistemas facilitada. A adoção a sistemas orgânicos também pode ser uma alternativa a escassez de insumos para a produção de leite, principalmente em fazendas distantes de centros comercias (ROSATI; AUMAITRE, 2004).

Pierce e Tilth (2020), afirmaram que dentre as razões para produzir e comprar os alimentos orgânicos, a maior parte se fundamenta em três bases: saúde, comunidade e meio ambiente. Para os autores o princípio do movimento orgânico é construído sobre ideal de que solos saudáveis levam a colheitas saudáveis, animais saudáveis, humanos saudáveis em ambiente sustentável. Os sistemas orgânicos também podem ser utilizados como alternativa para apoiar pequenas propriedades de caráter familiar, que não competem em igualdade com modelos de fazendas coorporativas e alto índice de tecnificação (HAMADANI; KHAN, 2015).

O mercado global de produtos lácteos orgânicos está crescendo a taxa de 8%, sendo estimado em US$ 18 bilhões em 2017, representando 20% do total da categoria de alimentos e bebidas orgânicos, perdendo apenas para o setor de frutas e vegetais. A categoria de leite líquido orgânico responde por 24% dos laticínios orgânicos e está avaliada em US$ 4,3 bilhões. Os mercados globais de varejo de leite orgânico permanecem concentrados em certas áreas geográficas, com os EUA com 54%, Alemanha com 11% e França com 7% do total de áreas destinadas a produção de leite orgânico. (OMSCO, 2019)

É importante compreender que sistemas de produção orgânicos, embora partam da mesma premissa, são regidos por diferentes regulamentações, controladas por órgãos competentes, de cada país. Nos EUA, a certificação de fazendas orgânicas foi unificada pelo Programa Orgânico Nacional (National Organic Program - NOP) do Departamento de Agricultura dos Estados Unidos (USDA). Desse modo, certificadores privados e estaduais, que adotavam diferentes regras de produção, manuseio e processamento de orgânicos, passaram a adotar padrão único a partir de outubro de 2002 (PIERCE; TILTH, 2020). No Brasil, a certificação é obtida através da contração de uma Certificadora por Auditoria, ou o produtor pode se ligar a um Sistema Participativo de Garantia – SPG, que por obrigação deve estar sob certificação de um Organismo Participativo de Avaliação da Qualidade Orgânica – OPAC credenciado junto ao Ministério da Agricultura, Pecuária e Abastecimento – MAPA. A Instrução Normativa 007/1999, de 17/05/1999, estabelece normas de produção, tipificação, processamento, envase, distribuição, identificação e certificação da qualidade para os produtos orgânicos de origem vegetal e animal, na qual são detalhadas as etapas de conversão e transição das propriedades rurais que os produzem. Embora a produção de leite orgânico represente uma parcela pequena em relação aos 845.7 milhões de litros de leite produzidos em 2020, constitui uma fatia promissora do mercado de leite e derivados, apresentando crescimento maior que 20% no número de propriedades certificadas entre 2018 e 2020 (BRASIL, 1999).

3.2.1 Padrões para produção de leite orgânico

Existem 88 países com seus próprios padrões para produção de orgânicos baseados em princípios semelhantes da produção orgânica. Para que seja permitida a exportação dos produtos, são utilizados acordos de equivalência entre métodos. As etapas envolvidas na certificação incluem o registro dos produtores e das indústrias de processamento, fornecimento de informações básicas sobre as safras e fazenda, e inspeção e verificação da fazenda, unidade de processamento, métodos de produção e práticas de produção pelo inspetor nomeado pela agência certificadora (HAMADANI; KHAN, 2015).

Nos EUA, o Programa Orgânico Nacional é relativamente novo. Mesmo com o amadurecimento do programa, existem pontos chaves comuns a todos os produtos lácteos orgânicos certificados pelo USDA. Os principais pontos do regulamento para certificação de produtos lácteos orgânicos são: vacas leiteiras devem ser alimentadas e manejadas organicamente por pelo menos um ano antes da produção de leite orgânico. A ração de bezerros e vacas devem ser 100% orgânicas. Os bezerros devem ser alimentados exclusivamente com leite orgânico. Todos os animais, incluindo aqueles em fase de crescimento, devem ter acesso a pastagens. Ingredientes do concentrado, feno e pastagens devem ser cultivados sem o uso de fertilizantes sintéticos ou pesticidas não aprovados para uso orgânico. A terra usada para essas culturas deve estar livre de todos os materiais proibidos por pelo menos três anos antes da primeira colheita orgânica e uso de transgênicos é estritamente proibido.

Os aditivos e suplementos alimentares como vitaminas e minerais, também devem ser aprovados para uso em produtos orgânicos. Somente produtos de saúde aprovados podem ser utilizados. Muitos deles são restritos em como e quando podem ser usados, e antibióticos não são permitidos. No entanto, não é permitido negar tratamento médico a uma vaca doente a fim de manter a condição orgânica do animal, o bem-estar dos mesmos deve ser preservado. O uso de hormônios de crescimento, engenharia genética e clonagem é proibido (PIERCE; TILTH, 2020).

Todas as operações de produção e processamento devem ser comprovadas por agências de certificação credenciadas pelo USDA. Uma vez que o rebanho é certificado, todos os animais que forem adicionados ao rebanho, devem ser gerados a partir de vacas criadas em sistemas orgânicos pelo menos a partir do último terço da gestação. Os animais devem ser alimentados e manejados organicamente em todas as etapas de desenvolvimento, para produzir leite orgânico. Registros detalhados de todos os alimentos, medicamentos e transações, devem ser mantidos na propriedade. A integridade orgânica deve ser protegida evitando que a vaca e os alimentos fornecidos entrem em contato com substâncias proibidas ou se misturem com produtos químicos. Todas as fazendas certificadas são inspecionadas e auditadas todos os anos, e qualquer fazenda pode ser inspecionada sem aviso prévio. O texto mais atualizado da regra final do NOP pode ser encontradono site do NOP, no site eletrônico do *Code of Federal Regulations* norte-americano (USDA, 2021).

4.2.1 Manejo nutricional e de pastagem

Em fazendas orgânicas, o uso de concentrado é limitado, o que pode comprometer os índices de produção de leite da propriedade. A limitação de concentrado, associada ao padrão orgânico de alimentação dos animais, com ingredientes produzidos na propriedade, resulta em menores taxas de lotação o (OFFERMANN; NIEBERG, 2000). O alto preço das safras de grãos orgânicos também pode significar que é mais lucrativo vende-las do que alimentar o gado, contribuindo para a necessidade de ajustes na taxa de lotação de acordo com a capacidade de terras e pastagens remanescentes que ficam disponíveis (NICHOLAS et al., 2004).

Mosimann e Suter (2003) descreveram dois sistemas de alimentação com diferentes níveis de produção na Suíça para mostrar que ambos podem ter sucesso e respeitar a legislação orgânica. No primeiro sistema, a dieta era composta por pasto, silagem e leguminosas; no segundo, os animais também recebiam concentrado. O sistema em que as vacas receberam concentrado apresentou média de produção compatível com sistemas convencionais. Os autores estimaram que em rebanhos convencionais, 20% do leite produzido é proveniente do concentrado, enquanto em rebanhos orgânicos, essa percentagem é de 15%.

A maior parte dos nutrientes para produção de pastagens em fazendas orgânicas precisa vir da fixação de N, oriundo da aplicação de chorume e esterco produzidos pelo próprio sistema, uma vez que os padrões orgânicos proíbem o uso de fertilizantes de nitrogênio sintético (CR, 1999). Uma estratégia utilizada para aumentar a entrada total de N no pasto e a incorporação de matéria orgânica é o uso de leguminosas, como o trevo vermelho e branco, em consórcio com gramíneas (JORGENSEN; LEDGARD, 1997). Kristensen et al. (1995) observaram que em sistemas orgânicos da Dinamarca, a proporção de trevo no pasto determinou a entrada total de N, que por sua vez pode influenciar o conteúdo de proteína bruta e rendimentos do pasto.

As exigências de proteína para vacas leiteiras orgânicas são essencialmente supridos pelo uso de leguminosas nas pastagens (ROSATI; AUMAITRE, 2004). Davies (1996) afirmou que o alto teor de proteína e baixo teor de fibra do trevo, melhora a digestão dos alimentos e contribui para taxas mais rápidas de decomposição de partículas e uso mais eficiente de nutrientes. Esses fatores podem contribuir para o aumento da ingestão voluntária de ração, que contribuem para o aumento da produção de leite. No entanto, a forte relação do alto consumo do trevo com problemas como timpanismo e dificuldades com ensilagem. Como as pastagens fornecem uma das fontes de alimentação mais baratas disponíveis em uma fazenda de leite orgânico, reduzir potencialmente o uso de concentrados de origem orgânica, produzir leite a partir da forragem pode ser uma forma de aumentar a eficiência da produção do leite orgânico. Para que esta seja opção viável, devem-se assegurar a máxima utilização e qualidade das pastagens, garantido pelo manejo adequado do pasto e de dejetos e a manutenção da leguminosa consorciada (NICHOLAS et al., 2004).

O maior uso de pasto para rebanhos orgânicos contribuiu para o bem-estar dos animais, porém o uso limitado de drogas em sistemas orgânicos pode comprometer a saúde das vacas (ROSATI; AUMAITRE, 2004). A composição da pastagem é influenciada pela frequência, intensidade, estação de pastejo, e o manejo de dejetos. A escolha da espécie deve ser baseada na condição e vida útil esperada do período, condições ambientais, compatibilidade entre espécies e o custo da semente. Forragens de enraizamento profundo são frequentemente incluídas, com intuito de melhorar a estrutura do solo. Além disso, a forma de crescimento dessas gramíneas, permite ampliar a área em que planta explora os nutrientes do solo, de forma a torna-los mais disponíveis para os animais em pastejo (YOUNIE, 2001).

Para garantir a eficiência geral da pecuária leiteira orgânica é preciso produzir leite com um mínimo de insumos externos possíveis (NICHOLAS et al., 2004). Para isso, é preciso maximizar a produção de leite baseada em forragem, e minimizar a compra de concentrados e grãos (IFOAM, 2005). Em sistemas orgânicos, é importante a utilização de raças adequadas para regimes de baixo concentrado. Kristensen e Kristensen (1998), observaram que o alto teor de volumoso na dieta de vacas em sistemas orgânicos reduziu o consumo voluntário de MS nos estágios iniciais da lactação, mas garantiu consistência de ingestão ao longo de toda lactação, sugerindo que esse seja um dos fatores que contribuem para a persistência da lactação em rebanhos orgânicos. Nesse sistema, a qualidade da forragem é importante, já que a demanda de nutrientes das vacas deve ser majoritariamente atendida pela ingestão de volumoso. Knaus et al. (2001) realizaram estudo sobre o balanço de energia e nitrogênio em rebanhos leiteiros orgânicos, dentro das limitações dos regulamentos da União Europeia (UE). Devido às limitações no uso de concentrado, somente a suplementação com silagem de alta qualidade permitiu a obtenção de desempenhos de lactação superiores a 7000 kg de leite de vaca/ano.

REFERÊNCIAS

AFRC. **AGRICULTURAL AND FOOD RESEARCH COUNCIL. Energy and requirements of ruminants.** Wallingford, UK.: CAB International, 1993.

ALVAREZ, A. et al. Does intensification improve the economic efficiency of dairy farms? **Journal of Dairy Science**, v. 91, n. 9, p. 3693–3698, 2008.

ANDRÉS, P. et al. Desempeño productivo y reproductivo de vacas F1 Gyr x Holstein en clima cálido colombiano Productive and reproductive performance of F1 Gyr x Holstein cows in Colombian warm climate. v. 6, n. 1, p. 17–23, 2012.

ARC. **Agricultural Research Council. The nutrient Requirements of ruminant livestock**. 1980.

ARMSBY, H. P. **The nutrition of farm animalsNature**, 1912.

BASSET-MENS, C.; LEDGARD, S.; BOYES, M. Eco-efficiency of intensification scenarios for milk production in New Zealand. **Ecological Economics**, v. 68, n. 6, p. 1615–1625, 2009.

BAUMAN, D. E. et al. **Sources of variation and prospects for improvement of productive efficiency in the dairy cow: a review.Journal of animal science**Oxford Academic, 1985.

BAVA, L. et al. How can farming intensification affect the environmental impact of milk production? **Journal of Dairy Science**, v. 97, n. 7, p. 4579–4593, 2014.

BELL, M. J. et al. The effect of improving cow productivity, fertility, and longevity on the global warming potential of dairy systems. **Journal of Dairy Science**, v. 94, n. 7, p. 3662–3678, 2011.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento – MAPA. Instrução normativa Nº 007, de 17 de maio de 1999. Brasilia, DF, 1995.

CABRERA, V. E.; KALANTARI, A. S. Economics of production efficiency: Nutritional grouping of the lactating cow. **Journal of Dairy Science**, v. 99, n. 1, p. 825–841, 2016.

CABRERA, V. E.; SOLÍS, D.; DEL CORRAL, J. Determinants of technical efficiency among dairy farms in Wisconsin. **Journal of Dairy Science**, v. 93, n. 1, p. 387–393, 2010.

CANAZA-CAYO, A. W. et al. Estrutura populacional da raça Girolando. **Ciencia Rural**, v. 44, n. 11, p. 2072–2077, 2014.

CAPPER, J. L.; CADY, R. A.; BAUMAN, D. E. The environmental impact of dairy production: 1944 compared with 2007. **Journal of animal science**, v. 87, n. 6, p. 2160–2167, 2009.

CARTER, M. R. Resource allocation and use under collective rights and labour management in Peruvian coastal agriculture. **Economic Journal**, v. 94, n. 376, p. 826–846, 1984.

CARVALHO, P. H. D. A. et al. Energy metabolism and partition of lactating Zebu and crossbred Zebu cows in different planes of nutrition. **PLoS ONE**, v. 13, n. 8, p. 1–10, 2019.

CHAOKAUR, A. et al. Effects of feeding level on methane emissions and energy utilization of Brahman cattle in the tropics. **Agriculture, Ecosystems and Environment**, v. 199, p. 225–230, 2015.

CR. Council Regulation (EC). 1804/1999 of 19 July 1999 supplementing Regulation (EEC). 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production. **Official journal L**, v. 222, n. 24, p. 08, 1999.

CSIRO. **Nutrient Requirements of Domesticated Ruminants**. CSIRO Publishing, 2007.

DIJKSTRA, J. et al. Review: Rumen sensors: Data and interpretation for key rumen metabolic processes. **Animal**, v. 14, n. S1, p. S176–S186, 2020.

FAO. **Feed requirements for dairy cows**, 2015. Disponível em: <http://www.fao.org/ag/againfo/themes/documents/PUB6/P620.htm>. Acesso em: 1 de abril. 2021

FERRELL, C.; OLTJEN, J. W. Net Energy Systems for Beef Cattle-Cattles, Application, and Future Models Cattles, Application, and Future Models. **J. Anim. Sci**, v. 86, p. 2779–2794, 2008.

FLATT, W. P.; COPPOCK, C. E.; MOORE, L. A. **Energy balance studies with dry, non-pregnant dairy cows consuming pelleted forages**. Proceedings of Energy Metabolism of Farm Animals, 3rd. **Anais**...1965

FONTES, C. A. DE A. et al. Uso do abate comparativo na determinação da exigência de energia de mantença de gado de corte pastejando capim-elefante: descrição da metodologia e dos resultados. **Revista Brasileira de Zootecnia**, v. 34, n. 5, p. 1721–1729, 2005.

FOX, D. G. et al. **Application of the Cornell Net Carbohydrate and Protein model for cattle consuming forages.Journal of animal science**, 1995. Disponível em: <https://academic.oup.com/jas/article-abstract/73/1/267/4564216>. Acesso em: 26 março. 2021

GARRETT, W. N.; MEYER, J. H.; LOFGREEN, G. P. The Comparative Energy Requirements of Sheep and Cattle for Maintenance and Gain1. **Journal of Animal Science**, v. 18, n. 2, p. 528–547, 1959.

HAMADANI, H.; KHAN, A. A. **Organic Dairy Farming-An OverviewJ of Livestock Sci**. v. 6, 2015.

HOF, G. et al. Milk Urea Nitrogen as a Tool to Monitor the Protein Nutrition of Dairy Cows. **Journal of Dairy Science**, v. 80, n. 12, p. 3333–3340, 1997.

IFOAM. **Basic standards for organic production and processing.**, 2005. Disponível em:<https://scholar.google.com.br/scholar?hl=pt-BR&as_sdt=0%2C5&q=Basic+Standards+for+Organic+Production+and+Processing.&btnG=>. Acesso em: 21 de abril. 2021

JAVALGI, R. R. G.; GROSSMAN, D. A. Aspirations and entrepreneurial motivations of middle-class consumers in emerging markets: The case of India. **International Business Review**, v. 25, n. 3, p. 657–667, 2016.

JIANG, N.; SHARP, B. Technical efficiency and technological gap of New Zealand dairy farms: a stochastic meta-frontier model. **Journal of Productivity Analysis**, v. 44, n. 1, p. 39–49, 27, 2015.

JØRGENSEN, F. V.; LEDGARD, S. F. Contribution from stolons and roots to estimates of the total amount of N2 fixed by white clover (Trifolium repens L.). **Annals of Botany**, v. 80, n. 5, p. 641–648, 1997.

KNAUS, W. F.; STEINWIDDER, A.; ZOLLITSCH, W. **Energy and protein balance in organic dairy cow nutrition - model calculations based on EU regulations**. 4th NAHWOA Workshop. Network for Animal Health and Welfare in Organic Agriculture. **Anais**...2001

KOMPAS, T.; CHE, T. N. Technology choice and efficiency on Australian dairy farms*. **The Australian Journal of Agricultural and Resource Economics**, v. 50, n. 1, p. 65–83, 2006.

KRISTENSEN, E. S.; HØGH-JENSEN, H.; KRISTENSEN, I. S. A simple model for estimation of Atmospherically-Derived nitrogen in Grass-Clover systems. **Biological Agriculture and Horticulture**, v. 12, n. 3, p. 263–276, 1995.

KRISTENSEN, T.; KRISTENSEN, E. S. Analysis and simulation modelling of the production in Danish organic and conventional dairy herds. **Livestock Production**

Science, v. 54, n. 1, p. 55–65, 1998.

LLANOS, E.; ASTIGARRAGA, L.; PICASSO, V. Energy and economic efficiency in grazing dairy systems under alternative intensification strategies. **European Journal of Agronomy**, v. 92, n. August 2016, p. 133–140, 2018.

LUIK-LINDSAAR, H. et al. How do herd's genetic level and milk quality affect performance of dairy farms? **Czech Journal of Animal Science**, v. 63, n. 10, p. 379–388, 2018.

LUIK-LINDSAAR, H.; PÕLDARU, R.; ROOTS, J. Estonian dairy farms' technical efficiency and factors predicting it. **Agronomy Research**, v. 17, n. 2, p. 593–607, 2019.

MA, W.; BICKNELL, K.; RENWICK, A. Feed use intensification and technical efficiency of dairy farms in New Zealand. **Australian Journal of Agricultural and Resource Economics**, v. 63, n. 1, p. 20–38, 2019.

MADALENA, F. E. et al. Evaluation of Strategies for Crossbreeding of Dairy Cattle in Brazil. **Journal of Dairy Science**, v. 73, n. 7, p. 1887–1901, 1990.

MARETH, T. et al. Analysing the determinants of technical efficiency of dairy farms in Brazil. **International Journal of Productivity and Performance Management**, v. 68, n. 2, p. 464–481, 2019.

MEIGS, E. B. The Energy Requirements of Dairy Cows: II. Is Net Energy or Metabolizable Energy the More Useful Index for Practical Purposes? **Journal of Dairy Science**, v. 8, n. 6, p. 523–536, 1925.

MELLADO, M. et al. Lactation performance of Holstein and Holstein x Gyr cattle under intensive condition in a subtropical environment. **Tropical and Subtropical Agroecosystems**, v. 14, n. 3, p. 927–931, 2011.

MIRANDA-DE LA LAMA, G. C. et al. Consumer Attitudes Toward Animal Welfare-Friendly Products and Willingness to Pay: Exploration of Mexican Market Segments.

Journal of Applied Animal Welfare Science, v. 22, n. 1, p. 13–25, 2019.

MIRANDA, J. E. C.; FREITAS, A. F. **Raças e tipos de cruzamentos para produção de leiteEmbrapa Gado de Leite, Circular Técnica**, 2009. Disponível em: <https://www.infoteca.cnptia.embrapa.br/bitstream/doc/737102/1/CT98Racasetiposdecruzamentos.pdf>. Acesso em: 27 de abril. 2021

MOE, P. W.; FLATT, W. P.; TYRELL, H. F. Net Energy Value of Feeds for Lactation. **Journal of Dairy Science**, v. 55, n. 7, p. 945–958, 1972.

MONTOYA, E. S.; CHARÁ, J. D.; BARAHONA-ROSALES, R. The nutritional balance of early lactation dairy cows grazing in intensive silvopastoral systems. **Ciência Animal Brasileira**, v. 18, n. 0, p. 1–12, 2017.

MOSIMANN, E.; SUTER, D. Autonomie en protéines et environnement: le comportement helvétique. **Actes des Journées AFP, Fourrages, Protéines, Environnement.**, p. 107–115, 2003.

MU, W. et al. Benchmarking nutrient use efficiency of dairy farms: The effect of epistemic uncertainty. **Agricultural Systems**, v. 156, p. 25–33, 2017.

MULDER, M.; ZOMER, S. Dutch Consumers' Willingness to Pay for Broiler Welfare. **Journal of Applied Animal Welfare Science**, v. 20, n. 2, p. 137–154, 2017.

NICHOLAS, P. K. et al. Organic dairy production: A review. **Biological Agriculture and Horticulture**, v. 22, n. 3, p. 217–249, 2004.

NRC. **Effect of Environment on Nutrient Requirements of Domestic Animals**. 1981.

NRC. **Nutrient Requirements of Beef Cattle**. 2000.

NRC. **National Research Council. Nutrient Requirements of Dairy Cattle: Seventh Revised Edition**. 7. ed. Washington, DC: The National Academies Press, 2001.

OFFERMANN, F.; NIEBERG, H. **Economic Performance of Organic Farms in Europe**. v. 5, 2000.

OLDHAM, J. D. Protein-Energy Interrelationships in Dairy Cows. **Journal of Dairy Science**, v. 67, n. 5, p. 1090–1114, 1984.

OLIVEIRA, A. S. Meta-analysis of feeding trials to estimate energy requirements of dairy cows under tropical condition. **Animal Feed Science and Technology**, v. 210, p. 94–103, 2015.

OMSCO. **Global Organic Dairy - Market Report 2019**. Disponível em: <https://www.omsco.co.uk/wp-content/uploads/2019/01/The-Global-Organic-Dairy-Market-Report-2019-APP.pdf>. Acesso em: 17 de abril. 2021.

ONDERSTEIJN, C. J. M.; GIESEN, G. W. J.; HUIRNE, R. B. M. Identification of farmer characteristics and farm strategies explaining changes in environmental management and environmental and economic performance of dairy farms. **Agricultural Systems**, v. 78, n. 1, p. 31–55, 2003.

PERISSINOTTO, M. وآخ. Influência das condições ambientais na produção de leite da vacaria da Mitra. **Revista de Ciências Agrárias**, v. 30, n. 1, p. 143–149, 2018.

PIERCE, JI.; TILTH, O. **Introduction to Organic Dairy Farming | eOrganic**. Disponível em: <https://eorganic.org/node/224>. Acesso em: 19 de abril. 2021.

RAMSBOTTOM, G. et al. Relationship between dairy cow genetic merit and profit on commercial spring calving dairy farms. **Animal**, v. 6, n. 7, p. 1031–1039, 2012.

RANGANATHAN, J. et al. Shifting diets for a sustainable food future. **Creating a Sustainable Food Future**, v. 11, n. April, p. 90, 2016.

ROBBINS, J. A. et al. Awareness of ag-gag laws erodes trust in farmers and increases support for animal welfare regulations. **Food Policy**, v. 61, p. 121–125, 2016.

RODRIGUES, M. T.; VIEIRA, R. A. M. Metodologias aplicadas ao fracionamento de alimentos: **Nutrição de ruminantes**. p. 29–56, 2011.

ROIBAS, D.; ALVAREZ, A. Impact of genetic progress on the profits of dairy farmers.

Journal of Dairy Science, v. 93, n. 9, p. 4366–4373, 2010.

ROIBAS, D.; ALVAREZ, A. The contribution of genetics to milk composition: evidence from Spain. **Agricultural Economics**, v. 43, n. 2, p. 133–141, 2012.

ROSATI, A.; AUMAITRE, A. **Organic dairy farming in Europe**. Livestock Production Science. **Anais**...Elsevier, 2004.

SILVA, R. R. **Respirometria e determinação das exigências de energia e produção de metano de fêmeas bovinas leiteiras de diferentes genótipos**. Tese. Universidade Federal de Minas Gerais (UFMG), 2011.

SORIANO, F. D.; POLAN, C. E.; MILLER, C. N. Supplementing pasture to lactating Holsteins fed a total mixed ration diet. **Journal of Dairy Science**, v. 84, n. 11, p. 2460–2468, 2001.

SPANGHERO, M.; KOWALSKI, Z. M. Critical analysis of N balance experiments with lactating cows. **Livestock Production Science**, v. 52, n. 2, p. 113–122, 1997.

TYRRELL, H. F.; REID, J. T. Prediction of the Energy Value of Cow's Milk. **Journal of Dairy Science**, v. 48, n. 9, p. 1215–1223, 1965.

USDA. **Código Eletrônico de Regulamentações Federais (eCFR)**. Disponível em: <https://efctppikdzc66rc6pz232qtltq-adv7ofecxzh2qqi-www-ecfr-gov.translate.goog/cgi-bin/text-idx?SID=a6a0935ddf00e166695f4c2138bd58d8&mc=true&node=pt7.3.205&rgn=div5>. Acesso em: 19 de abril. 2021.

VALADARES FILHO, S. DE C. et al. **Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE**. 2016.

VANDEHAAR, M. J. Efficiency of Nutrient Use and Relationship to Profitability on Dairy Farms. **Journal of Dairy Science**, v. 81, n. 1, p. 272–282, 1998.

VANDEHAAR, M. J.; ST-PIERRE, N. Major advances in nutrition: Relevance to the sustainability of the dairy industry. **Journal of Dairy Science**, v. 89, n. 4, p. 1280–1291, 2006.

VINICIUS, M. et al. Programa de Melhoramento Genético da Raça Girolando Teste de Progênie: Sumário de Touros 2010 Editores Técnicos. **infoteca.cnptia.embrapa.br**, 2010.

VON KEYSERLINGK, M. A. G. et al. Invited review: Sustainability of the US dairy industry. **Journal of Dairy Science**, v. 96, n. 9, p. 5405–5425, 2013.

WHITE, P. J. C. et al. Routes to achieving sustainable intensification in simulated dairy farms: The importance of production efficiency and complimentary land uses. **Journal of Applied Ecology**, v. 56, n. 5, p. 1128–1139, 2019.

WRIGHT, T. C. et al. Effects of Rumen-Undegradable Protein and Feed Intake on Nitrogen Balance and Milk Protein Production in Dairy Cows. **Journal of Dairy Science**, v. 81, n. 3, p. 784–793, 1998.

YOUNIE, D. Organic grassland: the foundation stone of organic livestock farming. **Organic livestock farming. Papers presented at conferences held at the Heriot-Watt University, Edinburgh and at the University of Reading 9-10 February 2001**, p. 75–102, 2001.

ZHLLIMA, E.; IMAMI, D.; CANAVARI, M. Consumer perceptions of food safety risk: Evidence from a segmentation study in Albania. **Journal of Integrative Agriculture**, v. 14, n. 6, p. 1142–1152, 2015.

Energy requirements for Holstein-Gyr F1 dairy cows

J. P. Sacramento[1], A. E. Leao[2], A. P. Fonseca[2], R. R. Silvi[6], J. P. P. Rodrigues[5], S. G. Coelho[2], T. R. Tomich[3], F. S. Machado[3], A. S. Oliveira[4], M. M. Campos[3], L. G. R. Pereira[3]

[1]Department of Bioengineering, Federal University of São João del Rei, 36307-352, São João dei Rei, Minas Gerias, Brazil
[2]Department of Animal Science, School of Veterinary Medicine, Federal University of Minas Gerais, 30161-970, Belo Horizonte, Minas Gerais, Brazil
[3]Brazilian Agricultural Research Corporation – Embrapa Dairy Cattle, 36038-330, Juiz de Fora, Minas Gerais, Brazil
[4]Instituto de Ciências Agrarias e Ambientais, Federal University of Mato Grosso, 78557-267, Sinop, Mato Grosso, Brazil
[5]Department of Animal Science, Federal University of Sul e Sudoeste do Pará, 31st Street, Block 7, 68557-335, Xinguara, Pará, Brazil
[6]Santa Cruz State University, Ilheus, Bahia, Brazil, 45662-900

ABSTRACT

The objective was to evaluate the requirement of maintenance and net and metabolizable energy of crossbred Holstein x Gyr cows throughout a whole lactation. Likewise, energetic efficiency parameters as the efficiency of utilization of the ME intake for milk production (k_L), tissue gain (k_G), and utilizing body stores for milk production (k_T). Twenty-nine Holstein x Gyr crossbred cows with an average initial weight of 563.40 ± 40.08 and 2.45 ± 0.09 years were used throughout a whole lactation. The animals were first-calving, already in an adult weight, and during the study, the cows were non-pregnant to decrease any confounding factor. Cows were fed immediately after milking twice a day in amounts to allow *ad libitum* intake (allowing for 5 to 10% orts). The intake and milk production were determined daily. Apparent digestibility assay followed by a calorimeter measurements was performed at the beginning and the end of each lactation period, approximately 45 to 45 days in milk (DIM). Subsequently completed the lactation, the dry cows were fed the diet used at the late lactation stage limited at 1.1% of BW of DMI to prevent change in body weight (maintenance level). After 21 days of diet adaptation, an apparent digestibility assay followed by calorimeter measurements was performed. Fasting measurement was performed later than the maintenance period where the cows were housed individually inside the chambers for 72 hours without access to food. Data were analyzed using the MIXED procedure of SAS (SAS version 9.4; SAS Inst. Inc., Cary, NC) according to a completely randomized design. Crossbred Holstein × Gyr dairy cow showed a ME_M of 0.588 MJ/$BW^{0.75}$ and NE_M of 0.395 MJ/$BW^{0.75}$. The efficiency of dietary ME utilizing for producing milk, gain, and the efficiency of utilizing body stores for milk production were 0.67, 0.77, and 0.81, respectively. Crossbred

Holstein × Gyr dry and non-pregnant cows showed a ME_M of 0.434 MJ/$BW^{0.75}$ and NE_M of 0.351 MJ/$BW^{0.75}$ for maintenance and a ME_M of 0.396 MJ/$BW^{0.75}$ and NE_M of 0.345 MJ/$BW^{0.75}$ for fasting metabolism. The efficiency of utilizing dietary ME for maintenance (k_M) was 0.80. The results confirmed that F1 crossbreed Holstein × Gyr dairy cows have differences in the requirement of energy throughout the lactation stages, however, comparing with data available in the literature, there are no differences between crossbred animals raised under tropical and purebred cows.

Key words: crossbreed, maintenance, efficiency

INTRODUCTION

The energy requirements of dairy cattle have two important key parameters, the metabolizable energy (ME) requirement for maintenance (ME_M) and the efficiency of utilization of the ME intake for milk production (k_L). In the current energy systems for dairy cattle, the net energy (NE) or ME requirements for maintenance and milk production, as a k_L was derived from indirect calorimetric trials mainly using Holstein or Holstein-Friesian cows under temperate climate conditions (AFRC, 1993; NRC, 2001; CSIRO, 2007; INRA, 2018).

Accurate estimates of energy requirements for dairy cattle allow nutritionists to formulate efficient diets, achieving the requirements for maintenance and production, and may decrease nutrient loss, such as nitrogen, which can reduce livestock environmental impacts. The Nutrient Requirements of Dairy Cattle (NRC, 2001) is the most used system to balance the diets of dairy cows. However, the equations to estimate NE or ME requirements do not consider tropical crossbreed, low production, and tropical climate conditions. Some studies have investigated energy requirements across crossbreed animals under tropical conditions (Oliveira, 2015; Oss et al., 2016; Sguizzato et al., 2020) and the result are inconclusive about the necessity for an adjustment in the energy requirement values.

Approximately 70% of the Brazilian dairy herd is composed of crossbred Holstein × Gyr cows. The F1crossbreed Holstein × Gyr (F1 H × G) has great milk production in tropical conditions as a result of heterosis, which incorporates the best characteristics of each breed; milk production from Holstein and adaptability to tropical climate from Gyr (Carvalho et al., 2019). Additionally, Oliveira (2015) using 60 studies, with 231 treatment and 752 cows, that was published in Brazilian journals in a meta-analysis approach, reported that crossbred cows (*Bos taurus* × *Bos indicus*) have lower maintenance requirements and lower efficiency for milk production when compared to *Bos taurus* cows. Nevertheless, any study has been published with crossbreed Holstein × Gyr cows during a whole lactation evaluating energy requirements.

Therefore, this study was conducted with the main objective of evaluating the requirement of maintenance and net and metabolizable energy throughout a whole lactation. Likewise, energetic efficiency parameters as the efficiency of utilization of the ME intake for milk production (k_L), tissue gain (k_G), and utilizing body stores for milk production (k_T). We expected that achieving this objective would provide the dairy industry useful information to calculate the requirement of net and metabolizable energy more efficiently. We hypothesized that (*i*) there are differences in the requirement of energy throughout the lactation, and (*ii*) the Holstein × Gyr dairy cattle crossbreed raised under tropical climate conditions have different requirements of energy than purebred dairy cattle raised under temperate climate conditions.

MATERIALS AND METHODS

The study was performed at the Bioenergetics Laboratory of the Brazilian Agricultural Research Corporation (Embrapa), at the Multi-use Livestock Complex of Bioefficiency and Sustainability at Embrapa Dairy Cattle, in Coronel Pacheco, Minas Gerais, Brazil, between April 10, 2017, and May 09, 2018. All animal care and handling procedures were approved by the Embrapa Dairy Cattle Animal Care and Use Committee (Juiz de Fora, Minas Gerais, Brazil; Protocol CEUA-EGL 9264220317).

Animals and Management

Thirty-two Holstein x Gyr crossbred cows with an average initial weight of 563.40 ± 40.08 and 2.45 ± 0.09 years were used throughout a whole lactation. The animals were first-calving, already in an adult weight, that is, no longer presenting nutritional requirements for growth. The Cows calved between April and June 2017, entered the experiment during the first week after calving, and were housed in a free-stall system according to the date of calving in 4 groups of 8 cows at the beginning of the experiment. During the study, the cows were non-pregnant to decrease the confounding factor. Three cows had to be removed from the experiment due to low milk production (n=2), and subclinical mastitis (n=1). The diet was the same for all animals, changing every lactation period (100 days in milk; DIM), in a total of three different diets throughout the experiment. Every 45 DIM a total tract nutrient digestibility assay was performed. Total feces were collected for 5 days and the urinary volume was estimated by spot collection according to Rodrigues et al. (2017). After the digestibility assay, the cows were confined in the respirometry chamber for 2 periods of 22 hours.

Diet, Feed Management and Intake Measurements

The diet (Table 1) was formulated according to NRC, (2001) to supply the nutritional requirements for maintenance, lactation, and average daily gain of 100 g/day for the early lactation of a 570 kg cow producing 25 kg/d of milk (3.8% fat) and consuming 18.0 kg of DM/d, and no average daily gain for middle and late lactation of a cow with 615 and 660 kg, producing 20 and 15 kg/d of milk and consuming 15 kg of DM/d, respectively. The diet base was based on 61:39 corn silage and concentrate a ratio for early lactation, 74:26 for middle lactation, and 81:19 for late lactation. The concentrate was composed of ground corn, soybean meal, and mineral supplement (table 1).

Table 1. Ingredients and chemical composition of experimental diet fed to F1 crossbreed Holstein × Gyr dairy cows throughout a first lactation.

	Early	**Middle**	**Late**
Ingredients (g/kg of DM)			
Corn Silage	593.0	726.3	809.2
Hay	23.0	--	--
Soybean meal	198.8	197.2	173.6
Ground corn	164.0	60.0	--
Mineral mix[1]	18.0	16.5	17.2
Urea	2.4	--	--
Chemical composition (g/kg of DM)			
DM	536.4	473.2	439.0
OM	928.0	923.7	916.3
CP	169.7	162.5	147.3
NDF	323.7	334.2	351.7
EE	33.5	33.0	31.3
Ash	71.9	76.3	80.0
NFDcp	300.3	313.5	331.6
Non-fibrous carbohydrates	400.7	394.1	383.2
GE Mcal of DM	4.4	4.4	4.4
DE Mcal of DM	3.0	3.1	3.0
ME Mcal of DM	2.6	2.7	2.6
q[2]	0.59	0.61	0.58

[1] Mineral Mix: 150g/kg of Ca, 60g/kg of P, 15g/kg of Mg, 1,300mg/kg of K, 57g/kg of Na, 30mg/kg Se, 100mg/kg of Cu, 1,300mg/kg of Mn, 3,000mg/kg of Zn, 1,500mg/kg of Fe, 100mg/kg of I, 220,000 IU/kg of vitamin A, 60,000 IU/kg of vitamin D, 1,000 IU/kg of vitamin E

[2] q = metabolizability (MJ of ME/MJ of gross energy)

Cows were housed in free-stall fitted with electronic feed bins and headgates (AF-1000 Master Gate, Intergado Ltd., Contagem, MG, Brasil), as well as electronic water bins (WD-1000, Intergado Ltd., Contagem, Minas Gerais, Brazil). Feed and water bins were attached to radio frequency identification (RFID) antennas that monitored individual feed and water intake, as well as feeding and drinking behavior (Chizzotti et al., 2015). The visit duration and the number of visits to feed and water bins, and fresh feed and water intake data were exported to Intergado® web software. Cows were fitted with an ear tag containing a unique passive transponder (FDX – ISO 11784/11785; Allflex, Joinville, SC, Brazil) in the right ear, and each feed bin was randomly assigned to a single cow. Each respiratory chamber was also fitted with the Intergado® feed technology.

Cows were fed immediately after milking twice a day at 8 a.m. and 4 p.m. in amounts to allow *ad libitum* intake (allowing for 5 to 10% orts). The intake was determined daily by the difference in weight between the feed offered and orts collected before the morning milking. Samples of 300 to 500 g of corn silage and orts were taken three times a week and pooled into 2 week periods for analysis of the chemical composition.Tthe concentrate was sampled once a week and pooled into 4 week periods for analysis. The samples were packed in plastic bags and frozen at -20º C until processing.

Milk and Body Weight sample procedure

Milking was performed twice a day (6:30 a.m. and 3:30 p.m.) in a fishbone milking parlor (2x4) equipped MPC 580/680 control unit, and automatic cluster removal system (DeLaval, Tumba, Sweden). Milk yield data were obtained by Alpro software (DeLaval, Tumba, Sweden). Milk samples were collected twice a week in the morning and afternoon (totaling four samples per week) for analysis of composition (protein, fat, lactose, dry extract, defatted dry extract content, and urea). The samples were stored at 5º C with the bronopol preservative and sent to the Milk Quality Laboratory at Embrapa Dairy Cattle within 24 hours for analysis in a Bentley Fourier-Transform Infrared Spectrometer System (Bentley FTS, Bentley Instruments, Chaska, MN) following the recommendations of the International Dairy Federation (IDF, 2000).

Bodyweight was measured every time that the cows visit the water bins (WD-1000, Intergado Ltd., Contagem, Minas Gerais, Brazil), obtaining more than one daily weighing per animal. Each water bin had a body weighing scale and the daily weight was considered as the average of all weighing performed during the day. Data resulting from errors in weighing were excluded, due to the presence of more than one animal on the scale of animal supported only with the front limbs on the scale, at the time of registration.

Digestibility and Calorimeter Measurements

An apparent digestibility assay was performed at the beginning and the end of each lactation period, approximately 45 to 45 DIM, the animals were divided into a group of 8 and transferred to a tie-stall system with individual feeders and water troughs. Total feces were collected for 5 consecutive days from all animals. At the end of each collection day, the feces of each animal were weighed. The feces were sampled after homogenization. The samples were then weighed, dried in a forced-ventilation oven (55°C) for 72 h, and ground through a 1-mm screen (Wiley mill; A. H. Thomas, Philadelphia, PA). One composite sample per animal, based on DM weight, for every collection day, was prepared for chemical analysis. The same chemical analyses that were performed for the experimental diet were performed for feces.

Spot urine samples (200 mL) were collected through stimulation of the pudendal nerve by massaging the area below the vulva or during voluntary urination at 8-time points with 3-h intervals split up in 3 consecutive days according to Rodrigues et al. (2017). After sampling, 2 aliquots were made: the first 10 mL was acidified with 40 ml of sulfuric acid (H_2SO_4, 1 mL/L) and the second was stored as obtained. Both urine samples were kept frozen at -20ºC.

During the sampling period, the cows were milked in their stalls in the morning and afternoon (at 7:30 a.m. and 2:30 p.m.) with a a portable milking machine (Weizur do Brazil Ltda, Sorocaba, Brazil). Individual milk yield was determined by weighing, and a sample from each animal was collected at each milking for analysis of milk composition.

Samples from the diet provided (concentrate and corn silage) and orts were taken during the five days of the assay to evaluate DM and nutrient intake. To determine the intake of DM (DMI) and other nutrients, the following equation was used: DMI% = (kg DM ingested x % nutrients) - (kgDM orts x % nutrients)*100.

Four open-circuit respiratory chambers were used according to the specifications and procedures described by Machado et al. (2016). The cows were housed individually inside the chambers after the first milking of the day. And the measurement was performed for 2 consecutive periods of 20-22 h and the data obtained were extrapolated to 24 h. The chambers were maintained under thermoneutral conditions for crossbred Gyr x Holstein cows (23 ± 1° C and relative air humidity of 65 ± 5%).

The animals were weighed before and after entering the chamber. The CH_4 emission was calculated according to Machado et al. (2016). To determine the energy partitioning, the gross energy of feed, orts, feces, and urine samples collected during the digestibility assay were previously determined in an adiabatic calorimeter (IKA - C5000, IKA Works, Staufen, Germany). From the quantification of gross energy intake (GEI), obtained by the difference between the dietary energy and that found in orts, the other energy fractions were calculated.

Sample processing and laboratory analysis

Samples of feed, orts, and feces were oven-dried at 55°C and milled through 1-mm screens for further analyses. The analyzes were performed to determine: DM by oven-drying at 105º C (AOAC, 1990; method 934.01), ashes (AOAC, 1990; method 942.05), CP by the Kjeldahl method (AOAC, 1990; method 984.13), GE by combustion in an adiabatic calorimeter (IKA - C5000, IKA Works, Staufen, Germany), NDF by the sequential method of Van Soest et al. (1991), adapted to the ANKOM220 device, FiberAnalyzer (Ankom Technology, Fairport, NY), with the addition of 500 μL/g DM of thermostable amylase without sodium sulfite and corrected for ashes and nitrogen EE (AOAC, 1990; method 920.39). For calculations of non-fibrous carbohydrates (NFC) was used the equation: NFC = 100 - (NDF% + CP% + EE% + ASH%) suggested by Mertens, (1997).

Samples of urine were analyzed for concentrations of creatinine (assay kit no. 500701, Cayman Chemical Co., Ann Arbor, MI) using a chromate microplate reader set at a wavelength of 492 nm (Awareness Technology Inc., Palm City, FL), allantoin (Chen et al., 1992), and uric acid (assay kit no. 1045–225; Stanbio Laboratory, Boerne, TX). Allantoin and uric acid were determined at wavelengths of 522, and 520 nm, respectively, in a UV/visible spectrophotometer (Beckman Coulter Inc., Pasadena, CA). Daily urine volume was estimated from urinary creatinine concentration assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urinary excretion of allantoin, uric acid, and total purine derivatives (**PD** = allantoin plus uric acid) were calculated by multiplying the concentration of each of these metabolites by the urinary volume. Gross energy was analyzed by combustion in an adiabatic calorimeter (IKA - C5000, IKA Works, Staufen, Germany).

Maintenance and fasting procedure

Subsequently completed the lactation the dry cows were fed the diet used at the late lactation stage limited at 1.1% of BW of DMI to prevent change in body weight (maintenance level). After 21 days of diet adaptation, the cows were transferred to a tie-stall system with individual feeders and water troughs to performed an apparent digestibility assay followed by calorimeter measurements as described above. Fasting measurement was performed later than the maintenance period where the cows were housed individually inside the chambers for 72 hours without access to food. The chamber was changed every 24h and maintained under thermoneutral conditions . The length of fasting period was sufficient to reduce respiratory quotient (RQ) to 0.74 and methane production to a negligible amount (<0.22/kg of $BW^{0.75}$ daily). All the cows during the maintenance and fasting procedure were dry and non-pregnant.

Data Analyses

The digestible energy (DE) was obtained by the difference between the gross energy (GE) consumed and the energy lost in feces. Subsequently, the metabolizable energy (ME) was determined by discounting urine and CH_4 energy losses (NRC, 2000). For quantification of energy lost as CH_4, the loss of 9.45 kcal/L of CH_4 produced (Brouwer, 1965) was adopted during the respiratory. The metabolizability (q) of the diet was calculated by the relation between the metabolizable energy and the gross energy intake, according to AFRC, (1993).

The net energy for lactation (NE_L, Mcal/day), defined as the energy contained in the milk produced, was calculated based on the equation proposed by NRC, (2001), which considers NE_L as the sum of the heats of combustion of individual milk components (fat, protein, and lactose), as follows:

NE_L = [(((0,0929 x morning fat content) + (0,0547 x morning protein content) + (0,0395 x morning lactose content)) x monring milk yield))) + (((0,0929 x afternoon fat content) + (0,0547 x afternoon protein content) + (0,0395 x afternoon lactose content)) x afternoon milk yield)))]

Heat production (HP, Kcal/day) was calculated according to Brouwer (1965):

$$HP\ (Kcal/day) = (3.866 \times VO_2) + (1.200 \times VCO_2) - (0.518 \times VCH_4) - (1.431 \times UN)$$

Where: VO_2 = volume of oxygen; VCH_4 = volume of methane; VCO_2 = volume of carbon dioxide (CO_2) (all in L/day) and UN = total urine nitrogen.

The values found were transformed to MJ (1 Mcal = 4.184MJ). Energy balance (EB) was calculated by the difference between MEI, NE_L, and HP, where: EB = MEI - NE_L - HP. The relationships between ME/DE, HP/ME, NE_L/ME, and EB/ME were also calculated as indicators of energy efficiency.

The ME_M, k_L, k_G, and k_T were estimated from a linear regression of the MEI and milk energy output adjusted for zero energy balance (EL(0)) for individual cows (Strathe et al., 2011). Herein, ME_M is the ME requirement for maintenance (MJ/kg of $BW^{0.75}$. day), k_L is the efficiency of utilizing dietary ME for milk production (MJ of milk/MJ of ME), k_T is the efficiency of utilizing body stores for milk production (MJ of milk/MJ of tissue), and k_G is the efficiency of utilizing dietary ME for tissue gain (MJ of tissue/MJ of ME). Four initial models were used to estimate the requirements of nutrients throughout the lactation and for each period (early, middle and late lactation), and the final model is described as

$$MEI = \beta_0 + (\beta_1 \times EL) + (\beta_2 \times PEB) + (\beta_3 \times NEB)$$

where MEI is the dietary ME intake (MJ/kg of $BW^{0.75}$. day), EL denotes the milk energy output (MJ/kg of $BW^{0.75}$. day), PEB is the positive energy balance (MJ/kg of $BW^{0.75}$. day), and NEB is the negative energy balance (MJ/kg of $BW^{0.75}$. day), note that PEB and NEB are zero if the cow is in negative or positive energy balance, respectively; β_0 is the intercept and, β_1, β_2, and β_3 are the parameters describing the change in MEI with unit changes in EL, PEB, and NEB, respectively. Under this model β_0 represents the ME_M, $k_L = 1/\beta_1$, $k_G = 1/\beta_2$, and $k_T = \beta_3/\beta_1$.

The energy requirement of maintenance was calculated using the following model:

$$HP = a \times \exp^{(b \times MEI)}$$

where HP is the heat production (MJ/kg of $BW^{0.75}$. day), MEI is the dietary ME intake (MJ/kg of $BW^{0.75}$. day), a is the intercept and b is the parameters describing the change in HP with unit changes in MEI. The efficiency of ME_M (k_M) was obtained from the ratio of NE_M to ME_M (NRC, 2001):

$$k_M = NE_M/ME_M$$

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS version 9.4; SAS Inst. Inc., Cary, NC) according to a completely randomized design. For the lactation data, milk energy output, positive energy balance, negative energy balance, lactation stage, and interactions between milk energy output-lactation stage, positive energy balance-lactation stage, and negative energy balance-lactation stage were included in the model as a fixed effect. Cow and chamber were included in the model as random effects. Values are presented as least squares means with standard errors of the mean. The following model was used:

$$Y_{ijklmn} = \mu + EL_i + PEB_j + NEB_k + LS_l + EL_i \times LS_l + PEB_j \times LS_l + NEB_k \times LS_l + A_m + C_n + e_{ijklmn}$$

where, Y_{ijklmn} = dependent variable, μ = overall mean, EL_i = fixed effect of milk energy output, PEB_j = fixed effect of positive energy balance, NEB_k = fixed effect of negative energy balance, LS_l = fixed effect of lactation stage, A_m = random effect of cow, C_n = random effect of chamber, $EL_i \times LS_l$ = interaction between milk energy output and lactation stage, $PEB_j \times LS_l$ = interaction between positive energy balance and lactation stage, $NEB_k \times LS_l$ = interaction between negative energy balance and lactation stage and e_{ijklmn} = residual error. Normality of residuals was checked with normal probability and box plots and homogeneity of variances with plots of residual versus predicted values. Outliers were removed from statistical analyses when studentized residuals were > 2.5 or < -2.5.

For the maintenance data, ME intake, feeding stage (lactation, maintenance, and fasting), and the interaction between ME intake and feeding stage were included in the model as a fixed effect. The cow was included in the model as random effects. Values are presented as least squares means with standard errors of the mean. The following model was used:

$$Y_{ijk} = \mu + MEI_i + F_j + MEI_i \times F_j + A_k + e_{ijk}$$

where, Y_{ijk} = dependent variable, μ = overall mean, MEI_i = fixed effect of ME intake, F_j = fixed effect of feeding stage, A_k = random effect of cow $MEI_i \times F_j$ = interaction between ME intake and feeding stage, and e_{ijk} = residual error. The normality of residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Outliers were removed from statistical analyses when studentized residuals were > 2.5 or < -2.5.

RESULTS

Data Description

The ingredients and chemical composition of the experimental diet used in the present study are presented in Table1. The cows were offered three different TMR diets throughout the lactation, representing various DM composition (536.4, 473.2, and 439 g/kg of DM) early to late lactation stage, a various in CP composition (169.7, 162.5, and 147.3 g/kg of DM), and fiber (NDF) contents (323.7, 334.2, and 351.7 g/kg of DM) early, mid, and late lactation stage, respectively. The range of GE, DE, and ME presented in Mcal of DM have not had a big difference throughout the diets with a GE composition of 4.4 Mcal of DM, DE (3.0 to 3.1 Mcal of DM), and ME composition (2.6 to 2.7 Mcal of DM) throughout lactation stage.

Energetic parameters for the whole lactation and each stage of lactation are summarized by posterior means and 95% credible intervals in Table 2. Crossbred Holstein × Gyr dairy cow throughout the lactation showed a ME_M of 0.588 $MJ/BW^{0.75}$ and NE_M of 0.395 $MJ/BW^{0.75}$. The efficiency of dietary ME utilizing for producing milk, gain, and the efficiency of utilizing body stores for milk production were 0.67, 0.77, and 0.81, respectively. When the energetic parameters were calculated for each stage of lactation, the cows in the early lactation showed requirements of ME_M and NE_M ($MJ/BW^{0.75}$) numerically higher than cows in the mid-lactation stage, 0.69 vs. 0.67 and 0.51 vs. 0.45, respectively. However, they do not differ between early and mid-lactation. Cow in the late stage of lactation had lower requirements compare with mid and early stages, 0.54 vs. 0.67 vs. 0.68, and 0.36 vs. 0.45 vs. 0.51, for late, mid, and early, and ME_M and NE_M ($MJ/BW^{0.75}$), respectively.

The efficiency of utilizing dietary ME for producing milk (k_L) was different between the stages with early lactation showing a k_L 9.5% higher than mid-lactation, and 11.2% higher than the late stage of lactation. The k_L did not differ between the mid and late stages. When k_L was calculated for the whole lactation compared with the stages of lactation, cows in the early stage demonstrated better utilization of dietary ME (0.74 vs. 0.67). However, k_L did not differ between mid and late stages, likewise between both and when was calculated for the whole lactation. The efficiency of body mass utilizing for milk production (k_T) was 0.85 to the early stage of lactation and 0.98 to mid-lactation. Using the whole lactation data, k_T was 0.81 and no difference to valuesfounded between whole lactation and early stage, however, the data differ between whole lactation and mid-lactation stage. The efficiency of dietary ME utilization for tissue gain (k_G) was 0.71, 0.79, and 0.75 for the whole lactation, early-stage, and mid-stage of lactation, respectively.

Table 2. Energetic parameters and 95% credible intervals (in parentheses) to Holstein x Gyr crossbred cows for the whole lactation and each lactation stage.

	ME_M	NE_M	k_L	k_G	k_T
Method	$MJ/BW^{0.75}$	$MJ/BW^{0.75}$			
Whole lactation	0.588 (0.566, 0.610)	0.395 (0.371, 0.421)	0.672 (0.655, 0.690)	0.771 (0.744, 0.800)	0.814 (0.779, 0.899)
Early	0.689 (0.635, 0.742)	0.511 (0.458, 0.567)	0.742 (0.722, 0.764)	0.792 (0.763, 0.823)	0.859 (0.818, 0.950)
Mid	0.672 (0.629, 0.715)	0.455 (0.403, 0.513)	0.677 (0.641, 0.717)	0.752 (0.717, 0.790)	0.982 (0.901, 1.179)
Late	0.544 (0.515, 0.574)	0.363 (0.330, 0.412)	0.667 (0.641, 0.696)		

Energetic parameters for maintenance and fasting are summarized by posterior means and 95% credible intervals in Table 3. Crossbred Holstein × Gyr dry and non-pregnant cows showed a ME_M of 0.434 $MJ/BW^{0.75}$ and NE_M of 0.351 $MJ/BW^{0.75}$ for maintenance and a ME_M of 0.396 $MJ/BW^{0.75}$ and NE_M of 0.345 $MJ/BW^{0.75}$ for fasting metabolism. The efficiency of utilizing dietary ME for maintenance (k_M) was 0.80.

Table 3. Energetic parameters and 95% credible intervals (in parentheses) to Holstein x Gyr crossbred cows during the maintenance and fasting period.

	ME_M	NE_M	k_M
Method	$MJ/BW^{0.75}$	$MJ/BW^{0.75}$	
Maintenance	0.434 (0.344, 0.523)	0.351 (0.278, 0.423)	0.809 (0.643, 0.975)
Fasting	0.396 (0.256, 0.535)	0.345 (0.223, 0.466)	

DISCUSSION

Several measures of energy requirement and their efficiency parameters have been presented in the literature (Agnew and Yan, 2000; Moraes et al., 2015; Guinguina et al., 2020). Traditionally, the energy requirement for dairy cows utilizes data from studies using Holstein or Holstein-Friesian cows under temperate climate conditions and has limited consideration for possible differences in metabolic rates between pure Holstein cows and their crossbreeds (AFRC, 1993; NRC, 2001). Holstein × Gyr crossbred dairy cows have a prominent contribution to milk production under tropical conditions. However, there is still a gap in knowledge regarding the energy requirements of Holstein × Gyr crossbred dairy cows (Oliveira, 2015).

The results of the current study indicate that there are differences in the energy requirement among the stages of lactation and between the data from whole lactation and the stages. Cows in the early stage of lactation exhibited higher requirement of ME and NE than the other stages, as was expected, since cows at the beginning of the lactation producing more amount of milk, have a potential change in energy balance, request more energy for production, and those factors can change the requirements of energy (Moe et al., 1972). The current study was based on respiration chamber data that most likely give a more accurate estimate of energy requirement than estimates from BW changes or comparative slaughter (Moraes et al., 2015).

In a study developed by Dong et al. (2015) using data derived from a meta-analysis of respiration chamber using two groups of cows, Holstein-Friesian and non-Holstein (Norwegian, and crossbred F1 Holstein × Norwegian and Holstein × Jersey) throughout the whole lactation. The authors demonstrated that the values of ME_M calculated from the linear regression of milk energy output adjusted to zero energy retention against ME intake were similar between the two groups. Nevertheless, these results are in line with the results presented the current study (Table 2), where the value of ME_M for the F1 Holstein × Gyr cows in early lactation is 0.689 $MJ/BW^{0.75}$ compare with 0.688 and 0.686 $MJ/BW^{0.75}$ for Holstein and non-Holsteins dairy cows, respectively (Dong et al., 2015).

The results from our study corroborate findings of Moraes et al. (2015), that using multivariate and univariate analysis to analyzing energy balance data from lactating cow and investigated potential changes in maintenance requirements and partial efficiencies of energy utilization by lactating cows established values of 0.57 and 0.36 to ME_M and NE_M respectively. Likewise, the ME_M presented in our study is within the range of estimates (0.49 to 0.67 MJ/BW$^{0.75}$) valued by Agnew and Yan (2000).

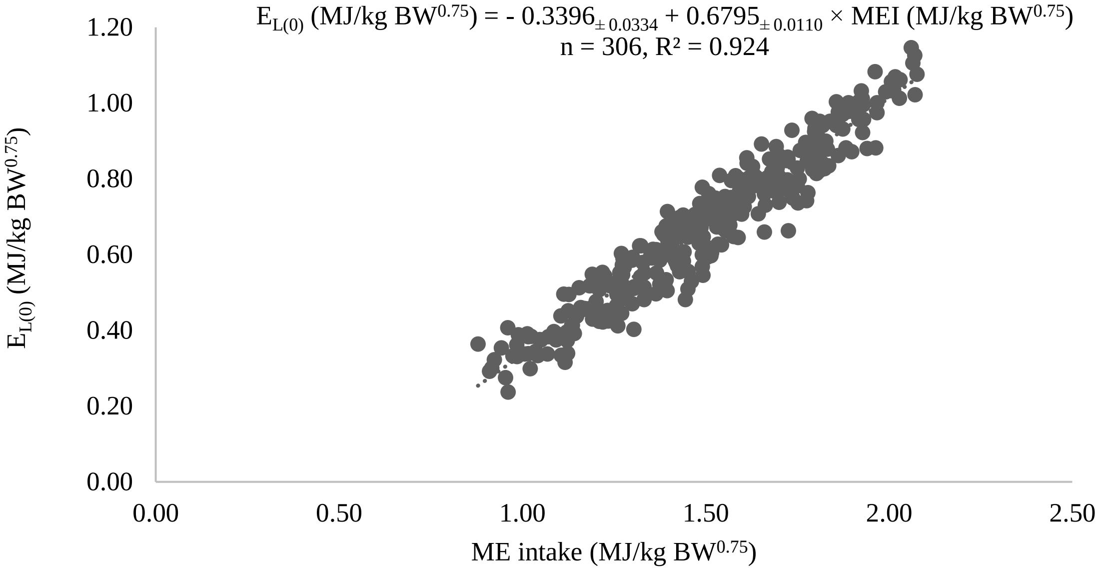

Figure 1. Linear relationships between ME intake and milk energy output adjusted to zero energy balance for F1 Holstein × Gyr dairy cattle crossbreed throughout lactation (experimental means, *n* = 29).

Measured fasting heat production in F1 Holstein × Gyr dry and non-pregnant dairy cows was 0.34 MJ/BW$^{0.75}$ thatis similar (0.33 MJ/BW$^{0.75}$) with the value proposed by NRC (2001) (Table 3). When the energy output in milk as a function of metabolic weight is similar, there was no evidence to suggest that energy requirements for maintenance or production differ between breeds (Tyrrell et al., 1991; Dong et al., 2015). These results indicate that crossbreeding of Holstein cows may have little effect on the basal metabolic rates of cows, and the maintenance energy requirement derived from Holstein cow under temperate climate conditions can be used to F1 Holstein × Gyr dairy cows.

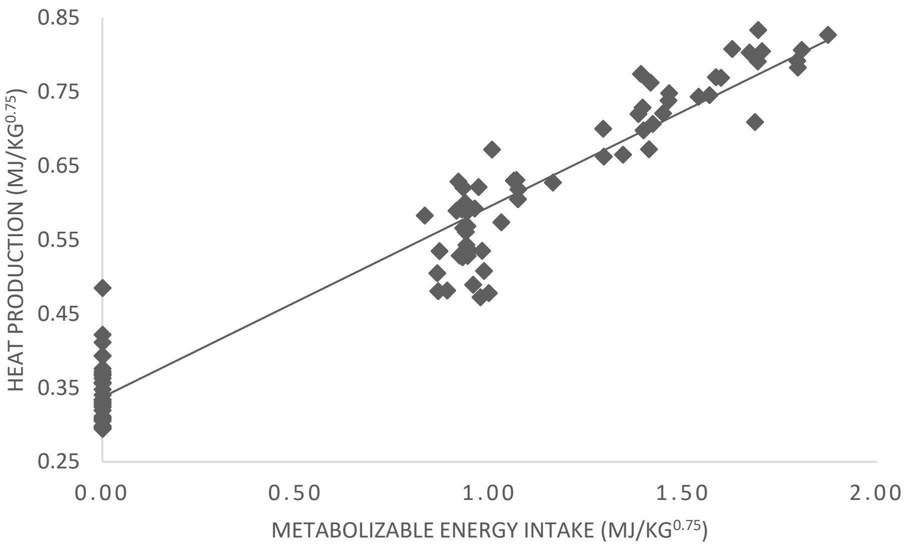

Figure 2. Linear relationships between ME intake and heat production for F1 Holstein × Gyr dairy cattle crossbreed throughout lactation, maintenance, and fasting stages.

The ME_M and k_L values obtained in this study did not corroborate with the values presents by Oliveira (2015) using crossbred cows under tropical conditions. The author concludes that crossbreed cows have lower ME_M requirements and lower k_L than pure breed cows. The values presented in our study were higher than the values indicated by Oliveira (2015) with ME_M of 0.588 vs. 0.558 MJ/BW$^{0.75}$ and k_L of 0.67 vs. 0.53 for whole lactation data compare with his complete data and ME_M of 0.588 vs. 0.519 MJ/BW$^{0.75}$ and k_L 0.67 vs. 0.50 for whole lactation data compared to data from grazing animals' data, respectively. On the other hand, Carvalho et al. (2019) using F1 Holstein × Gyr cows during the mid-lactation demonstrated values for NE_M similar to those established in our study (0.45 vs. 0.44 MJ/BW$^{0.75}$). Carvalho et al. (2019) used different nutritional plans and the crossbred F1 Holstein × Gyr had better performance with a 10% feed restriction of DMI. The authors justify the differences in energy requirement due to the lower milk production and how the energy requirements for maintenance can represent a larger share of the total net energy, and due to the lower viscera/liver size and activity, and lower body protein turnover of Gyr cows that could contribute to a lower endogenous energy expenditure of crossbred Holstein × Gyr dairy cows (Oliveira, 2015; Carvalho et al., 2019). F1 Holstein × Gyr cows are rustic animals and due to the dual fitness characteristic, they can often gain weight in the final lactation phase, when supplemented for increasing milk production, reaching high body condition scores, which can lead to complications following lactation.

In particular, estimates of the efficiency of utilizing dietary ME for tissue gain (k_G) and the efficiency of utilizing body tissues for milk production (k_T) were similar o the k_G and k_T proposed by Moraes et al. (2015). Their values of k_G were 0.75 and 0.70 compared with 0.77 using whole lactation data, and 0.79 and 0.75 for early and mid-lactation data, respectively, and their values of k_T were 0.80 and 0.89 compared with 0.81 using whole lactation data, and 0.85 for the early stage of lactation (Table 2). Moreover, Moraes et al. (2015) suggest that there may be substantial differences in the estimative of efficiencies between Europe and North America databases rather than differences in the models used to estimate energetic efficiencies. Differences in k_G could be an outcome of the gain composition and different degrees of cow maturity at the beginning of lactation and k_G is theoretically affected by diet composition and how differences in the nutrient fractions containing dietary ME have the potential to alter the efficiency of dietary energy utilization. Similarly, the estimates k_T from our study suggest a high efficiency of utilization of body stores for milk production, which is a good agreement with the NRC (2001). Two important points have to be considered while calculating energy efficiencies. First, is that the tissue energy balance calculations are subjective to cumulative errors from measurements of ME intake, heat production, and milk energy output. Second, is the instability in estimating energetic parameters from indirect calorimetry and the inherent correlation between energetic efficiencies within a model (Moe et al., 1971; Strathe et al., 2011).

Evidence indicates that the efficiency of utilizing dietary ME for producing milk (k_L) values remains relatively constant in a wide range of conditions such as dietary composition, animal, genotype, and production level (Agnew and Yan, 2000). The values presented in this current study are inconclusive to that theory since they were similar to values found by some authors (Agnew and Yan, 2000; Dong et al., 2015; Guinguina et al., 2020) and higher compare with other authors (Moraes et al., 2015; Oliveira, 2015).

Ferris et al. (1999) using Holsteins dairy cows with high and medium genetic merit and different concentrate proportion conclude that k_L decreases with the increasing proportion of concentrate in the diet. Even our results showing the opposite, the k_L demonstrated in this study decrease with lower concentrate proportion, our value of k_L for the whole lactation data were similar (0.67 vs. 0.67) compare with 37% of concentrate proportion included in the diet (Ferris et al., 1999). The decrease in k_L in this present study followed the lactation stages. Cow at the final lactation stage had lower k_L than cows at the early stage. The lower milk production at the late stage of lactation could influence the lower k_L presented in our study (Table 2). Likewise, Agnew and Yan (2000) suggested an average k_L of 0.66, derived from calorimetric data of dairy cows drawn from 42 studies from across the world, this value is also similar to the values presented in our study (Table 2).

Dong et al. (2015) presented a value of k_L to the non-Holstein cow of 0.64, this value is in our 95% credible intervals for k_L of mid and late lactation F1 Holstein × Gyr dairy cows (Table 2). These results support that dietary and animal factors have little effect on k_L values when accounting for both milk energy output and body tissue energy retention (Agnew and Yan, 2000). On the other hand, Oliveira (2015) suggested an average k_L of 0.53 for crossbred *Bos taurus* × *Bos indicus* dairy cows under tropical conditions, concluding that crossbred animals under tropical conditions had lower net energetic efficiency for milk production than purebred animals. This divergence in the data present in the literature highlights the need for further studies with animals crossed under tropical conditions.

CONCLUSION

The results support the hypothesis that F1 crossbreed Holstein × Gyr dairy cows have differences in the requirement of energy throughout the lactation stages, however, comparing with data available in the literature, there ae no differences between F1 animals raised under tropical and purebred cows. This study may contribute to adjustments in feeding system energy recommendations for dairy cows under tropical conditions. It is important to note that additional research with crossbred cows, especially throughout the whole lactation, is required to better elucidate the differences in the requirement of energy between crossbred and purebred dairy cows influenced by different climate condition.

REFERENCES

AFRC. 1993. AGRICULTURAL AND FOOD RESEARCH COUNCIL. Energy and Requirements of Ruminants. (Commonwealth Agricultural Bureaux International, Wallingford).

Agnew, R.E., and T. Yan. 2000. Impact of recent research on energy feeding systems for dairy cattle. Livest. Prod. Sci. 66:197–215. doi:10.1016/S0301-6226(00)00161-5.

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists. Arlington, VA.

Carvalho, P.H., A.L. Da Costa Cruz Borges, R. Reis E Silva, H.F. Lage, P.A.D. Vivenza, J.R.M. Ruas, E.J.F. Filho, R.L.A. Palhano, L.C. Gonçalves, I. Borges, E. De Oliveira Simões Saliba, D.G. Jayme, and A.Ú. De Carvalho. 2019. Energy metabolism and partition of lactating Zebu and crossbred Zebu cows in different planes of nutrition. PLoS One 13:1–10. doi:10.1371/journal.pone.0202088.

Brouwer, E. 1965. Report of sub-committee on constants and factors. London, UK.

Chen, X.B., and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivative - An overview of technical details. International Feed Research Unit, Rowett Research Institute, Aberdeen, UK.

Chizzotti, M.L., F.S. Machado, E.E.L. Valente, L.G.R. Pereira, M.M. Campos, T.R. Tomich, S.G. Coelho, and M.N. Ribas. 2015. Technical note: Validation of a system for monitoring individual feeding behavior and individual feed intake in dairy cattle. J. Dairy Sci. 98:3438–3442. doi:10.3168/jds.2014-8925.

CSIRO. 2007. Nutrient Requeriments of Domesticated Ruminants. Commonwealth, Scientific and Indystrial Research Organisation (CSIRO), Collingwood, Autralia.

Dong, L.F., T. Yan, C.P. Ferris, and D.A. McDowell. 2015. Comparison of maintenance energy requirement and energetic efficiency between lactating Holstein-Friesian and other groups of dairy cows. J. Dairy Sci. 98:1136–1144. doi:10.3168/jds.2014-8629.

Ferris, C.P., F.J. Gordon, D.C. Patterson, M.G. Porter, and T. Yan. 1999. The effect of genetic merit and concentrate proportion in the diet on nutrient utilization by lactating dairy cows. J. Agric. Sci. 132:483–490. doi:10.1017/S0021859699006553.

Guinguina, A., T. Yan, A.R. Bayat, P. Lund, and P. Huhtanen. 2020. The effects of energy metabolism variables on feed efficiency in respiration chamber studies with lactating dairy cows. J. Dairy Sci. 103:7983–7997. doi:10.3168/jds.2020-18259.

INRA. 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, the Netherlands.

IDF. 2000. INTERNATIONAL DAIRY FEDERATION. Determination of milfat, protein and lactose content – Guidance on the operantion of mid-infrared instruments. (IDF, Brussels), 15.

Machado, F.S., T.R. Tomich, A.L. Ferreira, L.F.L. Cavalcanti, M.M. Campos, C.A.V. Paiva, M.N. Ribas, and L.G.R. Pereira. 2016. Technical note: A facility for respiration measurements in cattle. J. Dairy Sci. 99:4899–4906. doi:10.3168/jds.2015-10298.

Mertens, D.R. 1997. Creating a System for Meeting the Fiber Requirements of Dairy Cows. J. Dairy Sci. 80:1463–1481. doi:10.3168/jds.S0022-0302(97)76075-2.

Moe, P.W., W.P. Flatt, and H.F. Tyrell. 1972. Net Energy Value of Feeds for Lactation. J. Dairy Sci. 55:945–958. doi:10.3168/jds.S0022-0302(72)85601-7.

Moe, P.W., H.F. Tyrrell, and W.P. Flatt. 1971. Energetics of Body Tissue Mobilization. J. Dairy Sci. 54:548–553. doi:10.3168/jds.S0022-0302(71)85886-1.

Moraes, L.E., E. Kebreab, A.B. Strathe, J. Dijkstra, J. France, D.P. Casper, and J.G. Fadel. 2015. Multivariate and univariate analysis of energy balance data from lactating dairy cows. J. Dairy Sci. 98:4012–4029. doi:10.3168/jds.2014-8995.

NRC. 2000. National Research Council Nutrient Requirements of Beef Cattle. 7th ed. Na. Washington, DC.

NRC. 2001. National Research Council. Nutrient Requirements of Dairy Cattle. 7th rev. e. Washington, DC.

Oliveira, A.S. 2015. Meta-analysis of feeding trials to estimate energy requirements of dairy cows under tropical condition. Anim. Feed Sci. Technol. 210:94–103. doi:10.1016/j.anifeedsci.2015.10.006.

Oss, D.B., F.S. Machado, T.R. Tomich, L.G.R. Pereira, M.M. Campos, M.M.D. Castro, T.E. da Silva, and M.I. Marcondes. 2016. Energy and protein requirements of crossbred (Holstein × Gyr) growing bulls. J. Dairy Sci. 100:2603–2613. doi:10.3168/jds.2016-11414.

Rodrigues, J.P.P., R.M. de Paula, L.N. Rennó, M.M.S. Fontes, A.F. Machado, S. de C. Valadares Filho, P. Huhtanen, and M.I. Marcondes. 2017. Short-term effects of soybean oil supplementation on performance, digestion, and metabolism in dairy cows fed sugarcane-based diets. J. Dairy Sci. 100:4435–4447. doi:10.3168/jds.2016-11725.

Sguizzato, A.L.L., M.I. Marcondes, J. Dijkstra, S.D.C.V. Filho, M.M. Campos, F.S.

MacHado, B. Castro Silva, and P.P. Rotta. 2020. Energy requirements for pregnant dairy cows. PLoS One 15. doi:10.1371/journal.pone.0235619.

Valadares, R. F., G. Broderick, S. Valadares Filho, and M. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivates. J. Dairy Sci. 82:2686-2696.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Strathe, A.B., J. Dijkstra, J. France, S. Lopez, T. Yan, and E. Kebreab. 2011. A Bayesian approach to analyze energy balance data from lactating dairy cows. J. Dairy Sci. 94:2520–2531. doi:10.3168/jds.2010-3836.

Effects of feeding legume-based forages on milk yield, nutrient digestibility, N utilization, and methane emissions in organic-certified dairy cows

J. P. Sacramento[*1,2], L. H. P. Silva[3], D.C. Reyes[1], Y. Geng[1], L.G.R. Pereira[4], A. F. Brito[1]

[1]Departmemt of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH., [2]Department of Bioengineering, Universidade Federal de São João del Rei, São João del Rei, MG, Brazil., [3]Department of Animal Science, Western Kentucky University, Bowling Green, KY, [4]Brazilian Agricultural Research Corporation. Embrapa Dairy Cattle. Juiz de Fora. MG. Brazil

ABSTRACT

Previous research showed that red clover (RC) improved N utilization in dairy cows, but data on RC mixed with other cool-season legumes is lacking. Sixteen multiparous and 4 primiparous organic-certified Jersey cows averaging 116 ± 52 DIM and 502.1 ± 52.2 kg of BW were used to investigate the effects of partially replacing red clover (RC) with a legume mix (LM) on DMI, milk yield and composition, apparent total-tract digestibility of nutrients, and CH_4 emissions. Cows were blocked in pairs by DIM or parity and, within pair, randomly assigned to treatments in a crossover design. Each experimental period lasted 24 d, with14 d for diet adaptation and 10 d for sample collection. Two fields were planted with RC- or alfalfa-grass mix consisting (% of total) of 79:14:7 legume:meadow fescue:timothy seeding rate. Forages were harvested as baleage, with second and third cuttings used in the study. Diets were fed twice daily as TMR, with a 60:40 forage:concentrate ratio. Based on the botanical composition of each field, the forage portion of the treatments contained (% of diet DM): (1) 41% RC, 5% white clover, 9% weeds, and 5% grasses (RC diet), and (2) 21% RC, 10% white clover, 12% alfalfa, 11% weeds, and 5% grasses (LM diet). Diets averaged 17.7 vs. 17.1% CP and 31.7 vs. 31.3 % aNDFom for RC and LM, respectively. Fecal grab samples were collected over 3 consecutive d and analyzed for iNDF. Spot urine samples were collected 5 times over 2 consecutive days. Data were analyzed using PROC MIXED of SAS with significance declared at $P < 0.05$. Cows fed RC had greater DMI (21 vs. 20.4 kg/d; $P = 0.01$) and a greater N intake (605 vs. 591 g/d, $P = 0.01$) than those fed LM, but no significant differences were observed for yields of milk (mean = 21.1 kg/d), 4% FCM (mean = 25.5 kg/d), ECM (mean = 27.6 kg/d), MUN (mean = 12 mg/dL) and PUN (mean = 16.3 mg/dL) concentrations, and milk N efficiency (mean = 20%). However, diets affect concentration of milk fat (5.21 vs. 4.46 %; $P = 0.02$) and a trend was observed to protein (3.66 vs.

3.71%; $P = 0.05$) with cows fed RC diet producing less than cows fed LM diet. In contrast, digestibilities of DM, OM, NDF, and ADF increased ($P < 0.05$) with feeding RC than LM (68.4, 70.4, 47.9, and 51.8% vs. 64, 66, 39.7, and 48.6%, respectively). Digestibility of CP did not differ between diets. Urinary excretion of urea N, expressed in g/d (mean = 162 g/d) or as a proportion of urinary N (mean = 72.5%) or N intake (mean = 27%), was not affected by diets. Uric acid excretion in urine was greater in RC than LM diet (80.4 vs. 73.5 mmol/d, $P = 0.01$), but no differences were observed for that of allantoin (mean = 376 mmol/d) and total purine derivatives (mean = 453 mmol/d). While cows fed RC had lower CH_4 yield (18.8 vs. 19.6, respectively; $P = 0.03$), no significant differences were observed for CH_4 production (mean = 393 g/d) and CH_4 intensity (mean = 14.6 g/kg of ECM). In summary, partially replacing RC with a LM did not affect N utilization in lactating dairy cows, and despite improved DMI and digestibility and decreased CH_4 yield, cows fed RC did not produce more milk possibly because additional energy was not partitioned into milk synthesis.

Key words: legume mix, nitrogen, clover, organic

INTRODUCTION

One of the major limitations of feeding high-forage organic rations, which are formulated to contain at least 60% of the total diet dry matter as stored feed, is the reduction in both milk production and nutrient use efficiency. For instance, feeding high-forage rations to lactating dairy cows generally increase the outputs of nitrogen (**N**) and carbon (**C**), as enteric methane (**CH_4**), to the environment (Brito et al., 2008; Gerber et al., 2013; Hristov et al., 2013a).

Manure-N can be converted to nitrous oxide (**N_2O**) and can be emitted as an intermediate product during nitrification and denitrification when chemical fertilizers or animal manure are applied to crops (Groffman et al., 2000). Both CH_4 and N_2O are potent greenhouse gases (**GHG**) with global warming potential 25 and 298 times greater than carbon dioxide (**CO_2**), respectively (IPCC, 2007).

Pasture and stored feeds used in high-forage rations have N excess relative to energy (Hafla et al., 2016), resulting in an unbalanced supply of water-soluble carbohydrates (**WSC**) and CP to the animal (NRC, 2001; Hristov et al., 2013a). When energy is limiting in pasture and stored feeds, but there is an excess of N in the form of peptides and amino acids of plant origin, the rumen microbiota uses amino acids for energy and releases ammonia through deamination processes (NRC, 2001). Therefore, forage-based diets must be formulated to prevent excessive protein degradation in the rumen, thereby increasing dietary and microbial amino acids supply to the small intestine to improve N use efficiency.

Feeding legume-based forages as tertiary legume-grass mixtures can improve productivity and achieve a better balance between energy and CP than binary mixtures (Silva et al., 2014). Red clover, white clover and alfalfa are commonly used in organic dairies as stored feeds and mixed pastures (Hafla et al., 2016). These three legume crops also have different concentrations of WSC (Pelletier et al., 2010). A nonprotein-N, a large proportion of alfalfa protein is broken down to ammonia, amino acids, and peptides, whereas that of red clover are protected against proteolysis due to the presence of enzyme polyphenol oxidase in red clover tissues (Jones et al., 1995). One of the biggest concern about using red clover as a sole forage to feed dairy cows is the negative effect on milk production (Brito et al., 2007; Johansen et al., 2018). Tertiary legume-grass diets may improve N use efficiency via increased rumen microbial protein synthesis and milk production and decreased N excretion to the environment and CH_4 emissions (Brito et al., 2008; Gerber et al., 2013).

Therefore, developing mixtures of perennial legumes and grasses selected to increase forage quality via energy-dense stored feeds (i.e., high concentration of WSC) have strong potential to increase milk production and decrease GHG emissions of dairy farms transitioning to organic agriculture. We aim to enhance milk production and energy-corrected milk by evaluating the effects of feeding stored feed consisted of different grass-legume tertiary mixtures on milk production, N use efficiency, and GHG emissions in certified organic dairy cows.

MATERIALS AND METHODS

The study was performed at the University of New Hampshire Burley-Demeritt Organic Dairy Research Farm in Lee, New Hampshire, from March 17th to May 05th, 2020. Care and handling of the animals used in the current study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee (Protocol no. 200304)

Animals, Experimental Design, and Diets

Sixteen multiparous Jersey cows averaging (mean ± SD) 122 ± 51 DIM, 516.2 ± 44.9 kg of BW, and 23.1 ± 3.47 kg of milk/d, and 4 primiparous Jersey cows averaging 91 ± 56 DIM, 445.9 ± 39.6 kg of BW, and 20.9 ± 2.62 kg of milk/d at the beginning of the study were used. Animals were blocked in pairs by DIM and parity and, within each pair, randomly assigned to 2 treatments in a crossover design with 2 experimental periods. Each period lasted 24 d (total of 48 d) and consisted of 14 d for diet adaptation and 10 d for data and sample collection. Treatments were fed as TMR (60:40 forage-to-concentrate ratio) formulated according to the NRC (2001) and balanced to meet the nutritional requirement of a Jersey cow producing 26 kg of milk/g, 5.0% milk fat, and 4.0% milk true protein.

The treatments consisted of Red Clover diet (RC) contain (DM basis) 60% second- and third-cut RC-grass (30% each cut) and 40% concentrate and Legume Mix diet (LM) contain 60% second- and third-cut ALF- and RC-grass (30% second-cut ALF-grass, 15% second-cut RC-grass, and 15% third-cut RC-grass), and 40% concentrate. Based on the botanical composition of each field, the forage portion of the treatments contained (% of diet DM): 41% RC, 5% white clover, 9% weeds, and 5% grasses (RC diet), and 21% RC, 10% white clover, 12% alfalfa, 11% weeds, and 5% grasses (LM diet). The ingredients and nutritional composition of the experimental diets are presented in Table1.

Two fields were planted with alfalfa (ALF)- or red clover (RC)-grass mixed with 79:14:7 legume:meadow fescue:timothy seeding rate (% total). Forages were harvested as baleage, with second- and third-cut legume-grass mixtures used in the study. The botanical composition (DM basis) of second-cut ALF-grass swards averaged 68% legume (40% alfalfa, 26% white clover, and 2% red clover), 9% grasses, and 23% weeds. The second-cut of RC-grass swards averaged 75% legume (red clover), 4% grasses, and 21% weeds and the third-cut RC-grass swards averaged 79% legume (62% red clover, and 17% white clover), 11% grasses, and 10% weeds.

Animals were housed in a bedded-pack barn with dried pine shavings as bedding and keep in a pen separated from the remaining lactating cows in the herd. The bedding area (132m^2) opens to a 478-m^2 concrete-floor outdoor lot (total pen area = 610m^2), allowing cows to walk freely in compliance with the USDA National Organic Program “Livestock living conditions” (USDA, 2010), which calls for year-round access to the outdoors for all ruminants among other regulations. Cows had access to a roof-covered feeding station equipped with electronic recognition Calan doors systems (American Calan Inc., Northwood, NH) located at the opposite end of the bedding area. Body weights were recorded after the milking time in the afternoon for two consecutive days at the beginning of the experiment as well as during the last two d of each period to determine BW and BW change.

Table 1. Botanical and nutritional composition (% of DM unless otherwise noted) of Red Clover and Legume Mix diet

	Diets	
Item	RC	LM
Botanical composition (% of DM)		
Grasses[1]	4.50	4.95
Legumes		
Red Clover	41.1	21.2
Alfalfa	0.00	12.0
White Clover	5.10	10.4
Weeds	9.30	11.6
Ingredients (g/kg of DM)		
Red Clover	602.7	300.8
Legume mix		300.8
Corn	224.1	224.6
Soybean	60.0	60.2
Barley Grain	60.2	60.4
Soybean seeds	26.2	26.3
Calcium Sulfate	1.5	1.5
Limestone	2.5	2.4
Magnesium Oxide	1.3	1.3
Manganese Sulfate	1.5	1.5
Salt	4.4	4.4
Sodium Bicarbonate	5.5	5.5
Vitamin premix[2]	2.4	2.4
Molasses	7.9	8.0
Chemical composition (g/kg of DM)		
DM	627.7	586.4
OM	907.2	909.3
CP	177.4	171.5
EE	47.2	34.0
Ash	97.7	99.0
Starch	187.6	181.3
ADF	227.4	242.7
aNDFom[3]	317.3	313.2
iNDF	87.7	108.6
Lignin	47.1	44.0
TDN	707.0	687.0

[1] Predominant grass species: timothy (*Phleum pratense* L.), Meadow Fescue (*Festuca pratensis*)

[2] Contained (as fed basis): 0.664% Ca, 0.454% P, 0.332% Mg, 0.719% K, 0.819% Na, 0.826% Cl, 0.591ppm Se, 18.7ppm Cu, 0.925ppm Mn, 114.8ppm Zn, 74.4ppm Fe, 1.78ppm I, 2,707 IU/kg of vitamin A, 960.6 IU/kg of vitamin D, 24.83 IU/kg of vitamin E.

[3] aNDFom = α-amylase, sodium sulfite-treated ash-free NDF.

Feeding and Feed Sampling and Analyses

All bales used in the current study were sampled before feeding using a Hilti model TE 7-A (Hilti North America, Tulsa, OK) fitted with a metal core sampler (40 cm long). Throughout the study, baleage samples (approximately 100 g each) obtained after 3 to 4 core samplings from each bale were composited to yield individual sample sizes of 300 to 400 g. Baleage samples were equally divided into 2 subsamples, dried (55℃, 72 h) in a forced-air oven (VWR Scientific, Radnor, PA), with the first set for determination of DM to adjust the TMR as-fed basis and to calculate DMI and the second set shipped for nutritional analyses. Concentrate samples (about 500g) were collected once weekly throughout the experiment. Prior to the morning feeding, baleages were weighed and chopped in a vertical mixer (Valmetal V-Mix 400; Saint-Germain-de-Grantham, QC, Canada) approximately 20 min every 2 d. Dietary ingredients, including water, were loaded in an A100 self-propelled mixer (Jaylor Fabricating Inc., East Garafraxa, ON, Canada) and mixed for at least 10 min to obtain TMR with a target DM of 60%. Diets were offered ad libitum twice daily at 0630 and 1600 h, with about 40% of the daily TMR allocation fed in the morning and the remaining 60% in the afternoon. The amount of TMR offered to the animals was adjusted daily to yield orts of approximately 5 to 10% of the as-fed intake. Orts were collected daily before the afternoon feeding and weighed as done for the TMR, and individual feed intake was recorded throughout the experiment. Animals had free access to clean water throughout the experiment. Body weights were recorded (Northeast Scale Co. Inc., Hooksett, NH) immediately after afternoon milking for 3 consecutive days before d 1 of the experiment and the last 3 d of each period.

Samples of concentrate used for nutritional analyses were collected on d 15, 20 and, 24 of each period. Baleages, TMR, and orts were collected daily from d 15 to d 24 of each period and individual composite over the 10-d period were made. Samples of

TMR were collected and composited by diet, whereas individual cow orts samples were collected and composited by treatment. All TMR, feeds, and orts were dried in a forced-air oven (55°C, 72 h) and ground through a 1-mm screen (Willey mill, Arthur H. Thomas Co., Philadelphia, PA). Baleages, orts, and concentrates were shipped to Dairy One Cooperative Inc., (Ithaca, NY) and analyzed for DM (method 930.15; AOAC International, 2016), CP (total N × 6.25; method 990.03; AOAC International, 2016), amylase and sodium sulfite treated NDF exclusive of ash [**aNDFom**; Ankom Technology method 6, Fair-port, NY; solutions as in Van Soest et al. (1991)], ADF [Ankom Technology method 5, solutions as in method 973.18 (AOAC International, 2016)] neutral detergent insoluble CP (Leco TruMac N Macro Determinator on a NDF residue), acid detergent insoluble CP (Leco TruMac N Macro Determinator on an ADF residue), ADL (Ankom Technology method 9 in a Daisy II Incubator), ether extract [extraction by a Soxtec HT6 System (Foss North America, Eden Prairie, MN) using anhydrous diethyl ether; method 2003.05; AOAC International, 2016], ethanol soluble carbohydrates (Hall et al., 1999), ash (method 942.05; AOAC International, 2016). Feed AA was analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) by cation-exchange chromatography-HPLC coupled with post-column ninhydrin derivatization using norleucine as the integral standard (method 982.30; AOAC International, 2016).

Triplicate samples (~0.5 g) of feces, feed, TMR, and orts were weighed into Ankom F57 bags (25 µm pore size; Ankom Technology, Macedon, NY), placed in a larger laundry nylon bag, and inserted in the rumen of 2 ruminally cannulated late-lactation Holstein cow for 12 d; a corn silage-based diet with a forage:concentrate ratio of 50:50 was fed to the ruminally cannulated cow. After removal from the rumen, bags were rinsed with tap water and analyzed in-house for aNDF using an Ankom^{2000} fiber analyzer [Ankom Technology method 6, solutions as in Van Soest et al. (1991)]. Feeds and fecal indigestible NDF (iNDF) was used as an intrinsic marker to estimate the fecal output of DM and apparent total tract digestibility of nutrients (Cochran et al., 1986; Huhtanen et al., 1994).

Milk Sampling and Analyses

Cows were milked twice daily at 0530 and 1530 h in a 4-stall step-up parlor equipped with headlocks (Agromatic, Fond du Lac, WI), automatic take-offs, and milk meters (Westfalia Surge, GEA Farm Technologies Inc., Naperville, IL). Milk weights were recorded daily throughout the experiment (DairyPlan C21 Version 5.2, GEA Farm Technologies Inc.). Milk samples were collected using automatic samplers during 4 consecutive milking starting in the afternoon milking of d 15 to 17 of each period. Milk samples were transferred into tubes preserved with 2-bromo-2-nitropropane-1,3 diol (Broad Spectrum Microtabs II; Advanced Instruments Inc., Norwood, MA) and stored at 4°C until shipped overnight to Dairy One Cooperative Inc. laboratory for determination of fat, true protein, lactose, and MUN by Fourier transform infrared spectroscopy using a MilkoScan FT+ (Foss Inc., Hillerød, Denmark).

Blood Sampling and Analyses

Blood samples were collected into vacutainer 15% EDTA tubes (Monoject, Mansfield, MA) from the coccygeal vessels approximately 4 h after the morning feeding on d 20 of each period. Tubes were immediately placed in a chill bucket with beads (Chemglass Life Sciences, Vineland, NJ) and transported to the laboratory for centrifugation (2,155 × g, 20 min, 4ºC) using an Eppendorf centrifuge (model 5810; Eppendorf, Hamburg, Germany). After centrifugation, the first set of plasma samples were stored at -20°C for later analysis of urea N (PUN), which was done colorimetrically using a UV/visible spectrophotometer (Beckman Coulter Inc., Brea, CA) at a wavelength of 540 nm with the diacetyl monoxime method of Rosenthal (1955). Additionally, 4 mL of plasma from the second set of samples were added to 40-mL glass culture tubes containing 1 mL of 15% 5-sulfosalicylic acid solution (wt/vol), homogenized using a vortex (Mini Vortexer; VWR International, Bridgeport, NJ), and kept for 10 min at 4°C. Next, tubes were centrifuged (3,300 × g for 20 min at 4°C), and 0.9-µL aliquots of supernatants were stored by cow per period in cryovials at –80°C until shipped to the University of Missouri Agricultural Experiment Station Chemical Laboratory for AA analysis, following the methods of Deyl et al. (1986) and Fekkes (1996) using cation-exchange chromatography-HPLC as reported above.

Fecal and Urinary Sampling and Analyses

Fecal grab samples were taken directly from the rectum or by stimulating defecation at 0600 h and 1500 h (d 18), 1100 h and 1800 h (d 19), and 2200 h (d 20) of the sampling week in each period. Fecal samples (~200 g/sampling) were collected into 100-mL specimen containers and transferred into 2-L plastic bags to generate composited samples by cow per period. Samples were dried in a forced-air oven (VWR Scientific) at 55°C for approximately 72 h and ground (Wiley mill; A. H. Thomas Co.) to pass through a 1-mm screen. Fecal samples were analyzed for DM, CP, aNDF, ADF, and ash at Dairy One Cooperative Inc. Indigestible NDF was used as the internal marker to estimate the fecal output of DM and apparent total-tract digestibility of nutrients (Huhtanen et al., 1994).

Spot urine samples were collected concurrently with fecal samples into 100-mL specimen containers through stimulation of the pudendal nerve by massaging the area below the vulva or during voluntary urination. After each sampling, 1.6 mL of urine was pipetted into 50-mL centrifuge tubes containing 32 mL of 0.072 *N* H_2SO_4 to obtain composited urine samples by cow per period and stored at −20°C until analyses. After thawing at room temperature, samples were analyzed for concentrations of creatinine (assay kit no. 500701, Cayman Chemical Co., Ann Arbor, MI) using a chromate microplate reader set at a wavelength of 492 nm (Awareness Technology Inc., Palm City, FL), allantoin (Chen et al., 1992), uric acid (assay kit no. 1045–225; Stanbio Laboratory, Boerne, TX), urea N (Stanbio Urea Nitrogen Kit 580; Stanbio Laboratory Inc.), and total N (micro-Kjeldahl analysis, AOAC, 1990; Dairy One Cooperative Inc.). Allantoin, uric acid, and urea N were determined at wavelengths of 522, 520, and 520 nm, respectively, in a UV/visible spectrophotometer (Beckman Coulter Inc., Pasadena, CA). Daily urine volume was estimated from urinary creatinine concentration assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Urinary excretion of urea N, total N, allantoin, uric acid, and total purine derivatives (PD = allantoin plus uric acid) were calculated by multiplying the concentration of each of these metabolites by the urinary volume.

Measurements of Gaseous Fluxes

Emissions of CO_2, H_2 and enteric CH_4 were measured during the entire experimental period. A portable open-circuit head chamber (GreenFeed system, C-Lock Inc., Rapid City, SD) was used to measure the respiratory gas exchange according to the methods of Huhtanen et al. (2015). Span gas (O_2, CO_2, and CH_4) and zero gas (N_2) calibrations were performed once a week and the standard gases consisted of 2 concentrations of O_2 (2,000 and 2,100 ppm), 1,500 ppm each of CO_2 and CH_4 for span gas, and 1005 N_2 (99,999% pure) for zero gas. A CO_2 recovery test was conducted every other week during the whole experiment; the mean (± SE) recovery was 100.9 ± 1.81. Airflow rates and gas concentrations were measured continuously, and using the gas sensor information, a volumetric flux (L/min) of gas emitted by the animal was calculated.

Following the manufacturer's recommendation, the airflow was maintained above 26 L/s by cleaning the air filter when the flow rate starts to approach this level.

The GreenFeed unit was placed at the corner at the barn, next to the calan gates. A pelletized bait concentrate (Morrison's organic 16% Dairy, Morrison's Custom Feeds Inc., Barnet, VT) contained 16% CP, 9% crude fiber, and 3.0% of crude fat (Formula Report, Morrison's Custom Feeds Inc.) was used in the GreenFeed unit during the experimental period. The system was configured to allow each animal to visit a minimum of 5 h intervals. During each visit, the animals were given 10 drops of 30 g bait feed every 40 s. The average bait feed intake from GF was 1.2 kg of DM/d.

Heat production (HP, Kcal/day) was calculated according to Brouwer (1965):

$$\text{HP (Kcal/day)} = (3.866 \text{ x } VO_2) + (1.200 \text{ x } VCO_2) - (0.518 \text{ x } VCH_4) - (1.431 \text{ x UN})$$

Where: VO_2 = volume of oxygen; VCH_4 = volume of methane; VCO_2 = volume of carbon dioxide (CO_2) (all in L/day) and UN = total urine nitrogen.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS version 9.4; SAS Inst. Inc., Cary, NC) according to a crossover design. Treatment (RC diet and LM diet), period, and interactions between period-treatment and treatment-block were included in the model as a fixed effect. Where the interaction term was not significant, it was excluded from the final model. Cow nested within a block, and block were included in the model as random effects. Values are presented as least squares means with standard errors of the mean. Statistical significance was assumed at a probability threshold value of $P \leq 0.05$, and a tendency toward significance was assumed at the value of $0.05 < P \leq 0.10$. The following model was used:

$$Y_{ijkl} = \mu + P_i + T_j + P_i \times T_j + T_j \times B_k + C_{l(k)} + B_k + e_{ijkl}$$

where, Y_{ijkl} = dependent variable, μ = overall mean, P_i = fixed effect of period, T_j = fixed effect of treatment, $C_{l(k)}$ = random effect of cow within block, B_k = random effect of block, $P_i \times T_j$ = interaction between period and treatment, $T_j \times B_k$ = interaction between treatment and block, and e_{ijkl} = residual error. Normality of residuals was checked with normal probability and box plots and homogeneity of variances with plots of residual versus predicted values. Outliers were removed from statistical analyses when studentized residuals were > 2.5 or < -2.5.

RESULTS

Feed Nutrient Composition

Overall, both diets were similar in forage:concentrate ratio (60:40) and chemical composition (g/kg of DM), although RC diet had higher DM (6.57%) CP, EE, Starch, and Lignin contents (3.32, 27.9, 3.3, 6.6%, respectively), and lower concentrations of ADF and iNDF (6.7 and 23.8%, respectively, Table 1). The fermentation profile and amino acids concentration of the constituents of the diet are present in Table 2. The pH between both baleage was similar with an average of 5.61. The LM diet presented higher concentrations of lactic, acetic, and propionic acid, and volatile fatty acids (VFA) than RC diet. Likewise, the values of soluble CP, and ammonia N (% of total N) were superior in the LM diet compare with RC diet (Table 2). Concentration of individual, essential, and total AA were all similar between the baleages (Table 2).

Table 2. Fermentation profile and amino acids concentration of the baleage and concentrate used in the diets and of the pellet

	Baleage			
Item	RC	LM	Concentrate	Pellet
pH	5.67	5.56		
% of DM				
Lactic acid	0.7	3.8		
Acetic acid	0.4	0.8		
Propionic acid	0.0	0.1		
Latic/Acetic ratio	2.1	4.9		
VFA	3.9	7.6		
% of total N				
Soluble CP	39.2	58.5		
Ammonia N	4.7	9.0		
EAA (% of CP)				
Arg	3.07	2.24	4.34	6.02
His	1.35	1.38	1.91	2.37
Ile	3.68	3.76	3.06	3.92
Leu	6.02	5.77	6.47	7.37
Lys	3.63	3.76	3.70	4.89
Met	1.14	1.16	1.10	1.24
Phe	3.98	3.65	3.64	4.57
Thr	3.34	3.32	2.72	3.33
Val	4.72	4.72	3.53	4.46
Total AA[1]	71.2	71.6	71.9	90.5

[1]Total AA = essential + nonessential AA.

Animal Performance, Milk Composition, and Blood Metabolites

The increasing in dietary legume diversity amounts affect total DMI, 21 vs. 20.4 kg/d (*P* = 0.02), without affect milk yield, 21.1 ± 0.767 kg/d, by cow fed RC diet with higher total DM intake than cows fed LM diet (Table 3). It is important to note, however, that milk fat (%) was lower to RC diet compare with the LM diet, 5.21 vs. 5.46 % (*P* = 0.03), and a tendency was observed for milk protein and lactose (%) with lower milk protein, 3.66 vs. 3.71 % (*P* = 0.06), and higher milk lactose, 4.74 vs. 4.70 % (P = 0.08) to cows fed RC diet. As a result, 4% FCM and ECM did not differ significantly across treatments, averaging 25.1 ± 0.858 and 27.2 ± 0.901 respectively (Table 3). Feed efficiency expressed as milk yield/DMI, 4% FCM/DMI, or ECM/DMI were not affected by increasing dietary legume diversity and averaged 1.0, 1.2, and 1.3 kg/kg, respectively (Table 3). Body weight change was not different between treatments, mean = -0.24 kg/d (Table 3). The concentration of MUN did not differ between treatments, however, a tendency was observed for PUN, 15.6 vs. 17 mg/dL (P = 0.06), with cows fed RC diet demonstrating a lower concentration of plasma urea nitrogen.

Table 3. Feed intake, milk yield and composition, and plasma urea N (PUN) in dairy cows fed Red Clover (RC) or Legume Mix (LM) diet

	Treatment			P-value
Item[1]	RC	LM	SEM	Treatment
DMI, kg/d	19.7	19.2	0.401	0.072
GF pellet DMI, kg/d	1.22	1.35	0.078	0.131
Total DMI, kg/d	21.0	20.4	0.390	0.017
Milk yield, kg/d	21.3	20.9	0.767	0.326
4% FCM, kg/d	25.0	25.3	0.868	0.455
ECM, kg/d	27.1	27.3	0.901	0.572
Milk fat, %	5.21	5.46	0.141	0.028
Milk fat, kg/d	1.10	1.13	0.041	0.241
Milk protein, %	3.66	3.71	0.064	0.057
Milk protein, kg/d	0.77	0.77	0.040	0.771
Milk lactose, %	4.74	4.70	0.036	0.085
Milk lactose, kg/d	1.01	0.99	0.059	0.213
Milk TS, %	14.7	14.9	0.205	0.121
Milk TS, kg/d	3.12	3.13	0.107	0.842
MUN, mg/dL	11.7	12.3	0.492	0.158
Milk yield/DMI, kg/kg	1.02	1.03	0.030	0.768
4% FCM/DMI, kg/kg	1.21	1.25	0.028	0.124
ECM/DMI, kg/kg	1.31	1.35	0.028	0.148
Milk N, % N Intake	20.6	21.0	0.373	0.332
PUN, mg/dL	15.6	17.0	0.480	0.056
BW change, kg/d	-0.25	-0.22	0.127	0.898

[1] 4% FCM = [0.40 × milk yield (kg/d)] + [15 × milk fat yield (kg/d)] (Gaines and Davidson, 1923); ECM = [0.327 × milk yield (kg/d)] + [12.95 × fat yield (kg/d) + [7.2 × protein yield (kg/d)] (Orth, 1992).

Nitrogen Utilization and Apparent Digestibility of Nutrients

Treatment effects on intake, apparent total-tract digestibility of nutrients, and urinary N excretion are presented in Table 4. Cows fed RC diet had greater intake for

DM, OM, CP, and EE (*P* = 0.05), and tended to have greater starch intake (*P* = 0.096) than cows fed LM diet. In contrast, ADF and iNDF intake were reduced (*P* = 0.005) by 4.8 and 17%, respectively, for the RC diet. Likewise, cows fed RC diet had greater apparent digestibility of nutrients for DM, OM, NDF, and ADF (*P* = 0.02) than LM diet and no difference was found for CP digestibility. Nitrogen intake was greater (*P* = 0.01) for cows fed RC diet but did not affect urinary daily excretions of total-N and urea-N as a proportion of N intake. Urinary concentrations of creatinine (mean = 3.07 mmol/d) and Allantoin (mean = 376 mmol/d) were not affected by increasing legume diversity. Whereas uric acid was greater (*P* = 0.003) and PD tended to be greater (*P* = 0.08) in cows fed RC diet (Table 4).

Table 4. Intake, total tract apparent digestibility, and urinary N excretion in dairy cows fed Red Clover (RC) or Legume Mix (LM) diet

	Treatment			P-value
Item	RC	LM	SEM	Treatment
Intake, kg/d				
DM	21.0	20.4	0.390	0.017
OM	19.0	18.5	0.352	0.014
CP	3.78	3.69	0.067	0.015
NDF	6.17	6.15	0.129	0.863
ADF	4.61	4.83	0.095	0.005
EE	0.90	0.88	0.036	0.050
Starch	4.39	4.32	0.076	0.096
iNDF	1.81	2.11	0.082	<.0001
Total tract apparent digestibility, % of intake				
DM	68.4	64.0	0.871	0.006
OM	70.4	66.0	0.805	0.004
CP	60.9	57.8	1.438	0.158
NDF	47.9	39.7	0.996	0.000
ADF	51.8	48.6	0.806	0.020
N intake, and urinary daily excretion				
N intake, g/d	605	591	11.02	0.017
Total-N, g/d	230	217	8.484	0.205
Total-N, % N intake	38.3	36.7	1.390	0.347
Urea-N, g/d	168	156	6.339	0.115
Urea-N, % N intake	28.0	26.2	1.015	0.166
Urea-N, % Total-N	73.0	72.0	1.662	0.685

Creatinine, mmol/d	2.95	3.18	0.153	0.154
Allantoin, mmol/d	389	363	21.59	0.142
Uric acid, mmol/d	80.4	73.5	3.605	0.003
PD, mmol/d	470	437	24.36	0.079

[1] Purine derivatives (PD) = allantoin + uric acid.

Gas Emissions

Emissions of CO_2, CH_4 and H_2 are presented in Table 5. No effect of treatment was observed for CO_2 emissions (mean = 10,224 g/d), H_2 emissions (mean = 2.79 g/d), CH_4 production (mean = 393 g/d), CH_4 intensity (mean = 14.6 g/kg of ECM), and for heat production (mean = 105 MJ/d). The CH_4 yield was lower for RC diet, a decrease of 4.3% compared with LM diet (18.8 vs. 19.6 g/kg of DMI, *P* = 0.04) (Table 5).

Table 5. Oxygen consumption, carbon dioxide, hydrogen, and methane production, and heat production in dairy cows fed Red Clover (RC) or Legume Mix (LM) diet

	Treatment			P-value
Item	RC	LM	SEM	Treatment
CO_2, g/d	10,193	10,254	205	0.633
O_2, g/d	7,079	7,170	134	0.263
RQ	1.04	1.04	0.01	0.547
H_2, g/d	2.85	2.74	0.15	0.379
CH_4				
g/d	389	397	11.7	0.352
g/kg of ECM	14.5	14.8	0.78	0.496
g/kg of DMI	18.8	19.6	0.80	0.036
HP, MJ/d	105	106	1.98	0.309

Plasma Concentration of AA

The plasma concentrations of individual EAA and NEAA, His-containing metabolites, and sum of AA are presented in Table 6. Leucine and Valine concentrations in plasma decreased (218 vs. 199, and 343 vs. 322 µ*M; P* = 0.02, respectively), by increasing legume diversity. However, no other treatment effect was observed for the plasma concentrations of all remaining individual EAA. There was a treatment effect for

Alanine and Proline (*P* = 0.02), with feeding LM diet decreased the plasma concentration of those NEAA. On the other hand, Glutamic acid tended to be high (*P* = 0.079) by increasing legume diversity. No other treatment effect was observed for the plasma concentrations of all remaining individual NEAA. Likewise, no treatment effect was observed for total EAA, total NEAA, and the sum of sulfur-containing AA, and uric acid-containing AA. However, branched-chain AA concentrations decreased 6.7% (*P* = 0.03) with feeding LM diet (Table 6).

Table 6. Plasma concentrations of AA and His-containing metabolites in dairy cows fed Red Clover (RC) or Legume Mix (LM) diet

	Treatment			P-value
Item	RC	LM	SEM	Treatment
EAA (µ*M*)				
Met	21.7	21.9	0.87	0.882
Lys	114	112	4.69	0.750
His	64.5	65.8	2.21	0.596
Arg	92.7	93.1	4.03	0.942
Leu	218	199	7.60	0.021
Ile	179	172	5.04	0.151
Phe	68.3	63.9	1.97	0.116
Thr	113	108	4.15	0.281
Trp	65.4	64.7	2.08	0.775
Val	343	322	11.7	0.028
NEAA (µ*M*)				
Ala	259	236	8.24	0.025
Asn	56.9	56.2	1.86	0.780
Asp	4.55	3.69	0.56	0.254
Cit	102	97.5	4.00	0.332
Cys	22.0	22.8	0.66	0.193
Gln	207	201	6.31	0.425
Glu	43.3	46.3	1.18	0.079
Gly	344	318	11.1	0.110
Hyp	6.10	6.49	0.44	0.494
Orn	62.4	63.2	2.66	0.824
Pro	104	94.0	3.62	0.008
Ser	111	103	4.34	0.191
Tau	35.3	34.1	1.75	0.409
Tyr	65.9	61.4	2.54	0.218
Sum and ratio of AA				

Σ EAA	1280	1222	36.4	0.165
Σ NEAA	1423	1344	33.0	0.122
Σ total AA	2703	2566	66.5	0.133
Σ BCAA[1]	740	693	23.8	0.031
Σ SAA[2]	83.3	83.3	2.49	0.997
Σ UCAA[3]	539	508	14.7	0.174
His:Met	3.01	3.12	0.17	0.473
Lys:Met	5.27	5.19	0.14	0.676
1-methyl-histine	6.98	7.07	0.35	0.763
3-methyl-histine	2.25	2.18	0.07	0.556
Carnosine	11.9	12.4	0.39	0.252

[1] BCAA = branched-chain AA (Ile + Leu + Val)

[2] SAA = Sulfur-containing AA (Met + Cys + Tau + Cystathionine/allocystathionine + Homocystine)

[3] UCAA = Uric acid-containing AA (Arg + Gly + Cit)

DISCUSSION

This study evaluated the inclusion of two different legumes in a red clover-based diet on the milk production responses, N use efficiency and CH_4 emission from certified organic dairy cows. Tertiary legume-grass diets might result in a better protein-energy balance supply and may improve N use efficiency via increased rumen microbial protein synthesis and milk production and decreased N excretion to the environment and CH_4 emissions (Brito et al., 2008; Gerber et al., 2013). Diets based on red clover forage can have negative impact in milk production (Brito et al., 2007) and the addition of other legumes forage could improve the dietary energy utilization.

The pH of baleage averaged 5.61 between treatments and it is similar to values reported by Brito et al. (2008) although they were higher than pH values reported for alfalfa and red clover silages, which averaged 4.80 and 4.50 across studies of Broderick et al. (2007) and Broderick (2018). This was expected due the greater DM concentration and particle size of the baleages compared with silages. The higher pH stabilization and the lower concentration of lactic, acetic, and propionic acids it is a consequence of the wilting process of grass and legume during the baleage production (Van Soest, 1994). The lower proportion of soluble N and ammonia N in the RC diet has been found consistently (Dewhurst et al., 2003; Vanhatalo et al., 2009) and is attributed to the endogenous presence and activity of polyphenol oxidase in red clover inhibiting the proteolysis in the bale (Jones et al., 1995).

Intake of DM was greater on RC diet compare with LM diet (Table 3) probably due the higher DM digestibility in RC diet, which has been influenced by the components of the diets. The cows fed LM diet had a greater proportion of second-cutting forages, especially the alfalfa field, and an effect in DM digestibility might be due to increased forage fiber content. Moreover, RC diet had higher NDF digestibility and that might have a consequence of increased DMI (Onetti et al., 2002). The DMI of RC diet in this study were similar to values presented by Antaya et al. (2015) for Jerseys cows fed red clover baleage. Brito et al. (2007) reported that cows fed diets based on red clover silage or alfalfa silage had no effect in DMI. Likewise, Johansen et al. (2018) using a meta-analysis approach to compare feed intake and milk production in dairy cows fed different grass and legume species demonstrate that there was no difference across alfalfa, red clover and white clover diets for DMI, however cow fed white clover diet had higher milk yield.

The higher DMI presented by cows fed RC diet was not sufficient to further higher milk production or BW changes in this present study. Milk yield, 4% FCM, and ECM (kg/d) were unaffected by treatments (Table 3). The inclusion of different legumes, as white clover and alfalfa were not enough to increase milk production. One of the major concerns in fed a diet with red clover base only is the effect in milk production, since studies have proved that cows fed RC diets produced less milk than cows fed diet with alfalfa, white clover or legume-mixed diets (Brito et al., 2007; Broderick et al., 2007b;

Johansen et al., 2018). The BW change did not differ between treatments, averaging -0.23 kg/d, and our values corroborate with values showed by Brito and Broderick (2006).

Lower milk fat and protein concentrations (%) for RC diet compared with LM diet were in agreement with previous studies (Broderick et al., 2007a; Johansen et al., 2018). Greater DMI reduced ruminal retention time and consequently decreased energy available for milk yield between treatments. Vanhatalo et al. (2009) attributed the lower molar proportion of butyric acid in the rumen of cows fed red clover compared with grass to the major cause of the reduce milk fat concentration. The reduced milk protein concentration for RC diet may be related to the presence of polyphenol oxidases in red clover, which can form complexes with plant proteins and protect proteins from degradation in the rumen (Lee, 2014).

Except for CP, total tract apparent digestibility was greater on RC diet. Compared with LM diet, apparent digestibility (% of intake) was increased by an average of 7% for DM, 6.5% for OM, 20% for NDF, and 7% for ADF on the RC diet (Table 4). The higher apparent digestibility of the nutrients for red clover have been observed in previously studies (Hoffman et al., 1997; Broderick et al., 2007b). Hoffman et al. (1993) evaluating ruminal degradation kinetics of different legume forage (alfalfa, birdsfoot trefoil, red clover) showed a greater *in situ* ruminal DM degradation for red clover forage and that was justified by differences in phenological growth characteristics. However, higher nutrient digestibilities did not consistently improve production as demonstrated in this study.

Regarding the N metabolism, cows fed RC diet had greater N intake than cows fed LM diet. The inclusion of alfalfa in the LM diet may have reduced the N intake. Some studies have demonstrated that cows fed alfalfa usually have low N intake, probably reflected by rapidly N availability with indigestible fiber in the rumen and lower energy supply (Broderick et al., 2000; Dewhurst et al., 2003). The less efficient of N use from LM diet was confirmed by the highest plasma urea N concentration (Table 3). Higher PUN values have been shown from cows fed diets with alfalfa-based or alfalfa-grass mixed (Broderick et al., 2007a). Additionally, the lower N intake, associate with a greater rumen microbial protein synthesis, which captures more N from rumen-degradable protein, reduces ruminal absorption of ammonium and thus urea-N excretion via urine (Dickhoefer et al., 2018). No difference was observed between treatments for urinary urea N, but it is important to note that urinary urea N is the major source of ammonia emissions from dairy systems. Therefore, strategies to reduce not only the amount but also the contribution of urea N to total urinary N are crucial to minimizing the negative environmental impact of dairy farms (Dijkstra et al., 2013). Moreover, forage legumes like alfalfa, white clove, and red clover have the advantage of biological N fixation as main N source. Their inclusion in dairy cattle diets may thus further lower environmental emissions due to reduced use of synthetic N fertilizers in feed crop cultivation (Lüscher et al., 2014).

Urinary excretion of allantoin and creatinine (mmol/d) did not differ between treatments. A difference for the uric acid excretion was observed for cows fed RC diet producing mor than cows fed LM diet, and a trend for the values of PD was observed in occurrence of the higher uric acid excretion (Table 4). These results are similar to the results reported by Broderick et al., (2007) where cows fed RC diet presented higher values of uric acid and purine derivates compared to cows fed Alfalfa diet.

Cows fed RC diet had lower CH_4 yield (g/kg of DMI) compare with cow fed LM diet (Table 5). Feed legumes diets can reduce CH_4 emissions due to the lower fiber content, higher DMI and faster ruminal passage rate when compare with grasses

(Beauchemin et al., 2008). Studies comparing CH_4 emission from organic dairy cows fed red clover or legume-mixed diets are scarce. Gidlund et al. (2017) feeding a red clover diet reported CH_4 emission much higher than the reported in the present study, within an average of 24 g/kg of DMI, with cows DMI average of 19 kg/d and milk yield average of 29.5 kg/d. Hammond et al. (2014) found no differences between red clover and perennial ryegrass fed as haylage or pasture grass in their effects on CH_4 production, and Dorland et al. (2007) concluded that white clover and red clover had similar effects on CH_4 emission. Despite the similar NFD intake between RC and LM diet, RC diet had a higher (20%) NDF apparent digestibility (Table 4). The passage rate of potentially digestible NDF in the rumen has been reported to be higher in cows fed diets based on red clover (Halmemies-Beauchet-Filleau et al., 2014) and that could explain the lower CH_4 yield by cows fed RC diet in the present study.

Vanhatalo et al. (2009) compared red clover versus grass silage diets in terms of individual plasma AA and demonstrated a higher concentration for cows fed red clover diet, suggesting an increased supply of absorbed AA. Their results are in line with the data presented in the current study (Table 6). Cows fed RC diet had a higher concentration of Leu, Val, Ala, and Pro compare with cows fed LM diet, and Leu has been proposed as a second-limiting AA for milk production after His (Kim et al., 2001). However, the higher concentration in AA, especially Leu, did not affect milk yield, and cows fed RC diet had a lower milk protein concentration.

CONCLUSION

Partially replacing RC diet with a LM diet in this current study did not affect N utilization in lactating dairy cows, and despite improved DMI and digestibility, and decreased CH_4 yield, cows fed RC diet did not produce more milk possibly because additional energy was not partitioned into milk synthesis. Therefore, developing mixtures of perennial legumes and grasses selected to increase forage quality via energy-dense stored feed have a strong potential to increase milk production and decrease greenhouse gas emissions of organic dairy farms.

REFERENCES

Antaya, N.T., R. Berthiaume, G.F. Tremblay, and A.F. Brito. 2015. Short communication: Feeding red clover cut in the afternoon or morning to late-lactation dairy cows. J. Dairy Sci. 98:7335–7339. doi:10.3168/jds.2015-9333.

AOAC International. 2016. Official Methods of Analyses, 20th ed. AOAC International, Gaithersburg, MD.

Beauchemin, K.A., M. Kreuzer, and T.A. Mcallister. 2008. Nutritional management for enteric methane abatement : 21–27.

Brito, A.F., and G.A. Broderick. 2006. Effect of Varying Dietary Ratios of Alfalfa Silage to Corn Silage on Production and Nitrogen Utilization in Lactating Dairy Cows. J. Dairy Sci. 89:3924–3938. doi:10.3168/jds.S0022-0302(06)72435-3.

Brito, A.F., G.A. Broderick, J.J.O. Colmenero, and S.M. Reynal. 2007. Effects of Feeding Formate-Treated Alfalfa Silage or Red Clover Silage on Omasal Nutrient Flow and Microbial Protein Synthesis in Lactating Dairy Cows. J. Dairy Sci. 90:1392–1404. doi:10.3168/jds.S0022-0302(07)71625-9.

Brito, A.F., G.F. Tremblay, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaud, H. Lapierre, C. Benchaar, H. V. Petit, D.R. Quellet, and R. Berthiaume. 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. J. Dairy Sci. 91:3968–3982. doi:10.3168/jds.2008-1282.

Broderick, G.A. 2018. Utilization of protein in red clover and alfalfa silages by lactating dairy cows and growing lambs. J. Dairy Sci. 101:1190–1205. doi:10.3168/jds.2017-13690.

Broderick, G.A., A.F. Brito, and J.J.O. Colmenero. 2007a. Effects of Feeding Formate-Treated Alfalfa Silage or Red Clover Silage on the Production of Lactating Dairy Cows. J. Dairy Sci. 90:1378–1391. doi:10.3168/jds.S0022-0302(07)71624-7.

Broderick, G.A., A.F. Brito, and J.J.O. Colmenero. 2007b. Effects of feeding formate-treated alfalfa silage or red clover silage on the production of lactating dairy cows. J. Dairy Sci. 90:1378–1391. doi:10.3168/jds.S0022-0302(07)71624-7.

Broderick, G.A., R.P. Walgenbach, and E. Sterrenburg. 2000. Performance of lactating dairy cows fed alfalfa or red clover silage as the sole forage. J. Dairy Sci. 83:1543–1551. doi:10.3168/jds.S0022-0302(00)75026-0.

Brouwer, E. 1965. Report of sub-committee on constants and factors. London, UK.

Chen, X.B., Y.K. Chen, M.F. Franklin, E.R. Orskov, and W.J. Shand. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep.. J. Anim. Sci. 70:1534–1542. doi:10.2527/1992.7051534x.

Deyl, Z., J. Hyanek, and M. Horakova. 1986. Profiling of amino acids in body fluids and tissues by means of liquid chromatography. J. Chromatogr. 379:177-250

Dewhurst, R.J., W.J. Fisher, J.K.S. Tweed, and R.J. Wilkins. 2003. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86:2598–2611. doi:10.3168/jds.S0022-0302(03)73855-7.

Dickhoefer, U., S. Glowacki, C.A. Gómez, and J.M. Castro-Montoya. 2018. Forage and protein use efficiency in dairy cows grazing a mixed grass-legume pasture and supplemented with different levels of protein and starch. Livest. Sci. 216:109–118. doi:10.1016/j.livsci.2018.08.004.

Dijkstra, J., O. Oenema, J.W. van Groenigen, J.W. Spek, A.M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N2O emissions.. Animal 7 Suppl 2:292–302. doi:10.1017/S1751731113000578.

Dorland, H.A. Van, H. Wettstein, H. Leuenberger, and M. Kreuzer. 2007. Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emission of dairy cows 111:57–69. doi:10.1016/j.livsci.2006.11.015.

Fekkes, D. 1996. State-of-the-art of high-performance liquid chromatographic analyses of amino acids in physiological samples. J. Chromatogr. B Biomed. Appl. 682:3-22

Gerber, P.J., A.N. Hristov, B. Henderson, H. Makkar, J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, A.T. Adesogan, W.Z. Yang, J.M. Tricarico, E. Kebreab, G. Waghorn, J. Dijkstra, and S. Oosting. 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: a review.. Animal 7 Suppl 2:220–234. doi:10.1017/S1751731113000876.

Gidlund, H., M. Hetta, and P. Huhtanen. 2017. Milk production and methane emissions from dairy cows fed a low or high proportion of red clover silage and an incremental level of rapeseed expeller. Livest. Sci. 197:73–81. doi:10.1016/j.livsci.2017.01.009.

Groffman, P. M., A. J. Gold, K. Addy. 2000. Nitrous oxide production in riparian zones and its importance to national emission inventories. Chemosphere - Global Change Sci. 1465-9972/00/S

Hafla, A.N., K.J. Soder, A.F. Brito, R. Kersbergen, F. Benson, H. Darby, M. Rubano, and S.F. Reis. 2016. CASE STUDY: Feeding strategy and pasture quality relative to nutrient requirements of dairy cows in the northeastern United States. Prof. Anim. Sci. 32:523–530. doi:10.15232/pas.2015-01500.

Halmemies-Beauchet-Filleau, A., A. Vanhatalo, V. Toivonen, T. Heikkilä, M.R.F. Lee, and K.J. Shingfield. 2014. Effect of replacing grass silage with red clover silage on nutrient digestion, nitrogen metabolism, and milk fat composition in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. J. Dairy Sci. 97:3761–3776. doi:10.3168/jds.2013-7358.

Hammond, K.J., D.J. Humphries, D.B. Westbury, A. Thompson, L.A. Crompton, P. Kirton, C. Green, and C.K. Reynolds. 2014. Agriculture , Ecosystems and Environment The inclusion of forage mixtures in the diet of growing dairy heifers : Impacts on digestion , energy utilisation , and methane emissions. "Agriculture, Ecosyst. Environ. 197:88–95. doi:10.1016/j.agee.2014.07.016.

Hoffman, P.C., D.K. Combs, N.M. Brehm, and D.A. Welch. 1997. Performance of Lactating Dairy Cows Fed Red Clover or Alfalfa Silage. J. Dairy Sci. 80:3308–3315. doi:10.3168/jds.S0022-0302(97)76306-9.

Hoffman, P.C., S.J. Sievert, R.D. Shaver, D.A. Welch, and D.K. Combs. 1993. In Situ Dry Matter, Protein, and Fiber Degradation of Perennial Forages. J. Dairy Sci. 76:2632–2643. doi:10.3168/jds.S0022-0302(93)77599-2.

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.

P. S., Adesogan, A. T., Yang, W., Lee, C., Gerber, P. J., Henderson, B., Tricarico, J. M., 2013. SPECIAL TOPICS - Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91, 5045-5069.

Huhtanen, P., Cabezas-Garcia, E. H., Utsumi, S., Zimmerman, S., 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J. Dairy Sci. 98, 3394-3409.

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48:211–227. doi:10.1016/0377-8401(94)90173-2.

Intergovernmental Panel on Climate Change (IPCC) 2007. Climate change 2007. The physical science basis. In Contribution of Working Group 1 to the Fourth Assessment report of the Intergovernmental Panel on Climate Change (ed. S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor and HL Miller), pp. 20-91. Cambridge University Press, Cambridge, UK and New York, USA.

Johansen, M., P. Lund, and M.R. Weisbjerg. 2018. Feed intake and milk production in dairy cows fed different grass and legume species: A meta-analysis. Animal 12:66–75. doi:10.1017/S1751731117001215.

Jones, B.A., R.E. Muck, and R.D. Hatfleld. 1995. Red clover extracts inhibit legume proteolysis. J. Sci. Food Agric. 67:329–333. doi:10.1002/jsfa.2740670309.

Kim, C.H., J.J. Choung, and D.G. Chamberlain. 2001. Responses of milk production to the intravenous infusion of amino acids in dairy cows given diets of grass silage and cereal-based supplements. J. Anim. Physiol. Anim. Nutr. (Berl). 85:293–300. doi:10.1046/j.1439-0396.2001.00317.x.

Lee, M.R.F. 2014. Forage polyphenol oxidase and ruminant livestock nutrition. Front. Plant Sci. 5:1–9. doi:10.3389/fpls.2014.00694.

Lüscher, A., I. Mueller-Harvey, J.F. Soussana, R.M. Rees, and J.L. Peyraud. 2014. Potential of legume-based grassland-livestock systems in Europe: A review. Grass Forage Sci. 69:206–228. doi:10.1111/gfs.12124.

NRC. 2001. National Research Council. Nutrient Requirements of Dairy Cattle. 7th rev. e. Washington, DC.

Onetti, S.G., R.D. Shaver, M.A. McGuire, D.L. Palmquist, and R.R. Grummer. 2002. Effect of supplemental tallow on performance of dairy cows fed diets with different corn silage:alfalfa silage ratios. J. Dairy Sci. 85:632–641. doi:10.3168/jds.S0022-0302(02)74117-9.

Pelletier, S., G.F. Tremblay, A. Bertrand, G. Bélanger, Y. Castonguay, and R. Michaud. 2010. Drying procedures affect non-structural carbohydrates and other nutritive value attributes in forage samples. Anim. Feed Sci. Technol. 157:139–150. doi:10.1016/j.anifeedsci.2010.02.010.

Rosenthal, H. L. 1995. Determination of urea in blood and urine with diacetyl monoxime. Anal. Chem. 27:1980-1982

Silva, M.S., G.F. Tremblay, G. Bélanger, J. Lajeunesse, Y.A. Papadopoulos, S.A.E.

Fillmore, and C.C. Jobim. 2014. Forage energy to protein ratio of several legume–grass complex mixtures. Anim. Feed Sci. Technol. 188:17–27. doi:10.1016/j.anifeedsci.2013.11.006.

United States Department of Agriculture (USDA) 2010. National Agricultural Statistics. Retrieved November 12, 2010, from http://www.usda.gov/wps/portal/usda/usdahome

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. Nutr. Ecol. Rumin. 1–122. doi:10.7591/9781501732355.

Valadares, R.F.D., G.A. Broderick, S.C. Valadares Filho, and M.K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6.

Vanhatalo, A., K. Kuoppala, S. Ahvenjärvi, and M. Rinne. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J. Dairy Sci. 92:5620–5633. doi:10.3168/jds.2009-2249.