MARCOLINO FREDERICO MIGUEL

SUPLEMENTAÇÃO COM SILAGEM DE MILHO PARA VACAS LEITEIRAS EM PASTEJO: CONSUMO DO PASTO, PRODUÇÃO E COMPOSIÇÃO QUÍMICA DO LEITE

Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, Universidade do Estado de Santa Catarina, como requisito parcial para a obtenção do grau de Doutor em Ciência Animal.

Orientador: Henrique Mendonça Nunes Ribeiro Filho

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RESUMO

MIGUEL, Marcolino Frederico. **Suplementação com silagem de milho para vacas leiteiras em pastejo: consumo do pasto, produção e composição química do leite**. 2016. 170f. Tese (Doutorado em Ciência Animal – Área: Produção Animal). Universidade do Estado de Santa Catarina. Programa de Pós graduação em Ciência Animal, Lages, 2016.

A suplementação com silagem de milho é uma da ferramentas de manejo disponíveis para manter a produção individual de vacas leiteiras em pastejo quando a quantidade de pasto disponível aos animais é reduzida. No entanto, os efeitos da suplementação com forragens no consumo do pasto e na resposta produtivas destes animais tem sido pouco estudados. Os objetivos desta tese foram identificar e compreender os principais fatores que afetam o consumo do pasto de vacas em lactação quando suplementadas com forragens conservadas. Para isso, foram realizados 3 experimentos e um estudo da literatura na forma de meta-análise. No primeiro buscamos compreender os efeitos do níveis da suplementação (0, 4 e 8 kg de MS de silagem de milho) no consumo do pasto e resposta produtiva de vacas manejadas em uma oferta diária de pasto de 35 kg de MS acima no nível do solo. No segundo foram testados os efeitos do aumento da oferta do pasto na taxa de substituição e resposta produtiva. Neste caso trabalhos com dois níveis de suplementação (0 e 4 kg de MS de silagem de milho) e duas ofertas contratantes de pasto (25 e 40 kg de MS/ dia em nível do solo). No terceiro experimento foram testados os efeitos da suplementação com 5 kg de MS de silagem de milho em duas ofertas de pasto contrastantes (15 e 30 kg de MS acima de 3,0 cm do solo) e também uma estratégia de manejo que visa a redução da altura de saída dos pastos de vacas suplementadas com silagem, afim de reduzir os efeitos negativos da taxa de substituição. Os dois primeiros experimentos foram conduzidos no setor de Bovinocultura Leiteira do CAV/UDESC em Lages – SC, nos invernos de 2011 e 2012. O terceiro experimento foi realizado na fazenda experimental do INRA em Méjusseaume na França durante a primavera de 2014. Nos três experimentos foram feitas medidas semelhantes sobre os animais. Em todos eles foram medidos o consumo de pasto, o consumo de suplemento, produção e composição de leite, além do comportamento alimentar. Foram realizadas também medidas sobre o pasto, buscando caracterizá-lo antes e após o pastejo. Pode-se afirmar que a quantidade de pasto disponível, bem como a sua estrutura são determinantes para a resposta à suplementação o com silagem de milho para vacas leiteiras em pastejo. Em situações de oferta restrita, a suplementação com silagem promove o aumento do consumo de MS total e consequentemente da produção de leite. Entretanto, ao adotarmos estratégias de manejo que visam a redução da altura residual do pasto de vacas suplementadas com forragens conservadas, a taxa de substituição aumenta e o consumo de MS total não é afetado pela suplementação, mesmo em situações de baixa oferta diária de pasto.

Palavras-chave: taxa de substituição, resposta leiteira, suplementação com forragens.

ABSTRACT

MIGUEL, Marcolino Frederico. Supplementation with corn silage for grazing dairy cows: pasture intake, milk production and milk composition. 2016 170f. Tese (Doutorado em Ciência Animal - Area: Produção Animal). Universidade do Estado de Santa Catarina. Programa de Pós-graduação em Ciência Animal, Lages, 2016.

Supplementing grazing dairy cows with corn silage is a practice for increase the individual milk production on periods with low available pastures. However, the effects of supplementation with conserved forages on pasture intake and productive responses of grazing dairy cows has been little studied. The objectives of this study were understand the main factors with effect on pasture intake and milk production responses of grazing dairy cows supplemented with conserved forages. For this, three experiments and a literature study in the form of meta-analysis were performed. At first experiment we tested the effects of the levels of supplementation (0, 4 and 8 kg DM of corn silage) on pasture intake and productive responses of cows grazing at medium pasture allowance (PA), with on average 35 kg DM/d above the ground level. In the second experiment, the effects of increasing the PA on substitution rate and productive response were tested. In this case, we worked with two supplementation levels (0 and 4 kg DM silage maize) and two PA (25 and 40 kg DM/d above ground level). In the third experiment, we tested the effects of supplementation with 5 kg DM of corn silage in two contrasting PA (15 and 30 kg DM above 3.0 cm from the ground) and also a management strategy, with objective to target a similar post-grazing sward height of unsupplemented cows The first two experiments were performed at Lages, SC, Brazil in the winters of 2011 and 2012. The third experiment was conducted at the experimental farm of INRA in Méjusseaume, France during the spring of 2014. In the three experiments similar measures on animals were made. Were measured pasture and supplement intake, milk production and composition, and the animal behaviour. The individual pasture intake and milk production and composition were measured on both experiments Were also carried out measures on pre- and post-grazing pasture characteristics. The amount of available pasture and the pasture structure are crucial to the response to supplementation with corn silage for grazing dairy cows. At low available pastureas, the supplementation with corn silage can increase the total DM intake and the individual milk production However, by adopting management strategies to reduce the post-grazing sward, the substitution rate increases and the total DM intake is not affected by supplementation, even in low PA.

Key-words: substitution rate, milk response, supplementation with forages

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1 INTRODUÇÃO

Os sistemas de produção de leite em pasto são conhecidos principalmente pelos baixos custos de produção e otimização da produção de leite por hectare. Comparado a sistemas de produção onde os animais são parcial ou totalmente confinados, a produção de leite em pasto mostrou-se mais rentável principalmente em situações de baixos preços pagos ao produtores para o leite e altos custos na aquisição de alimentos (TOZER et al., 2003). Além da redução nos custos de alimentação destacam-se a redução do capital investido e dos custos operacionais deste sistema, aumentando a eficiência do trabalho (DILLON et al., 2005). Em pastos de boa qualidade e bem manejados, vacas no terço médio de lactação tem produção de leite similar a animais que recebem suplementação. Entretanto, em situações de restrição na oferta do pasto ou baixa qualidade do mesmo, a produção individual tende a ser inferior, devido às limitações no consumo total de matéria seca, principalmente em vacas de alto mérito genético (KOLVER e MULLER, 1998; BARGO et al., 2002; PEYRAUD e DELAGARDE, 2013). Neste caso, alguns métodos de manejo podem ser adotados para aumentar o consumo individual de MS, afetando positivamente a produção de leite, tais como o aumento da oferta diária de pasto (RIBEIRO-FILHO et al., 2009; PÉREZ-PRIETO e DELAGARDE, 2013), inclusão de leguminosas nas pastagens (RIBEIRO-FILHO et al., 2003), suplementação com alimentos concentrados (DELAGARDE et al., 1999; STOCKDALE, 2000) e a suplementação com forragens conservadas (PHILLIPS, 1988).

A elevação na oferta diária de pasto (MS/vaca), em decorrência da diminuição das taxas de lotação (vacas/hectare), promove o aumento da ingestão das vacas em pastejo, afetando positivamente a produção individual de leite. Segundo o modelo proposto por Pérez-Prieto e Delagarde (2013), o consumo de pasto seria 1,8 kg de MS/dia superior em situações de alta oferta quando comparamos duas ofertas contrastantes (40 vs. 20 kg de MS/dia alta e baixa oferta medidas em nível do solo, respectivamente). Este aumento no consumo do pasto leva ao aumento do aporte energético e consequentemente na produção de leite dos animais em alta oferta diária de pasto (PEYRAUD et al., 1996; WALES et al., 1999). Em pastos de azevém anual, os efeitos da oferta diária sobre o consumo e a produção de leite de vacas em lactação foram testados por Ribeiro-Filho et al. (2009). Nesse experimento foram observados efeitos semelhantes aos obtidos em pastos de azevém perene, sendo que o aumento da oferta diária de pasto de 23 para 37 kg de MS, promoveu um aumento de 28% na ingestão de pasto e aumento a produção individual em 2,7 kg de leite por dia. Entretanto, o aumento da oferta reduz a proporção de foragem colhida em relação a biomassa inicial (BAUDRACO et al., 2011), ou seja aumenta a biomassa residual, o que pode afetar a qualidade do pasto também nos ciclos de pastejo subsequentes (PURCELL et al., 2011). Ao otimizar o consumo e a produção individual em pastejo, com diminuição da taxa de lotação, a produção de leite por hectare pode cair 24%, sendo acompanhada por uma queda de 19% da forragem colhida (BAUDRACO et al., 2011). Assim, o sucesso dos sistemas de produção a base de pasto está associado a capacidade dos animais em colher o máximo de pasto sem afetar a taxa de acúmulo nos rebrotes subsequentes, uma vez que ao favorecer exclusivamente o consumo individual todo o sistema pode ser penalizado.

Otimizar os sistemas de produção de leite à pasto implica em aumentar a quantidade de forragem colhida por hectare (PEYRAUD e DELAGARDE, 2013), desde que, a quantidade de pasto residual não afete a capacidade de rebrota do mesmo. De outra forma, com o constante aumento no número de animais por rebanho, as áreas disponíveis para o pastejo tornam-se um limitado recurso para estes sistemas (RAMSBOTTOM et al., 2015). Para Dillon et al. (2008), quando a área disponível para os animais pastejarem diminui, tem-se uma proliferação de sistemas de confinamento, com altos custo de produção e consequências indesejáveis do ponto de vista ambiental. Como estratégia para aumentar a lucratividade, a inclusão de forragem conservada como suplemento na dieta pode proporcionar um aumento na produção de leite individual e também por hectare (RAMSBOTTOM et al., 2015). Nestes casos, o uso de forragens conservadas poderia reduzir o efeito da variação no consumo do pasto e aumentar a eficiência do uso da terra quando em altas taxas de lotação (PHILLIPS, 1988).

O sucesso da suplementação com forragens conservadas está diretamente relacionado ao aumento no consumo de MS total de vacas quando em situações de oferta restrita de pasto. Elas são geralmente utilizadas em períodos de baixo crescimento do pasto, como no outono e inverno, ou até mesmo para evitar a degradação de pastagens em períodos chuvosos, onde os animais permanecem presos durante um período do dia recebendo o suplemento (PÉREZ-RAMIREZ et al., 2008; PÉREZ-PRIETO et al., 2011; PEYRAUD e DELAGARDE, 2013). A suplementação durante um período maior do ano surge da necessidade de aumentar a produção individual quando as pastagens não são suficientes para atender as demandas energéticas dos animais, principalmente vacas de alto mérito genético. Forragens suplementares caracterizam-se como fontes de energia mais baratas (KOLVER et al., 2001), sendo que no sul do Brasil grande parte dos produtores de leite utilizam a suplementação com silagem de milho durante todo o ano (KUHNEN et al., 2015). Este efeito na produção individual de leite promovido pela suplementação pode ser chamado de resposta leiteira. Vacas em pastejo não suplementadas que produziam 22,0 kg de leite/dia, ao consumirem em média 5 kg de silagem de milho passaram a produzir 24,4 kg de leite/dia, o que representa uma resposta leiteira de 0,5 kg de leite por 1,0 kg de MS de silagem consumida (STOCKDALE, 1997a).

As respostas leiteiras variam de acordo com o consumo de MS total dos animais suplementados. Para um mesmo nível de suplementação com silagem ela pode variar de 0,5 a 0,8 dependendo do efeito da suplementação no consumo total de matéria seca (STOCKDALE e DELLOW, 1995; WOODWARD et al., 2006). Para Mayne (1991) baixas respostas leiteiras são decorrentes da ineficácia do suplemento em possibilitar aumentos no consumo de MS total por vacas em pastejo e estão relacionadas aos altos efeitos substitutivos das forragens suplementares. Estes efeitos substitutivos são quantificados na forma de taxa de substituição, a qual é definida pela queda no consumo de MS do pasto por kg de MS de forragem suplementar consumido. Altas taxa de substituição fazem com que aumente a biomassa residual do pasto (STOCKDALE, 2000) e são maiores quando se utiliza forragens conservadas em comparação à suplementação com alimentos concentrados (INRA, 2007; DELAGARDE et al., 2011). Este aumento na taxa de substituição com forragens conservadas está ligado principalmente a maior capacidade destes suplementos em preencher o rúmen em relação aos alimentos concentrados (INRA, 2007).

A taxa de substituição é afetada por fatores relacionados à pastagem (oferta, altura, espécie, biomassa), ao suplemento (quantidade, valor nutritivo e tipo) e ao animais (nível de produção, fase de lactação) fatores bem descritos quanto ao efeito da suplementação com concentrados porém pouco estudados quando utiliza-se forragens conservadas (STOCKDALE, 2000; BARGO et al., 2003). Segundo o modelo GrazeIn proposto por Delagarde et al. (2011), ela pode variar de 0,4 a 1,1 de acordo com a oferta diária de pasto, sendo em média 0,8 para animais suplementados com silagem de milho. Este valor é similar ao observado por Phillips (1988) de 0,9. Porém, segundo Muller et al. (1988) este valor seria de 1,0, independentemente do tipo da forragem suplementar. Entretanto, diversos experimentos com forragens conservadas apresentam valores de taxas de substituição inferiores aos acima citados. Com uma oferta média de pasto de 22 kg de MS/dia, a taxa de substituição pode variar de 0,12 a 0,48 para vacas suplementadas com 5 kg de silagem (STOCKDALE e DELLOW, 1995; STOCKDALE, 1997a; 1997b; CHAVES et al., 2002; WOODWARD et al., 2006). Quando a oferta aumenta para 40 kg de MS/dia, o valor médio da taxa de substituição, para um nível de suplementação 4,4 kg de MS por dia de silagem de milho, aumenta para 0,61 (MORAN e STOCKDALE, 1992; STOCKDALE, 1996; MORRISON et al., 2007; PÉREZ-PRIETO et al., 2011; BURKE et al., 2008). Observa-se, portanto que em ambos os casos, alta e baixa oferta, as taxas de substituição são menores do que a citadas nos modelos existentes, evidenciado suas limitações para predizer a resposta produtiva de vacas leiteira em pasto quando suplementadas com forragem conservada.

Para Delagarde et al. (2011), o nível de suplementação com forragens conservadas também interfere na taxa de substituição em uma mesma oferta de pasto, de maneira similar ao que ocorre com alimentos concentrados. Entretanto, se simularmos este efeito em uma mesma altura do pasto, o aumento do nível de ingestão da forragem conservada não altera a taxa de substituição (INRA, 2007), o que deixa claro que este efeito da nível de suplementação não está bem estabelecido na literatura. Ao aumentar o nível de suplementação de 2,5 para 7,5 kg de MS por dia de silagem de milho para vacas em pastos de baixa qualidade, a taxa de substituição passou de -0,16 (aumento na ingestão de pasto dos animais suplementados) para 0,41 (MORAN e CROKE, 1993). Moate et al. (1984), não encontraram diferença na taxa de substituição ao aumentar de 3,0 para 6,0 kg de MS a oferta de silagem, sendo em média 0,30. Em ambos os experimentos a oferta diária de pasto medida em nível de solo era inferior a 25 kg de MS por dia. O modelo GrazeIn (DELAGARDE et al., 2011) estima que em uma mesma oferta diária 12 kg de MS, medida à 5,0 cm do solo, a taxa de substituição passaria de 0,54 para 0,76 se a oferta de silagem de milho passasse de 3,0 para 9,0 kg de MS. Para Delagarde e

O'Donovan (2005) são poucos os trabalhos que estudaram os efeitos da suplementação com forragens no consumo do pasto, em comparação aos observados quanto ao consumo de concentrados, resultando em poucos modelos de previsão de consumo que contemplem a utilização pasto + forragem suplementar na literatura.

Os objetivos do nosso estudo foram identificar e compreender os principais fatores que afetam o consumo do pasto de vacas em lactação quando suplementadas com forragens conservadas. Foram estudados também os efeitos que a suplementação com forragens conservadas provocam no consumo total de MS de vacas em pastejo e consequentemente no aporte energético e na resposta leiteira destes animais quanto a suplementação. Os resultados deste trabalho permitirão: melhorar a formulação da dieta de animais em pastejo a fim de assegurar melhores níveis zootécnicos em sistemas mistos de produção (pastagens + forragens conservadas); implementar novas técnicas de suplementação com forragens, evitando os efeitos deletérios das taxas de substituição principalmente quanto a ineficiência da colheita dos pastos.

Testamos a hipótese de que a suplementação com silagem de milho, para vacas leiteiras, provoca reduções no consumo do pasto, as quais variam com o manejo da pastagem e com o nível de oferecimento da silagem. Em alta oferta diária, o consumo de pasto se altera na mesma proporção em que aumenta a ingestão de silagem, entretanto, o desempenho animal não se altera com a suplementação. Em baixa oferta diária, a taxa de substituição diminui e o desempenho animal melhora com o fornecimento da silagem. Testamos também a hipótese que ao reduzir a oferta diária de pasto de vacas suplementadas, é possível controlar os efeitos adversos da taxa de substituição nas pastagens, devido a maior capacidade do animal ingerir o suplemento sem que este afete de maneira significativa o consumo do pasto. Entretanto, nestes casos ocorre o aumento da taxa de substituição em comparação a animais suplementados em uma mesma oferta diária que os não suplementados.

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2 QUESTÕES E ESTRATÉGIA DE PESQUISA

Diversas foram as situações apresentadas na introdução que apontam a necessidade da suplementação com forragens para vacas em lactação. Poucos, porém, são os trabalhos que mostram os efeitos deste tipo de suplementação em boas condições de pastejo. A maioria dos experimentos concentra-se em situações de baixa oferta ou no final da estação de crescimento do pasto, onde sua qualidade diminui.

A apresentação desta tese visa compreender e quantificar os efeitos da suplementação com silagem de milho sobre o consumo de pasto e o desempenho produtivo de vacas em lactação. Esta tese foi concebida com a proposta de responder três questões de ordem prática:

1. Quais são os efeitos do nível de suplementação com silagem de milho sobre a taxa de substituição e de que maneira esta variável afeta a resposta produtiva de vacas leiteiras em pastejo?

2. A oferta diária de pasto afeta a taxa de substituição e a resposta produtiva de vacas leiteiras quando suplementadas com silagem de milho?

3. Será que forma como é medida a taxa de substituição em pesquisas, permite prever a taxa de substituição e as respostas produtivas com a suplementação com silagem de milho em propriedades comerciais, onde o produtor normalmente busca bem valorizar o uso do pasto?

Para responder a primeira questão optamos em realizar um experimento com três níveis de forragem suplementar em uma mesma oferta de pasto (35 kg MS/dia). Com esta oferta diária de pasto, buscamos uma situação que não fosse extremamente limitante ao consumo de pasto por vacas no terço médio de lactação. Há poucos dados na literatura sobre este efeito, sendo que normalmente o nível de suplementação varia de acordo com as necessidades do produtor ao longo do ano, devido a sazonalidade na produção do pasto.

Para responder a segunda questão foram realizados dois experimentos. Em ambos foram adotadas duas ofertas diárias de pasto contrastantes, sendo uma limitante e outra não limitante ao consumo dos animais, e dois níveis de suplementação. A escolha das ofertas contou com o auxílio de modelos como sugerido por Pérez-Prieto e Delagarde (2013), o qual estima o efeito da oferta do pasto sobre o consumo de vacas em pastejo sem suplementação.

Para responder a terceira questão a estratégia de manejo adotada teve como meta a obtenção de uma altura residual do pasto nos piquetes das vacas suplementadas similar a altura residual dos piquetes das vacas não suplementadas. A estratégia tem como meta reduzir a oferta do pasto dos animais suplementados em comparação aos não suplementados afim de evitar os efeitos adversos da substituição sobre a colheita do pasto. Esta estratégia foi testada em um terceiro experimento, junto com o efeito da oferta do pasto. Neste caso, testou-se também a interação entre a estratégia e a oferta do pasto. Pelo nosso conhecimento, esta é a primeira vez que este tipo de protocolo foi testado em animais suplementados com forragens conservadas.

O estudo de literatura da tese foi concebido na forma de meta-análise e será apresentado após os artigos originados dos experimentos citados. Esta opção deu-se pelo fato de que algumas informações geradas nestes trabalhos foram incorporadas à base de dados analisada. A meta-análise teve como objetivo principal auxiliar nas respostas das questões desta tese, gerando modelos que visam a predição dos efeitos do nível de suplementação, oferta do pasto e estratégia de manejo na taxa de substituição e resposta produtiva de vacas suplementadas com forragens conservadas. Foram avaliados os efeitos da suplementação com forragem sobre a taxa de substituição global, quando o nível baixo de suplementação (testemunha ou controle) é igual a 0. Para isso foram criadas duas bases. Na primeira, vacas leiteiras suplementadas foram comparadas com vacas não suplementadas em uma mesma oferta diária de pasto. Na segunda vacas não suplementadas foram comparadas a vacas suplementadas com oferta diária de pasto reduzida em comparação aos animais não suplementados. O resumo da estratégias adotadas para responder nossas questões foi apresentado na Tabela 1.

Tabela 1 - Estratégias de pesquisa adotadas para compreender os efeitos do nível de suplementação, oferta do pasto e estratégia de manejo na taxa de substituição e resposta produtiva de vacas em pastejo suplementadas com silagem de milho.

	Experimentos			Meta-análise
	1	2	3	
Nível de suplementação	×			×
Oferta do pasto		×	×	×
Estratégia de manejo			×	×

- O experimento 1 foi conduzido em Lages, SC, Brasil no ano de 2011. Objetivo foi determinar o efeito do aumento do nível de suplementação com silagem de milho na taxa de substituição e resposta produtiva de vacas pastejando azevém anual em uma oferta diária de pasto não limitante. Os animais foram suplementados com 0, 4 e 8 kg de MS de uma mistura de silagem de milho mais farelo de soja (7:1 na MS) em uma oferta diária de 35 kg de MS medida acima do nível do solo.
- O experimento 2 também foi conduzido em Lages, SC, Brasil no ano de 2012. O objetivo foi determinar o efeito da suplementação com silagem de milho na taxa de substituição e a resposta produtiva de vacas pastejando azevém anual em duas ofertas diárias de pasto, bem

como os possíveis efeitos da interação entre a suplementação e a oferta. Os animais foram suplementados com 0 e 4 kg de MS de uma mistura de silagem de milho mais farelo de soja (7:1 na MS). As ofertas diárias foram de 25 e 40 kg de MS medida acima do nível do solo.

- O experimento 3 foi conduzido na região da Bretanha, França no ano de 2014. O objetivo foi determinar o efeito de diferentes estratégias de manejo do pasto (oferta ou altura de saída) em animais suplementados com silagem de milho sobre a taxa de substituição e a resposta produtiva de vacas pastejando azevém perene em duas ofertas diárias de pasto. Os possíveis efeitos da interação entre as estratégias adotadas e as ofertas de pasto também foram testados. Os animais foram suplementados com 0 ou 5 kg de MS de silagem de milho por dia. As ofertas diárias foram de 15 e 30 kg de MS medidos acima de 3,0 cm do solo. Para obtenção da mesma altura pós-pastejo dos animais suplementados em comparação aos não suplementados, a oferta diária de pasto dos primeiros foi reduzida em comparação aos últimos.

Nos três experimentos foram feitas medidas semelhantes sobre os animais. Em todos eles foram medidos o consumo de pasto, o consumo de suplemento, produção e a composição química do leite, além do comportamento alimentar. As medidas de consumo visam responder a principal questão da tese: qual o efeito da suplementação com silagem de milho sobre consumo de pasto? As respostas produtivas visam quantificar os efeitos da ingestão do pasto e do suplemento sobre o desempenho animal. O comportamento alimentar foi medido para auxiliar na compreensão de respostas advindas de possíveis modificações no tempo de pastejo e/ou taxa de ingestão após o aporte do suplemento e de acordo com as ofertas diárias de pasto.

Em todos os experimentos o consumo de pasto foi medido pela técnica de *n*-alcanos (MAYES et al., 1986). A escolha do método baseou-se na experiência de parte do grupo de trabalho com o mesmo e a possibilidade de utilizar um mesmo método nos dois países. Trata-se de um método confiável para estimar o consumo de animais em pastejo suplementados com silagem de milho, o qual foi testado tanto na França (PÉREZ-RAMIREZ et al., 2012) como no Brasil (OLIVEIRA et al., 2008) em animais recebendo silagem de milho como alimento suplementar.

O comportamento alimentar foi medido pelo método de visualização por observadores treinados nos experimentos conduzidos no Brasil. A escolha deste procedimento se deu pelo fato de não se dispor de nenhum dispositivo eletrônico, além da experiência do grupo de trabalho em experimentos anteriores (RIBEIRO-FILHO et al., 2011; ANDRADE et al., 2014). Na França optou-se em utilizar o dispositivo eletrônico Lifecorder plus (LCP, Suzuken Co. Ltd., Nagoya, Japão), o qual foi validado no ano de 2013 por Delagarde e Lamberton (2015).

As medidas sobre o pasto buscaram caracterizar o mesmo antes e após o pastejo. As principais medidas foram a altura comprimida, com o uso de prato ascendente, e de perfilho estendido. A biomassa pré-pastejo foi medida em todos os experimentos. Na França, optou-se em considerar a biomassa acima de 3.0 cm de altura para o cálculo da oferta do pasto. Esta escolha teve como objetivo evitar possíveis efeitos adversos de piquetes com diferentes quantidades de biomassa no cálculo da oferta de forragem acessível a colheita do animal em pastejo (PÉREZ-PRIETO et al., 2012).

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3 ARTIGO I

3.1 PASTURE INTAKE AND MILK PRODUCTION OF DAIRY COWS GRAZING ANNUAL RYEGRASS WITH OR WITHOUT CORN SILAGE SUPPLEMENTATION

3.2 INTRODUCTION

Feeding systems based on mixed diets comprising grazed pasture and conserved forages are useful during times of low pasture availability, such as periods of low pasture accumulation rate or in an area with low grazing availability on a per cow basis. However, in pasture-based systems, even at high pasture allowance (PA), energy intake is the primary determinant of milk production (DELABY et al., 2001), which is limited by physiological, behavioral and management constraints to the consumption of grazed pasture (WALES et al., 2005). In this context, the use of forage supplements can improve the total DM intake (PHILLIPS, 1988). Additionally, annual ryegrass is a major forage species in dairy and crop-livestock systems in many subtropical regions of the world, including southern Brazil. In these cases, it is common to supplement dairy cows grazing temperate pastures with cereal grains. However, interest in using mixed diets including grazing pasture and conserved forage is increasing.

Information regarding pasture DM intake (PI) regulation when conserved forages are fed is necessary to accurately predict cow performance in these feeding systems. The values of substitution rate (SR), which is the kilogram decrease of PI per kilogram of supplement DM intake, can vary from 0.3 to 1.1 kg DM/kg DM in situations with restricted and *ad libitum* PA, respectively, (PHILLIPS, 1988) and with the level of forage supplementation (MOATE et al., 1984; STOCKDALE, 1994). An increase in SR is know to have a negative effect on milk production response, which is the kilograms extra milk produced per kilogram of supplement DM intake (MAYNE, 1991), but the quality of the pasture and of the forage supplement can also affect this response (MOATE et al., 1984; STOCKDALE, 1997). When the pasture has similar or better nutritive value than the silage, the inclusion of the supplement in the diet generally decreases the milk production at high PA (PHILLIPS, 1988; DELAGARDE et al., 2011b). At low PA, supplementation with silage generally improves milk production, and milk response ranges from 0.44 to 0.82 kg milk/kg supplement, depending on the supplement quality (WOODWARD et al., 2006; DELAGARDE et al., 2011b). However, studies on PI regulation and SR when various amounts of conserved forages are fed to dairy cows at medium PA are scarce.

The objective of this study was to determine the effect of level of corn silage supplementation on pasture/forage substitution rate and milk production response to dairy cows grazing annual ryegrass at medium PA. The hypothesis tested in this study was that SR will increase with increasing level of corn silage soybean meal supplementation. To explain these results, grazing behaviour and a detailed characterization of the pasture were described.

3.3 MATERIALS AND METHODS

3.3.1 Treatments and experimental design

Three supplementation levels (0, 4 and 8 kg DM/d, namely WS, S4 and S8 treatments, respectively) were compared on lactating dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Common) at a medium daily pasture allowance (PA) of 35 kg DM/cow (cut to ground level). This PA level was choses considering the typical range of PA in grazing dairy cows (from 20 to 60 kg DM/cow, WALES et al., 1999), and the known PI to PA exponential relationship (PÉREZ-PRIETO and

DELAGARDE, 2013). The supplement was a 7:1 mixture based on the DM of corn silage and soybean meal, which was balanced such that the rumen microbial protein synthesis (EMPS) was not limited, as recommended by INRA (2007). The chemical composition, energetic and proteic value of supplements are presented in Table 2.

Table 2 - Chemical composition and nutritive value of supplements (corn silage and soybean meal)

	Supplement	
Item	Corn Silage	Soybean meal
DM (g/kg)	330	818
Chemical composition (g/kg DM)		
OM	962	931
CP	59	560
NDF	526	288
ADF	277	130
ADL[1]	27	02
Nutritive Value		
NE_L (MJ/kg DM)[2]	6.0	8.6

[1]ADL, acid detergent lignin.
[2]NE_L, net energy for lactation estimated according to INRA (2007)

The supplement was offered individually twice daily for 60 and 75 min for treatments S4 and S8 respectively, after the morning and afternoon milkings. After this time, the remaining supplement was considered refusals and removed from the barn. The treatments were compared according to a 3 × 3 Latin square design replicated three times. Each experimental period was for 12 days, with an 8-day adaptation and a 4-day measurement period.

3.3.2 Animals

Nine multiparous Holstein cows were separeted into three homogeneous groups according to milk production (23.0 ± 4 kg/day), lactation stage (175 ± 59 days) and live weight (506 ± 59 kg) measured 1 week before the start of the experiment. Fourteen days before starting the experiment, the cows grazed as one herd non-experimental pastures and were supplemented daily with 4 kg of corn silage DM and 0.6 kg soybean meal DM. Cows were milked twice daily at 0700 and 1600 hours.

3.3.3 Grazing management and pasture

The study was performed at Lages, SC, Brazil (50.18°W, 27.47°S and 920 m altitude). The experiment was conducted in winter from 20 July to 24 September, 2011. The ryegrass pastures were seeded in 20 April 2011, after the corn crop harvest (*Zea mays*). Thirty days before starting the experiment and immediately after each cycle, the experimental area was fertilized with 50 kg N/ha supplied as ammonium nitrate. The grazing method was strip grazing, and the area allocated daily to each treatment group was calculated from a daily estimate of pre-grazing pasture mass (see below) to give 35 kg DM/com.day. One uniform 2.4-ha paddock was split into three 0.8-ha paddocks, and each one was assigned to one experimental treatment. The same paddock was grazed thrice, once per period. After each period, the entire area was mowed to standardize the characteristics of pasture regrowth between treatments. Between period intervals (two periods of 15 days), the cows grazed non-experimental pastures and were supplemented daily with 4 kg corn silage DM and 0.6 kg soybean meal DM. Water and minerals were continually available at grazing. During the experiment, the mean temperature was 16.6°C and the total rainfall 239 mm, and the climatic averages over 10 years were 14.5°C and 161 mm, respectively.

3.3.4 Animal measurements

The milk production was recorded for each cow at each milking. The milk fat and protein concentrations were measured on the last 4 of each period by infrared spectrophotometry (International IDF Standard 141C:2000). The liveweight was measured at the beginning and the end of each experimental period.

The PI was measured from day 9 to day 12 of each period using the *n*-alkane technique (MAYES et al., 1986) with the ratio of pasture C_{33} (tritriacontane) to dosed C_{32} (dotriacontane). Throughout each period, all cows were dosed twice daily with a cellulose stopper (Carl Roth, GmbH, Karlsruhe, Germany) containing 293 mg of C_{32}, beginning on day 1 before milking. During the 4 days of measurements, faecal grab samples were collected from each cow after each milking. The faeces were oven-dried at 60°C for at least 72 h, then composited by period and cow and ground through a 1-mm screen for subsequent chemical analyses. The pastures samples were collected on day 9 and day 11 as described in the following section.

The daily pattern of grazing time was measured individually by visual observations every 5 minutes from 0700 hours to 1900 hours and every 10 minutes from 1900 hours to 0700 hours on day 10 and day 12. Ruminating time was determined with the same methodology and at the same time as grazing time, i.e. only when cows were at grazing. No behavior was recorded indoors when the cows were milked or fed the supplement. Consequently, because the observation duration differed between treatments, ruminating time was expressed in percentage of total time of observation. The pasture intake rate (g DM/min) was calculated by dividing daily PI by daily grazing time.

The energetic balance was calculated as the ratio between NE_L supply and NE_L requirements. The NE_L requirements for lactation and maintenance were calculated from 4% FCM production and liveweight, respectively, using the equations from INRA (2007). The NE_L supply was calculated from the pasture, silage and soybean meal DM intake and the NE_L concentrations of each feed, accounting for the negative digestive interactions between concentrate and forages (INRA, 2007). Energy balance was calculated because the experimental periods were too short to measure any changes in body condition score or liveweight change.

3.3.5 Feed and sward measurements

The amounts of corn silage and soybean meal offered and refused were weighed at each meal, after morning and afternoon milkings, and subsampled from day 9 to day 12 of each experimental period. All samples were oven-dried at 60°C for at least 72 h and ground through a 1-mm screen for chemical analyses.

The pre-grazing pasture mass above ground level was estimated twice per period on day 0 and day 7 by means of sward height, measured by means of a rising plate meter (Farmworks®, F200 model, New Zealand), and DM amount present within the plate area (0.1m^2). During each grazing cycle, regression equations were obtained for pasture mass estimation (kg DM/ha) as a function of sward height (t'MANNETJE, 2000), and five points for each treatment were cut with scissors at ground level. After manual removal of soil and roots, the samples were dried in an oven for 72h at 60ºC. The pre-grazing extended height of the leaf blade and the highest sheath were measured on 100 tillers at random on days 9 and 11. The post-grazing leaf and sheath extended heights were measured on days 10 and 12, on 200 tillers per treatment.

The morphological and chemical compositions of the swards for each treatment were determined on days 9 and 11. Twenty handfuls of randomly selected herbage (~ 800 g fresh) was cut with scissors close to the ground in the strip to be grazed the following day, then manually sorted. This material was cut at the average post-grazing extended tiller height. This was considered as representing pasture selected by cows and was separated into two smaller subsamples. One subsample was dried in an oven for 72 h at 60°C with forced ventilation and stored for chemical analyses, and the another subsample was used for morphologic classification (ryegrass only). The ryegrass separated into leaf blades, pseudostems, including stems, flowers and dead tissues if any. Each constituent was dried in an oven for 72 h at 60°C to determine morphological composition on a DM basis. The *n*-alkanes concentration was determined on each morphological constituent. The proportion of *n*-alkanes in the selected pasture was then calculated from the *n*-alkanes concentration of each constituent and the known proportion of constituents into the selected pasture.

3.3.6 Chemical analyses

The DM concentration was determined by drying at 105°C for 24 hours. The ash was determined by combusting in a muffle furnace at 550°C for 4 h. and the organic matter (OM) by mass difference. The total N was assayed by the Kjeldahl method (Method 984.13; AOAC 1997). The neutral detergent fibre (NDF) concentration was assessed according to Mertens (2002) except that samples were weighed into filter bags and treated with neutral detergent in ANKOM equipment (ANKOM Technology, Macedon NY, USA). This analysis included α-amylase but did not include sodium sulfite. The concentration of acid detergent fibre (ADF) and sulfuric acid detergent lignin (ADL) were analyzed according to Method 973.18 of AOAC (AOAC, 1997). The ether extract (EE) was determined in a reflux system with ethyl ether, at 180º C during 4 h (Extrator de Óleos e Graxas MA491, Marconi, Brazil). The *n*-alkanes were determined according to Dove and Mayes (2006).

3.3.7 Statistical analyses

The data was subjected to variance analysis using the PROC MIXED of Statistical Analysis Systems (SAS Institute 1999) including the random effects of cow and period, and the fixed effect of supplementation level. The linear and quadratic effects of supplementation level were tested by using polynomial orthogonal contrasts, where the quadratic component was equivalent to lack of fit sum of squares for linearity. Each *F*-value was a ratio of contrast mean square to the residual (experimental error) mean square.

The animal variables, averaged per cow and period (n = 27) were analyzed by using the following model:

$$Y_{ijk} = \mu + cow_i + period_j + supplementation\ level_k + e_{ijk}$$

where: Y_{ijk}, μ, cow_i, $period_j$, supplementation $level_k$ and e_{ijk}, represent, respectively, the analyzed variable, the overall mean, the random effect of cow, the random effect of period, the fixed effect of supplementation level and the residual error.

The pasture variables were averaged per treatment and period (n = 9) and analyzed by using the following model:

$$Y_{jk} = \mu + period_j + supplementation\ level_k + e_{jk}$$

3.4 RESULTS

3.4.1 Pasture characteristics

The pre-grazing pasture mass was greater by 165 kg DM/ha for S8 than on the other treatments. The area offered to cows was smaller by 18 m² for S8 than on the other treatments, enabling to adjustment to the area such that the pasture allowance were similar between treatments (34.3 kg DM/cow.d). The green material and live lamina PA showed a tendency to increase with increasing supplementation level. The post-grazing pasture mass was greater by 270 kg DM/ha on S8 than the other treatments (Table 3).

The pre- and post-grazing height, measured with rising plate meter, and the pre-grazing extended sheath height, were greater for S8 than the other treatments. The pre-grazing extended tiller and the post-grazing extended lamina, increased linearly with increasing supplementation level.

The chemical composition and nutritive value of selected pasture was similar for all treatments. Pasture quality was high, with an average of 18.0% of CP, 47.4% of NDF, and 23.0% of ADF in the DM.

Table 3 - Pre- and post-grazing pasture characteristics of annual ryegrass (*Lolium multiflorum* Lam.) swards when grazed by dairy cows receiving different levels of corn silage and soybean meal supplementation

	Treatment[1]			SEM	*P*-value	
Item	WS	S4	S8		Linear	Quadr.
Pre-grazing pasture mass (kg DM/ha)	1722	1773	1912	19.6	0.025	0.073
Post-grazing pasture mass (kg DM/ha)	1307	1371	1609	7.13	0.001	0.003
	Pre-grazing sward height (cm)					
Rising plate meter	10.9	11.1	12.4	0.14	0.022	0.040
Extended tiller	40.6	42.4	43.1	0.13	0.005	0.749
Extended sheath	21.6	21.8	23.1	0.09	0.010	0.015
Extended lamina	18.9	20.6	19.9	0.21	0.047	0.067
	Post-grazing sward height (cm)					
Rising plate meter	7.6	7.8	9.3	0.06	0.003	0.004
Extended tiller	21.2	25.1	24.8	0.36	0.015	0.106
Extended sheath	15.2	16.3	17.4	0.16	0.012	0.130
Extended lamina	5.6	6.7	7.4	0.26	0.041	0.491
	Pasture allowance (kg DM/day)					
Above ground level	34.2	34.4	34.3	0.04	0.073	0.252
Green material	23.3	26.1	27.0	0.67	0.057	0.855

Live lamina	11.6	12.6	13.4	0.37	0.073	0.594
Offered area, m²/cow per day	199	195	179	2.07	0.026	0.065
		Chemical composition (g/kg DM)				
DM (g/kg)	195	189	196	4.64	0.973	0.362
OM	922	924	927	1.42	0.121	0.366
CP	168	194	177	3.94	0.120	0.058
NDF	471	472	479	6.02	0.529	0.565
ADF	248	219	224	7.14	0.111	0.329
ADL[2]	22	17	21	1.26	0.291	0.127
Nutritive value						
NE_L, MJ/kg DM[3]	6.9	7.0	7.0	0.07	0.282	0.562

[1]Treatments: WS = without supplementation; S4 = supplementation with 4 kg of corn silage and soybean meal DM/day; S8 = supplementation with 8 kg of corn silage and soybean meal DM/day.
[2]Acid detergent lignin.
[3]Net energy for lactation estimated according to INRA (2007)

3.4.2 Animal performance

Supplement intake was lower than expected, being 3.0 and 4.3 kg DM/d for treatments S4 and S8, respectively. The cows ate only 75% and 54% of supplement offered on S4 and S8, respectively. The PI averaged 14.0 kg DM/d and was unaffected by treatment. Total DMI increased linearly with increasing supplementation level, on average by 0.82 kg DM/kg DM of supplement consumed. Consequently, the average substitution was estimated to be 0.18 kg decrease in PI/kg supplement DM consumed. The NE_L supply increased linearly by 4.5 MJ NE_L/d per kg of supplement DM (Table 4).

The milk production and 4% FCM production increased linearly with increasing supplementation level, on average by 0.52 kg and 0.47 kg, respectively, per kilogram of supplement DM intake. The same effect of supplementation was observed for daily milk protein production. The milk protein concentration was similar between S4 and S8 and was greater for the supplemented than the unsupplemented cows.

The daily grazing time was considerably shorter for the supplemented than for the unsupplemented cows (-112 min/d, i.e. -20%). On average, grazing time decreased by 31 min/d for each kg of DM of supplement eaten. This reduction of grazing time occurred mainly during the daytime, and particularly during morning and afternoons (Table 4). The grazing time during the evening and night were unaffected by supplementation level. The pasture intake rate increased linearly with increasing supplementation level. During their time at pasture, the cows on S4 spent a greater proportion of their time ruminating.

Table 4 - Effect of corn silage and soybean meal supplementation on DM intake, milk production and composition, energy balance, and grazing behavior of dairy cows strip-grazing annual ryegrass (*Lolium multiflorum* Lam.) at medium pasture allowance.

	Treatment[1]				*P*-value	
Parameter	WS	S4	S8	SEM	Linear	Quadr.
DM intake (kg/d)						
Pasture	14.4	13.6	14.0	0.47	0.423	0.406
Corn silage	-	2.58	3.75	0.11	-	-
Soybean meal	-	0.37	0.54	0.02	-	-
Total	14.4	16.5	18.3	0.49	<0.001	0.287
Milk production (kg/d)	20.1	21.7	22.2	0.45	0.006	0.883
4% FCM production (kg/d)	18.7	20.3	20.4	0.48	0.021	0.565
Milk fat concentration (%)	3.58	3.61	3.47	0.06	0.390	0.168
Milk protein concentration (%)	3.01	3.13	3.09	0.02	0.007	0.035
Milk fat production (g/d)	710	772	766	21.6	0.058	0.460
Milk protein production (g/d)	600	672	683	15.2	0.001	0.537
LW (kg)	503	508	507	3.14	0.261	0.586
NE_L supply (MJ/d)	99.5	111	121	3.74	0.002	0.464
NE_L balance[2]	1.07	1.11	1.18	0.04	0.093	0.490

Grazing time (min/d)						
Total	569	472	444	10.7	<0.001	0.592
Morning (06:00-12:00h)	169	133	118	4.30	<0.001	0.886
Afternoon (12:00-18:00h)	193	141	120	5.01	<0.001	0.910
Evening (18:00-24:00h)	123	134	126	10.0	0.685	0.458
Night (24:00-06:00h)	84	63	80	5.49	0.255	0.020
Pasture intake rate (g DM/min)	25.5	29.5	32.3	1.43	0.005	0.617
Ruminating (% time) [3]	41.9	45.0	42.3	0.73	0.246	0.010

[1]Treatments: WS = without supplementation; S4 = supplemented with 4 kg of corn silage and soybean meal DM/day; S8 = 4 supplemented with 8 kg of corn silage and soybean meal DM/day.
[2]Proportion of NE_L requirements satisfied by NE_L supply
[3]Percentage of total time of observation spent ruminating

3.5 DISCUSSION

One of the primary objectives of this study was to determine the effect of increasing silage supplementation level on the substitution rate and milk production response of grazing dairy cows. Unfortunately, because of the large number of refusals of corn silage at the highest level of supplementation, the actual silage DM intake differed only by 1.3 kg DM between the two supplementation levels. This range too narrow to correctly interpret any difference between these two treatments. As a consequence, and because no effect of supplementation level was detected for PI, the corn silage supplementation effects will be assessed on the basis of the average values observed in S4 and S8.

Several reasons may explain the high amount of silage refusals. In grazing systems, partial refusals of silage given to dairy cows have previously been observed. The proportion of silage refused appears dependent on the ratio between silage and pasture quality, and also to pasture restriction severity

(PHILLIPS, 1988). At low PA, cows grazing pastures with greater quality than that of the supplement refused 28% of silage offered in the study of Woodward et al. (2006), similar to the 26% of refusals observed in the S4 treatment. When the silage is offered *ad libitum* in the paddocks, cows optimised the diet quality by selecting pastures with high energy and protein values, decreasing 25% of the silage intake, particularly at high pasture availability (HERNANDEZ-MENDO and LEAVER, 2010). In our study, the high nutritive value of pasture offered, associated with a relatively low quality of corn silage, and the greater pre- and post-grazing pasture heights in S8 treatment, facilitating PI, may explain the high rate of refusals of corn silage in this treatment.

The increase in total DMI and milk production for supplemented cows support the hypothesis tested in this study. A high milk production response is commonly observed in cows with restricted pasture allowance when the SR is low, which increases the total DM and energy intake (PHILLIPS and LEAVER, 1985; WOODWARD et al., 2006). It has also been shown that cows with high PA can improve their total DMI with silage supplement when the pastures have low PM and low nutritive value (PÉREZ-PRIETO et al., 2011). In the present study, the low pasture intake level of unsupplemented cows may be explain the low substitution rate, which was most likely limited more by pasture characteristics than by PA level. The offered pasture had good nutritive value but very low PM at ground level, most likely associated with low pasture tiller density (not measured). With a similar pre-grazing sward height, Ribeiro-Filho et al. (2009) observed a PM of 2.6 t DM/ha in annual ryegrass pastures and this value being 45% higher than the average PM observed in the present study. Even in good-quality pastures, decreasing the pre-grazing PM at a similar PA measured at ground level have negative effect on the PI (PÉREZ-PRIETO et al., 2013). When the PM is measured at ground level, the ungrazable stratum (below 2-3 cm) is greater at low PM because of the larger area offered, which decrease the actual pasture availability and pasture intake (PÉREZ-PRIETO and DELAGARDE, 2012). In this study , 70% of the PA was below 2 cm [calculated as proposed by Delagarde et al. (2011a)] and only 10 kg DM/d was available (i.e. above 2 cm) for cows, indicating that the pasture intake was limited by sward structure rather than by PA *per se*. The unsupplemented cows, with such low PA above 2 cm has a predicted pasture intake of only 11 kg DM/day (DELAGARDE et al., 2011b).

The low intake of pasture associated with the low PM is most likely to be the main factor explaining the low substitution rate. At similar PAs, pastures with low PM also provide low substitution rates (STOCKDALE and DELLOW, 1995) because pasture intake is restricted. When the PA is restricted, the use of forage supplements did not affect the intake of good-quality pasture and the values of SR averaged 0.16 (STOCKDALE, 1997; WOODWARD et al., 2006), which is similar to the values obtained in this study.

The milk response to silage supplementation is consistent with the increase in total DMI. The intake of supplements allowed higher energy supply, through increasing total DM intake and not energy concentration because nutritive value of pasture is superior to that of the supplement. At low PA, the milk production response of 0.57 kg/kg DM silage obtained by Woodward et al. (2006) is similar to that obtained in this study. In the study of Woodward et al. (2006), grazing conditions were very similar to our study, with an average SR of 0.14 kg DM/ kg DM and a pasture of greater nutritive value than the silage. The increase in milk protein production and concentration also support the significant increase in energy intake when cows were supplemented. Consistent with the review of Coulon and Rémond (1991), variations of milk protein concentration are positively correlated with the level of energy supply in dairy cows.

In this study, supplementation with silage largely affected grazing time despite any effect on PI, which was unexpected. Previous studies showed that a reduction of grazing time ~ 35 min per kg of silage DM intake may be observed mainly when SR is high (PHILLIPS and LEAVER, 1985). The use of forage supplements at grazing generally decreases pasture intake rate (PHILLIPS and LEAVER, 1985; PÉREZ-PRIETO et al., 2011), most likely indicating less motivation for grazing when part of the diet is easily fed indoors. In this present study, the large decrease of grazing time with silage supplementation and the absence of variation of PI resulted in 21% increase in pasture intake rate. We feel that this could not result from inaccurate estimate of PI because the *n*-alkanes method has been showed to be very accurate for measuring pasture intake on mixed diets, including pasture and corn silage (PÉREZ-RAMIREZ et al., 2012).

It is known that low sward and leaf heights affect negatively pasture intake rate through smaller bite mass, (BARRETT et al., 2001; BARRE et al., 2006), and because cows spend more time grazing to compensate for the low intake rate (RIBEIRO-FILHO et al., 2011; PÉREZ-PRIETO et al., 2011). In our study, the greater pre- and post-grazing tiller and leaf heights, particularly at high supplementation level, may explain the higher pasture intake rate. This hypothesis is supported by the strong relationship between post-grazing height and pasture intake rat The increase in grazing time of unsupplemented cows may observed through variations of PA (PÉREZ-PRIETO and DELAGARDE, 2013). The increase in grazing time of unsupplemented cows may thus be a consequence of the low pasture intake rate, allowing to maintain PI compared with the supplemented cows.

3.6 CONCLUSIONS

Under good-quality sward conditions of southern Brazil, dairy cows grazing annual ryegrass at medium pasture allowance ate a maximum 4 kg DM of a supplement based on corn silage and soybean meal, making it not possible to determine the effect of increasing supplementation level on substitution rate and milk production response. On average, cows increased their total DM intake and milk production when supplemented with corn silage. The low pasture mass limited the pasture intake and promoted low substitution rates even if pasture allowance was not limiting.

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4 ARTIGO II

4.1 CORN SILAGE SUPPLEMENTATION TO DAIRY COWS GRAZING ANNUAL RYEGRASS AT TWO PASTURE ALLOWANCES

4.2 INTRODUCTION

Annual ryegrass (*Lolium multiflorum* Lam.) is a major forage species used in dairy systems in many subtropical regions, where annual forage species are useful in mixed crop-livestock systems. These systems produce half of the world's food (HERRERO et al., 2010) and are considered more profitable and sustainable than more specialized production systems (RUSSELE et al., 2007). Some additional reasons for using annual ryegrass are the possibility of natural reseeding and high nutritional value (BARTH NETO et al., 2013).

In these systems, the use of corn silage supplementation can be a tool for increasing the stocking rate and milk production per hectare (MOATE et al., 1984; PHILLIPS, 1988). At cow level, the effects of this silage supplementation at grazing on milk production are variable and unclear. This is because the milk production response (MR – increase in milk production per kilogram of supplement DM intake) is dependent of substitution rate [SR – reduction on pasture DM intake (PI) per kilogram of DM supplement intake] (PHILLIPS, 1988; KOLVER et al., 2001), which is the result of many interactions between pasture management, pasture traits and supplement characteristics (MOATE et al., 1984; PHILLIPS, 1988; DELAGARDE et al., 2011).

The SR between pasture and a forage-based supplement can vary from 0.3 to 1.1 kg DM/kg DM in situations from restricted to *ad libitum* pasture allowance (PA – kg DM/cow.day), respectively, affecting total DM intake and MR (PHILLIPS, 1988; DELAGARDE et al., 2011). Nevertheless, even at high PA, relatively small values of SR have been described in the case of low pasture mass (STOCKDALE, 1996; MIGUEL et al., 2014). This occurs because a low pre-grazing pasture mass per se may restrict PI, independently of PA. Considering that low pasture mass is commonly observed in annual ryegrass pastures, mainly in the first grazing cycles following sowing (MIGUEL et al., 2014), studies to evaluate SR and MR as a function of grazing management are necessary to better predict the nutritional advantages of corn silage supplementation of grazing dairy cows.

The aim of this work was to investigate the effect of corn silage supplementation to dairy cows grazing annual ryegrass on total DM intake and milk production response at two contrasting levels of PA. We hypothesized that, even with low pasture mass, any increase of PA would increase SR and reduce MR in dairy cows grazing annual ryegrass and supplemented with corn silage.

4.3 MATERIAL AND METHODS

4.3.1 Treatments and experimental design

A 2 × 2 factorial design was studied, with two PA (low - 25; high - 40 kg DM/day above ground level) and two supplementation levels (0 and 4 kg DM/day). The four treatments were compared on lactating dairy cows grazing annual ryegrass (*Lolium multiflorum* cv. Common). The supplement was a 7:1 mixture based on DM of corn silage and soybean meal, which was balanced such that the rumen microbial protein synthesis (EMPS) was not limited, as recommended by INRA (2007). The chemical composition and energetic value of the supplements are presented in Table 5.

The supplement was offered individually twice daily after morning and afternoon milkings, for 60 min at each time (2 hours/day). After this time, the remaining supplement was considered refusals and removed from the barn. During the periods of feeding the supplement, unsupplemented cows were on pasture. The treatments were compared according to an incomplete 4×3 Latin square design replicated three times and balanced for carryover effects (JONES and KENWARD, 1989). Each experimental period was of 12 days, with an 8-day adaptation period and a 4-day measurement period.

Table 5 - Chemical composition and nutritive value of supplements (corn silage and soybean meal)

	Supplement	
Item	Corn silage	Soybean meal
Dry matter (g/kg)	303	878
	Chemical Composition (g/kg DM)	
Organic matter	951	942
Crude protein	54	492
Neutral detergent fiber	533	163
Acid detergent fiber	251	88
	Nutritive value	
NE_L (MJ/kg DM)[1]	6.2	8.5

[1]Net energy for lactation estimated according to INRA (2007)

4.3.2 Animals

Twelve multiparous Holstein dairy cows were separated into four homogeneous groups according to milk production (22.1 ± 5.4 kg/day), lactation stage (129 ± 61 DIM) and live weight (591 ± 115 kg) measured one week before the start of the experiment. Two weeks before the start of the experiment and between experimental periods cows grazed as one herd on non-experimental pastures of annual ryegrass and were supplemented daily with 4 kg corn silage DM and 0.6 kg soybean meal DM. Cows were milked twice daily at 0730 and 1600 h throughout the experiment.

4.3.3 Grazing management and pasture

The study was performed in Lages, SC, Brazil (50.18°W, 27.47°S and 920 m altitude). The experiment was conducted in the winter and spring from 25 August to 11 October, 2012. The ryegrass pastures were seeded after the corn crop harvest (*Zea mays*) in May 02, 2012. Thirty days before the start of the trial and immediately after each grazing cycle, the experimental area was fertilized with 50 kg of N/ha, supplied as ammonium nitrate. One uniform area including paddock 1 (2.4 ha) and paddock 2 (5.0 ha) was used for the experiment. For the first period, paddock 1 was split into four sub-paddocks, one for each group. Paddock 2 was divided into 8 sub-paddocks, and each one was assigned to one experimental treatment in the second or third period. The grazing method was strip-grazing, and the area allocated daily to each treatment group was calculated from a daily estimate of pre-grazing pasture mass (see Feed and Measurements section) to give 25 or 40 kg DM/cow, according to the treatment. Fresh pasture was allocated once daily after morning milking, the area allocated daily being adjusted by means of electric fences. Water and minerals were continually available at grazing.

4.3.4 Animal measurements

The milk production was recorded for each cow at each milking. The milk fat concentration and milk protein concentration were measured on the four last days of each period by infrared spectrophotometry (International IDF Standard 141C:2000). The live weight was measured at the beginning and end of each experimental period.

The individual PI was measured from days 9 to 12 of each period using the *n*-alkane technique (MAYES et al., 1986) with the ratio of pasture C_{31} (hentriacontane) to dosed C_{32} (dotriacontane). Throughout each period, all cows were dosed after morning and afternoon milking with a cellulose stopper (Carl Roth, GmbH, Karlsruhe, Germany) containing 164 mg of C_{32}, beginning on day 1. During the 4 days of measurements, faecal grab samples were collected from each cow after each milking. The faeces were oven-dried at 60°C for at least 72 h, then composited by period and cow, and ground through a 1-mm screen for subsequent chemical analyses. The pasture samples were collected on days 9 and 11 as described in the following section.

The net energy balance was calculated as the ratio between NE_L supply (MJ/day) and the NE_L requirements (MJ/day) according to INRA (2007). The NE_L requirements for lactation and maintenance were calculated from 4%-fat corrected milk (FCM) production and live weight, respectively. The NE_L supply was calculated from the pasture, silage and soybean meal DM intake and the NE_L concentrations of each feed, accounting for the negative digestive interactions between the concentrate and forages (INRA, 2007).

The daily pattern of grazing was measured individually by visual observations of trained observers every 10 min for 24 h on days 10 and 12. Daily grazing time was calculated as the number of grazing observations multiplied by 10. The pasture intake rate (g DM/min) was calculated by dividing the daily PI (kg DM) by the daily grazing time (min). No behaviour was recorded indoors when the cows were milked or fed the supplement.

4.3.5 Feed and sward measurements

The amounts of corn silage and soybean meal offered and refused for each cow were weighed at each meal and subsampled from days 9 to 12 for each experimental period. All samples were oven-dried at 60°C for at least 72 h and ground through a 1-mm screen for subsequent chemical analyses.

A rising plate meter (Farmworks®, F200 model, New Zealand) was used to estimate the pre and post-grazing pasture mass as an indirect method (‘t MANNETJE, 2000). On day 7 of each period, five points per treatment - from the lowest to highest point to pasture - were cut at ground level with scissors in the plate area (0.1 m^2) after the height measurement. After manual removal of soil and roots, the samples were dried in an oven for 72 h at 60°C. One equation was generated for each period, and used to calculate PA during measurements days (day 9 to day 12). In periods of adaptation, the pre-grazing pasture mass was calculated with the equations generated by Miguel et al. (2012; 2014) at same experimental station. For analytical purposes pasture mass were recalculated with all measurements per period, using one equation to estimate the pre-grazing pasture mass and one equation to estimate the post-grazing pasture mass, as following:

$$\text{Pre-grazing PM (kg DM/ha)} = 107 + 75\ (\pm 5.1) \times \text{pre-grazing sward height (cm)}\ (n = 60,\ R^2 = 0.83,\ \text{r.s.d.} = 421)$$

$$\text{Post-grazing pasture PM (kg DM/ha)} = 101 + 72\ (\pm 5.2) \times \text{post-grazing sward height (cm)}\ (n = 60,\ R^2 = 0.80,\ \text{r.s.d.} = 426)$$

The pre-grazing extended height of the tiller (upper lamina) and of its highest sheath were measured on 100 tillers per treatment taken at random on days 9 and 11. The post-grazing tiller and sheath extended heights were measured on days 10 and 12 on 200 tillers taken at random per treatment.

The morphological and chemical compositions of the sward for each treatment were determined on days 9 and 11 at each period. Twenty handfuls of randomly selected herbage

(~800 g fresh) was cut with scissors at ground level and stored at freezer (-8°C), maintaining carefully the vertical structure of pasture. After this, the sward was cut at the average post-grazing extended tiller height of the corresponding treatment, with the objective to represent the pasture selected by grazing cows. The upper part was separated into two subsamples. One subsample was dried in an oven for 72 h at 60°C with forced ventilation and stored for chemical analyses. The other subsample was used for morphological classification (ryegrass only). Annual ryegrass composed 98% of pasture available to cows. The ryegrass was separated into green leaves, stems + pseudostems and dead tissues if any. Each constituent was dried in an oven for 72 h at 60°C to determine the morphological composition on a DM basis.

4.3.6 Chemical analyses

Ash was determined by combustion in a muffle furnace at 550°C for 4 h, and the organic matter (OM) was determined by mass difference. Total N was assayed using Kjeldahl method (Method 984.13; AOC 1997). Neutral detergent fibre (NDF) concentration was assessed according to Mertens (2002), except that the samples were weighed into filter bags and treated with neutral detergent in a ANKOM equipment (ANKON Technology, Macedon NY, USA). This analysis included α-amylase but did not include sodium sulfite. The concentration of acid detergent fibre (ADF) was analysed according to Method 973.18 of AOAC (AOAC 1997) after the NDF analyses. N-alkanes were determined according to Mayes et al. (1986). The net energy concentration of feeds were calculated from their chemical composition according to INRA (2007).

4.3.7 Statistical analyses

All data were subjected to variance analysis using the PROC MIXED of the Statistical Analysis Systems (SAS Intitute, 1999). Animal data averaged per cow and period (n = 32) were analyzed using the following model:

$$Y_{ijkl} = \mu + \text{cow}_i + \text{period}_j + \text{pasture allowance}_k + \text{supplementation level}_l + [\text{pasture allowance}_k \times \text{supplementation level}_l] + e_{ijkl};$$

Where Y_{ijkl}, μ, cow_i, period_j, $\text{pasture allowance}_k$, $\text{supplementation level}_l$, [$\text{pasture allowance}_k \times \text{supplementation level}_l$] and e_{ijkl} represent, respectively, the analyzed variable, the overall mean, the random effect of the cow, the random effect of the period, the fixed effect of PA, the fixed effect of supplementation, the fixed effect of the interaction $\text{allowance}_k \times \text{supplementation}_l$ and the residual error.

Pasture data averaged per treatment and period (n = 12) was analyzed using the following model:

$$Y_{jkl} = \mu + \text{period}_j + \text{pasture allowance}_k + \text{supplementation level}_l + [\text{pasture allowance}_k \times \text{supplementation level}_l] + e_{jkl};$$

Where Y_{ijkl}, μ, period_j, $\text{pasture allowance}_k$, $\text{supplementation level}_l$, [$\text{pasture allowance}_k \times \text{supplementation level}_l$] and e_{ijkl} represent, respectively, the analyzed variable, the overall mean, the random effect of the period, the fixed effect of PA, the fixed effect of supplementation, the fixed effect of the interaction $\text{allowance}_k \times \text{supplementation}_l$ and the residual error.

4.4 RESULTS

4.4.1 Pasture characteristics

There was no interaction between pasture allowance and supplementation level for any pasture characteristics or for grazing management (Table 6). The pre-grazing pasture mass and platemeter sward height were similar between treatments averaging 1,902 kg DM/ha and 12 cm, respectively. The extended sheath height averaged 0.71 of extended tiller height. The post-grazing pasture mass and post-grazing extended sheath and lamina height did not differ with supplementation level, but were lower by 16 and 33% at low PA when compared with high PA respectively. The green leaves allowance was similar between supplementation levels and increased from 4.9 to 8.5 kg DM/d from low to high PA. The crude protein and neutral detergent fibre of selected pasture were similar between treatments, averaging 140 and 567 g/kg DM, respectively. The net energy value of selected pasture averaged 6.3 MJ NE_L/kg DM.

4.4.2 Dry matter intake and animal performance

The DM supplement intake was lower than expected, averaging 2.0 kg DM/d, i.e. approximately 50% of supplement offered, with slightly more refusals at high than at low PA (Table 7). The pasture DM intake was unaffected by PA, but was lower by 2.0 kg DM/d in supplemented than in unsupplemented cows. The total DM intake, milk production and milk protein production increased with silage supplementation at low PA, and were similar between supplemented and unsupplemented cows at high PA (interaction: $P<0.05$). The NE_L supply, FCM production and milk fat production increased with supplementation at low PA and decreased with supplementation at high PA (interaction: $P<0.05$). The daily grazing time and pasture intake rate decreased respectively by 59 min/d and 2.5 g DM/min in supplemented cows compared with unsupplemented cows, and were unaffected by PA.

Table 6 - Effects of pasture allowance (PA) and corn silage supplementation (S) on pre- and post-grazing pasture characteristics, grazing management and chemical composition of selected pasture by dairy cows grazing on annual ryegrass (*Lolium multiflorum* Lam.)

	Low PA		High PA		r.s.d[1]	*P* - value		
Item	0[2]	4[2]	0[2]	4[2]		PA	S	PA×S
Pasture mass (kg DM/ha)								
Pre-grazing	1796	1946	1910	1955	123	0.427	0.219	0.496
Post-grazing	1134	1169	1327	1325	97.9	0.021	0.775	0.755
	Pre-grazing sward height (cm)							
Rising plate meter	11.5	12.0	12.2	12.3	1.14	0.508	0.633	0.732
Extended tiller	42.3	44.4	45.6	44.8	1.38	0.055	0.430	0.124
Extended sheath	30.1	31.6	32.6	32.0	1.34	0.116	0.587	0.211
Extended lamina[3]	12.1	12.7	13.0	12.9	0.41	0.076	0.301	0.205
	Post-grazing sward height (cm)							
Rising plate meter	7.1	7.5	8.3	8.4	0.71	0.039	0.593	0.654
Extended tiller	25.6	26.7	31.4	33.6	3.27	0.015	0.419	0.770
Extended sheath	21.6	22.5	25.6	27.0	2.14	0.014	0.403	0.850
Extended lamina[3]	4.0	4.2	5.7	6.6	1.15	0.021	0.445	0.589
	Pasture allowance (kg DM/d)							
Above ground level	25.0	24.9	39.9	39.8	0.08	0.001	0.180	0.483
Green material[4]	22.5	24.1	38.6	38.6	1.42	0.001	0.380	0.396
Leaves	4.9	4.9	8.6	8.4	1.84	0.014	0.951	0.951
Offered area (m²/cow/d)	142	130	210	207	8.94	0.001	0.186	0.439
	Chemical composition (g/kg DM)							
DM (g/kg)	263	265	268	275	13.4	0.399	0.586	0.784
OM	941	940	942	940	05.2	0.912	0.675	0.826
CP	134	139	141	145	14.1	0.483	0.580	0.919
NDF	569	566	578	556	23.3	0.979	0.383	0.505
ADF	284	285	293	276	19.0	1.000	0.515	0.433
Energetic value (MJ/kg DM)	6.31	6.31	6.27	6.46	0.28	0.774	0.582	0.582

Low PA = 25 kg DM/d, High PA = 40 kg DM/d

[1]Residual standard deviation

[2]Level of supplementation: 0 = 0 kg of supplement DM/day; 4 = 4 kg of supplement DM/day
[3]Difference between extended tiller height and extended sheath height
[4]stem + pseudostem and leafs

Table 7 - Effects of pasture allowance (PA) and corn silage supplementation (S) on dry matter intake (DMI), energy balance, milk production, milk composition, and grazing behavior of dairy cows strip-grazing annual ryegrass (*Lolium multiflorum* Lam.)

	Low PA		High PA		r.s.d[1]	*P*-value		
Item	0[2]	4[2]	0[2]	4[2]		PA	S	PA×S
DMI (kg/d)								
Pasture	8.10	6.51	8.60	6.20	0.755	0.712	0.001	0.165
Corn silage		2.05		1.55	0.358	0.049	0.001	0.101
Soybean meal		0.29		0.22	0.051	0.049	0.001	0.101
Total	8.09[b]	8.85[a]	8.54	7.98	0.749	0.462	0.718	0.027
Substitution rate		0.68		1.35				
NE_L supply (MJ/day)	57.5[b]	61.2[a]	60.9[a]	55.5[b]	5.26	0.537	0.649	0.030
NE_L balance[c]	0.69	0.66	0.62	0.59	0.086	0.039	0.374	0.968
Milk production (kg/d)	13.2[b]	15.9[a]	16.3[a]	14.7[a]	2.44	0.296	0.572	0.027
4 % FCM production (kg/d)	11.6[b]	15.1[a]	15.5[a]	13.0[b]	2.66	0.367	0.612	0.007
Milk fat concentration (g/kg)	33.0	36.1	36.1	32.9	3.59	0.984	0.953	0.028
Milk protein concentration (g/kg)	34.5	34.6	34.3	34.5	1.03	0.748	0.736	0.896
Milk fat production (g/d)	421[b]	585[a]	601[a]	476[b]	118.5	0.420	0.653	0.004
Milk protein production (g/d)	444[b]	533[a]	556[a]	502[a]	76.5	0.154	0.538	0.021
Live weight (kg)	561	560	561	555	8.8	0.542	0.325	0.486
Grazing time (min)								
Total	561	491	537	494	37.4	0.450	0.001	0.321
Morning (0600-1200 h)	177	159	156	158	13.4	0.027	0.098	0.064
Afternoon (1200-1800 h)	187	155	198	156	11.6	0.202	0.001	0.249
Evening (1800-2400 h)	99.0	109.0	90.7	89.0	23.59	0.108	0.635	0.524
Night (2400-0600 h)	97.5	67.2	91.8	92.2	21.71	0.229	0.069	0.070
Pasture intake rate (g DM/min)	14.6	12.8	16.2	12.2	1.35	0.301	0.001	0.031

Low PA = 25 kg DM/d, High PA = 40 kg DM/d; When the interaction PA × S is significant ($P < 0.05$), means followed by same letters into the same PA are not different ($P > 0.10$).
[1]Residual standard deviation

[2]Supplementation level: 0 = 0 kg of supplement DM/d; 4 = 4 kg of supplement DM/d
[c]NE_L, net energy for lactation estimated according to INRA (2007).

4.5 DISCUSSION

4.5.1 Pasture characteristics

The aim of this work was to investigate the effect of corn silage supplementation to dairy cows grazing annual ryegrass on total DM intake and milk production at two levels of PA, annual ryegrass pastures being characterized by low pre-grazing pasture mass (<2,000 kg of DM/ha). However, beyond pre-grazing pasture mass, DM green leaves (DMGL) allowance was also very low, (< 10 kg DM/d), which may be associated to severe grazing conditions (DELAGARDE et al., 2001). Additionally, the energetic value of selected pasture ranged between 6.0 and 6.4 MJ NE_L/kg DM, which is indicative of pastures of low to medium quality (PEYRAUD and DELAGARDE, 2013).

The very low pre-grazing pasture mass in annual ryegrass is a consequence of short time between the sowing and entrance of cows on pasture at first grazing cycle. Annual ryegrass pastures has lower pasture bulk density when compared with perennial ryegrass pastures (210 *vs* 317 kg DM/ha.cm – RIBEIRO-FILHO et al., 2005; 2009; MIGUEL et al., 2012), but pasture bulk density averaging only 157 kg of DM/ha.cm has already been found (MIGUEL et al., 2014). In the present study, pasture bulk density averaged 159 kg DM/ha.cm.

The low- to medium- pasture quality was associated to crude protein content lower than 150 g/kg DM and NDF content greater than 550 g/kg DM, which were a consequence of a small proportion of leaves on the grazing layer. This result may be explained by an higher elongation rate of annual ryegrass tillers and presence of plants growing by self-seeding. Annual ryegrass pastures has a mechanism of increasing elongation rate to compensate a poor tiller density (DUCHINI et al., 2014), and it is able to re-establish annually by self-seeding (BARTH NETO et al., 2014). Plants re-etablished by self-seeding present reproductive tillers much earlier than that ones sown in the year of utilization, decreasing the nutritive value of pasture. In this study, the proportion of sheath was 20% greater than that observed in a previous experience conducted in the same area (MIGUEL et al., 2014).

4.5.2 Effect of pasture allowance on DM intake and animal performance

The very low PI at high PA and the lack of PA effect on PI may be, at least partially, explained by the severe grazing conditions, as a consequence of very low DMGL allowance and very low pre-grazing pasture mass, that are two well-known factors limiting intake even at high PA. Delagarde et al. (2001) has shown in a literature review that PI is stronger related to DMGL allowance than to total DM allowance. These authors found, when DMGL allowance was lower than 10 kg DM/d as in this experiment, a PI of around 8 kg DM/d. In the present study, PI observed in cows grazing at high PA without supplementation was close to 8.6 kg/d, for a DMGL allowance of less than 9 kg/d. In the same way, Ribeiro-Filho et al. (2009) observed that dairy cows grazing annual ryegrass increased PI from 11.9 to 16.6 kg when DMGL increased from 8.8 to 12 kg/d (24 to 37 kg of total DM/d). However, with a pasture mass lower than 2,000 kg DM/ha, even with a DMGL around 12.5 kg DM/d (35 kg of total DM/d) the PI was not higher than 14.5 kg DM/day (MIGUEL et al., 2014).

Grazing behavior parameters was also an evidence of severe grazing conditions. It is known that, with increasing grazing severity, daily grazing time generally increases and PI rate generally decreases in dairy cows grazing both annual (RIBEIRO-FILHO et al., 2011) or perennial ryegrass (BARRETT et al., 2001). When PA is estimated at ground level (as in the present work), values of PI rate close to 16 g DM/min has been found in the lowest threshold of the literature and daily grazing time close to 545 min has been found in the highest threshold of the literature (PÉREZ-PRIETO and DELAGARDE, 2012). In the present work, unsupplemented cows showed average PI rate and daily grazing time of 15 g DM/min and 550 min, respectively, which indicates very low PI rate and very high daily grazing time, supporting difficulties for grazing.

The lack of PA effect on milk production was a consequence of similar PI in both PA and the average milk production was drastically affected by the very low PI. Considering milk production two weeks before the start of the experiment (22 kg/d) and assuming a theoretical persistence of 98% per week (DELABY et al., 1999), the expected milk yield (eMY in kg/d) in the mid point of experiment (4 weeks delay) would be on average 19.6 kg/d. This value is close to that observed in a previous experiment using dairy cows from the same herd, with similar lactation stage and grazing annual ryegrass without supplementation (RIBEIRO-FILHO et al., 2009). In the current study, the milk production observed at high PA without supplementation was not higher than 16.5 kg/d. As consequence, NE_L supply averaged only 0.6 of NE_L requirements, supporting low feeding level in this experiment.

4.5.3 Effect of corn silage supplementation on DM intake and animal performance

The DM corn silage intake lower than expected may be, at least partially, explained by the nutritive value of corn silage. Substantial amounts of supplement refusals have already been observed (WOODWARD et al., 2006; MIGUEL et al., 2014) when the nutritive value of silage was similar or lower than that of grazed pasture. In the same way, grazing cows refused more corn silage in spring than in autumn, due to higher nutritive value of selected pasture (HERNANDEZ-MENDO and LEAVER, 2004). In the present study, crude protein content and NE_L of selected pasture at high PA were, respectively, 142 g/kg DM and 6.4 MJ/ kg DM, whereas the supplement showed lower CP content (111 g/kg DM) and similar NE_L (6.5 MJ/kg DM). Compared with the values of INRA (2007), the nutritive value of corn silage used in the present study was similar to that obtained from maize growing under poor vegetation conditions.

The negative MR (-0.90 kg of milk per kg DM of supplement) at high PA is in disagreement with previous studies which observed that at high PA the milk response to corn silage supplementation varied from to 0.1 to 0.7 kg per kg of supplement DM consumed (MORAN and STOCKDALE, 1992; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011; MIGUEL et al., 2014). It is however supported by the high SR (1.35), that may be associated to low motivation for grazing after the corn silage consumption, affecting the pasture intake rate of supplemented cows. The effect of silage supplementation on the loss of motivation to graze has been observed (PHILLIPS and LEAVER, 1985; PÉREZ-PRIETO et al., 2011), and is most evident at restrictive grazing conditions, as observed in our study, when compared with no restrictive grazing conditions (PHILLIPS and HECHEIMI, 1989). When grazing cows are supplemented with corn silage, reduction of PI is generally associated to a reduction of PI rate, ranging from 0.6 to 1.5 g DM/min per kg DM supplement intake (PHILLIPS and LEAVER, 1985; GRAF et al., 2005; PÉREZ-PRIETO et al., 2011). In the current study, PI rate decreased on average by 2.8 g DM/min per kg DM corn silage intake. Additionally, daily grazing time decreased by 29 min/kg of DM supplement intake. According to Phillips and Leaver (1986), forage supplements decrease daily grazing time by 27 min/kg of supplement DM intake.

4.6 CONCLUSIONS

Annual ryegrass pastures had specific structure that made difficult for the cows to achieve their potential intake, even at high PA. In these conditions, corn silage supplementation improved total DM intake and milk production of dairy cows grazing temperate annual pastures, but only at low PA. At high PA, the relationship between nutritive value of pasture and supplementary forage should be studied.

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5 ARTIGO III

5.1 HOW THE SUPPLEMENTING STRATEGY CAN AFFECT THE SUBSTITUION RATE AND PRODUCTION RESPONSES TO GRAZING DAIRY COWS SUPPLEMENTED WITH CORN SILAGE UNDER LOW AND HIGH PASTURE ALLOWANCES?

5.2 INTRODUCTION

The abolition of milk quotas in Europe demand the optimization of production of dairy farmers (CHATELLIER et al., 2013). Grazing production systems are interesting because the low productions costs associated to decrease of labour expense and capital investment (DILLON et al., 1995). Productivity per hectare is considered the most import factor to increase the profitability of these systems, however the land becomes a limited resource (RAMSBOTTON et al., 2015). The intensification of sector promote the decline of number of milk farmers increasing the number of cows per herd (CHATELLIER et al., 2013), forcing the adoption of forage supplements for more months per year, mainly because the low area available to graze. Forage supplements are a good option to maintaining the energy supply for grazing dairy cows during periods when the pasture production decrease [heavy rainfall, low temperature, drought seasons (CHÉNAIS et al., 1997)] but can play an important role to supplementing dairy cows at pasture to break the pasture feed barrier with high stocking rates (KOLVER et al., 2001).

With increase of stocking rate, the surface available to graze decrease, with negative effects on pasture allowance (PA). Supplementing grazing cows with 3 kg DM of corn silage allows decrease the daily area available to graze by 24% whiteout effects on milk production in comparison to unsupplemented cows at high PA (BURKE et al., 2008). However, at similar low

PA, corn silage supplemented cows can increase the total DM intake and milk production (STOCKDALE, 1997a; PHILLIPS, 1988). This milk production response is related to substitution rate [i.e. decrease of pasture DM intake (PI) per kg DM supplement intake] that affects total DM intake and energy intake. Increase the use of supplements can affect the capacity of pasture harvest per hectare (RAMSBOTTOM et al., 2015), mainly because the substitutive effects (KOLVER et al., 2001; STOCKDALE, 2000). Even with very low PA, the substitutive effects can be observed with forage supplements (MOATE et al., 1984) and the difficult to estimate de the total DM intake for supplemented cows is a challenger on pasture based dairy systems (McGILLOWAY and MAYNE, 1996). For permit to dairy farmers develop grazing and supplementing strategies to avoid the negative effects of substitution rates with forage supplements, is necessary understand the effects of corn silage supplementation on PI.

For predict the effects of supplements on PI, it is necessary to know the PI of unsupplemented cows (STOCKDALE, 2000). For many years, studies were conducted to compare unsupplemented cows vs. forage supplemented cows on rotational stocked grazing with similar PA (MOATE et al., 1984; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011; MIGUEL et al., 2014) or rotational grazing decreasing PA for supplemented cows in order to increase the milk production per hectare (CHAVES et al., 2002; WOODWARD et al., 2006; BURKE et al., 2008). For Reid et al. (2015) it is not possible to discuss substitution rate when different PA is offered to either supplemented or unsupplemented cows. However, different factors can affect the pasture intake, not only the forage supplement. At similar PA comparisons, the substitution rate increases from low to high PA (STOCKDALE, 1996) and the effects of forage supplementation on total DM intake and milk production varied according the pasture characteristics (STOCKDALE, 1997a; PÉREZ-PRIETO et al., 2011) and supplement quality (MOATE et al., 1984; WOODWARD et al., 2006). The substitution rate averaged 1.1 with decrease of the PA on grazing cows supplemented with silage when compared of unsupplemented cows at high PA on rotational stocked grazing, leading a similar post-grazing sward height (BURKE et al., 2008; PÉREZ-PRIETO et al., 2011; REID et al., 2015). The decrease of PI with corn silage supplementations can also be related with the lower PA of supplemented cows, not only by substitutive effects or pasture and supplement characteristics. However, dairy farmers adjust stocking rate to maintain a high pasture utilization rate and low post-grazing sward height, to compensate the adverse effects on residual pastures, reducing the PA of forage supplemented cows. That supplement strategy is empirically utilized, but the effects of reduce the post-grazing sward height on PI of corn silage supplemented cows are unknown. To compare this strategy on PI and milk production, it is necessary to compare unsupplemented and supplemented cows at same PA, considered the scientific method of estimate the substitution rate.

The aim of this study was to evaluate the effects of three corn silage supplementation strategies (unsupplemented, supplemented with similar PA and supplemented with similar post-grazing sward height) on pasture intake, milk response and grazing behaviour of dairy cows. Moreover, these three corn silage supplementation strategies were studied at low and high PA. We hypothesized that corn silage supplementation at similar post-grazing sward induces a greater substitution rate than compared to corn silage supplementation at similar PA. We also hypothesized that substitution rate is lower at low than at high PA.

5.3 MATERIAL AND METHODS

5.3.1 Treatments and Experimental Design

Six treatments were compared in a 2 × 3 factorial arrangement, with two PA [low PA (L) =15 and high PA (H) =30 kg DM/d, above 3.0 cm] and three supplementation strategies (U = unsupplemented treatment; A = 5 kg DM of corn silage supplement offered at similar PA than U, and H = 5 kg DM of corn silage supplement offered at similar post-grazing sward height than U). The treatments were thus: LU, LA, LH, HU, HA and HH. The six treatments were compared on 36 cows according to an incomplete switchback design. The cows were separated in two homogenous groups, one for low PA and one for high PA and affected for all experiment to the same PA group. Within each PA group, corn silage supplementation strategy was studied according to a 3 × 3 Latin square was replicated 6 times. Throughout the experiment, 3 groups switched between periods from U, A and H strategies and other 3 groups switched between periods from U, H and A strategies to balance the potential residual effects of treatments, with 3 periods of 14 days.

The experiment was carried out at the INRA experimental farm of Méjusseaume (longitude –1.71º, latitude +48.11º; Brittany, France) during spring from April 25 to June 20, 2014. Each experimental period was of 14 days, with an 7-d adaptation and a 7-d measurement period. The first two experimental periods were consecutive. An interval of 14 days was necessary for establish the last period. During this period, cows grazed as a one herd a non-experimental pasture and receiver 5 kg DM of corn silage.

Table 8 - Chemical composition and nutritive value of corn silage

Item	Corn Silage
Chemical composition, g/kg DM	
DM, g/kg	353
OM	950
CP	72
NDF	427
ADF	222
ADL[1]	23
Nutritive value	
OM digestibility[2]	0.731
NEL MJ/kg of DM	6.44
PDIN[3], g/kg of DM	44
PDIE[4], g/kg of DM	80

[1]Acid detergent lignin.
[2]Calculed from pepsin-cellulase digestibility (Aufrère and Michalet-Doreau, 1988)
[3]Protein truly digested in the intestine, with energy-limiting microbial synthesis in the rumen (INRA, 2007)
[4]Proportion of NE_L requirements satisfied by NE_L supply

5.3.2 Animals

Thirty-six Holstein-Friesian dairy cows at mid-lactation, including 12 primiparous, were allocated into 12 homogeneous groups of three animals. The cows were selected according to parity (one primiparous per group), lactation stage (175 ± 43 days), milk production (23.6 ± 3.8 kg/d), milk fat concentration (38.1 ± 3.1 g/kg), milk protein concentration (29.1 ± 1.7 g/kg), body weight (590 ± 56 kg) and body condition score (1.57 ± 0.3, scale 0-5) measured from 7 to 20 April 2014. During this period, cows grazed as a one single herd a non-experimental pasture and received 5 kg DM of corn silage and 2 kg DM of a commercial concentrate. Cows were milked twice daily at 0700 and 1600 h. The corn silage (Table 8) was offered individually after morning. milking (between 0800 and 0900 h) but the cows have access to same offered silage after afternoon milking (between 1630 and 1730 h).

5.3.3 Grazing Management and Pasture

The study was conducted in a plot of 7.3 ha with perennial ryegrass (*Lolium perenne* L., cv. 'Ohio'). Thirty days before the start of the experiment, non-experimental cows grazed the entire plot, with a residual plate meter height of 4.7 cm. After this grazing, area was mowed and fertilized with 30 kg N/ha supplied as ammonium nitrate. The plot was then divided into 12 sub-paddocks, one per treatment for the periods 1 and 2. For the period 3, the cow returns on paddocks of period 1, after a delay of the 35 days of regrowth.

Cows were managed under strip-grazing, and daily area to be offered to each treatment was calculated as a function of pre-grazing pasture mass (see below), estimated every day for each sub-paddock. In treatments LH and HH, PA was adjusted daily in order to achieve the same post-grazing sward height than in treatments LU and HU, respectively. Fresh pasture was allocated once daily, after morning milking, and the access to pasture was from 0900 to 1530 h and from 1800 to 0630 h (i.e., ~19 h/d). Water and mineral block were continually available at grazing, except during measurement of grazing time where mineral block was removed.

5.3.4 Feed, Sward and Grazing Measurements

Pre and post-grazing sward heights were measured using a electronic platemeter (30 × 30cm, 4.5kg/m², AGRO-Systèmes, France) on 30 measurements per strip for each treatment per day. Pre-grazing extended tiller and highest sheath were measured with a ruler on 50 tillers per treatment, on day 11 and day 12. Post-grazing tiller and sheath extended heights were measured on days 13 and 14, on 50 tillers per treatment. Each day, the pre-grazing pasture height (minus 3.0 cm) was multiplied by sward bulk density above 3.0 cm and used to calculate the PA for each strip.

Pre-grazing pasture mass above 3.0 cm was measured using the sward height, measured with an electronic platemeter, by the pasture bulk density. To estimate de bulk density two strips of 6 × 0.57 m were cut at 3.0 cm above ground level per treatment were cutting with a motorscythe (Agria) on days 1, 8 and 11 of each period. The pasture height was measured on each strip before and after cutting (12 measurements per strip). A 500g sub-sample of fresh pasture per strip was oven-dried at 60ºC for 48 h in order to determine the pasture DM concentration. The chemical composition of the offered pasture (> 3.0 cm) was determined on samples taken on Days 8 and 11, washed with fresh water, oven-dried at 60º for 72h and composited by treatment and period.

Stubble mass was estimated at ground level twice per period, on Days 8 and 11. In each strip cut under 3.0 cm, a quadrat of 0.1 m^2 was cut to ground level using scissors. These samples were washed with fresh water and then oven dried at 60ºC for 72h.

Botanical and chemical compositions of selected pasture were determined for each treatment on days 10 to 12. Each day, a 600 g sample of fresh pasture was manually collected (~20 handfuls) taken at random, imitating the post-grazing sward height of the previous day. A first subsample was oven dried for 72 h at 60º, then composited by treatment and period for chemical and *n*-alkane analyses. A second subsample was stored at 4ºC and composited by treatment at the end of the experimental period. This subsample was manually sorted in ryegrass, clover and others species. Each constituent of pasture was oven-dried at 60ºC for 48 h in order to determine the botanical composition of pasture on DM basis.

5.3.5 Animal Measurements

Milk production per cow was measured at each milking. Milk fat and protein concentration were determined on the last 4 days of each period by near infrared spectrophotometry using a Milkoscan instrument (Foss Electric, Hillerød, Denmark). The body weight was measured on the three last days of each period.

Individual PI was measured from Day 9 to Day 13 of each period from the *n*-alkane technique (MAYES et al., 1986), using the ratio of pasture C31 (hentriacontane) to dosed C32 (dotricontane). Throughout the experiment, cows were dosed twice daily, after milking, with a cellulose stopper (Carl Roth, GmbH, Karlesruhe, Germany) containing 410 ± 13 mg of C32. From afternoon milking on Day 10 to morning milking on Day 14, fecal grab samples were collected manually and stored at 4ºC. On Day 14, these samples were composited by period and by cow and oven-dried at 60º for 72 h, then milled through a 0.8 mm screen for chemical analyses.

Energy and protein truly digested in the intestine, with energy-limiting microbial synthesis in the rumen (PDIE) balances were calculated according to INRA (2007) and expressed as a proportion of requirements satisfied by supply. Net energy, protein truly digested in the intestine, with nitrogen-limiting microbial synthesis in the rumen (PDIN) and PDIE supplies were calculated from intake of pasture and corn silage and their concentrations of energy, PDIN and PDIE, respectively.

Grazing time was measured with Kenz Lifecorder Plus devices (LCP, Suzuken Co. Ltd., Nogoya, Japan) placed in a small waterproof plastic box and attached to the cow's neck by means of a simple collar (DELAGARDE and LAMBERTON, 2015). The Lifecorder Plus is an electronic portable device, based on a uniaxial accelerometer that records average physical activity level (range 0-9) every 2-min periods. The Lifecorder Plus detects primarily head movements rather than whole body movements and locomotion activity and is very precise for detecting grazing activity (mean prediction error = 5 % per day; DELAGARDE and LAMBERTON, 2015). At each period 24 cows (4 per treatment) were equipped simultaneously for at least three consecutive days, between day 8 and day 14, in order to obtain three complete day recordings per cow and period. The daily pasture DM intake rate was estimated by dividing the daily PI by the daily grazing time.

5.3.6 Chemical Analyses

The ash was determined by calcination at 550ºC for 5 h on a muffle furnace (Association Française de Normalisation, 1997). The N concentration was measured by the method of Dumas (Association Française de Normalisation, 1997) using a Leco instrument (Leco Corp., St. Joseph, MI). Pepsin-cellulase digestibility was determined according to Aufrère and Michalet-Doreau (1988). The concentrations of NDF, ADF, and acid detergent lignin were measured according to Van Soest et al. (1991) on a Fibersac extraction unit (Ankon Tchnology Corp., Fairport, NY). The *n*-alkanes were determined according Mayes et al. (1986) following direct saponification (VULICH et al., 1991). The net energy and PDI value of feeds were calculated from their chemical composition according to INRA (2007).

5.3.7 Statistical analyses

Animal and pasture data were analysed as a incomplete switchback design. The animal variables, averaged per cow and period (n = 108), were analysed using a following model (PROC GLM; SAS Institute, 1999):

$$Y_{ijkl} = \mu + \text{period}_i + \text{allowance}_j + \text{cow (allowance)}_k + \text{supplementation}_l + [\text{pasture allowance}_j \times \text{supplementation}_l] + [\text{pasture allowance}_j \times \text{period}_i] + e_{ijkl};$$

where Y_{ijkl}, μ, period_i, pasture allowance_j, cow_k, supplementation_l, [pasture allowance_j × supplementation_l], [pasture allowance_j × period_i] and e_{ijkl} represent, the analysed variable, the overall mean, the fixed effect of the period, the fixed effect of the pasture allowance, the fixed effect of cow within PA level, the fixed effect of supplementation, the fixed effect of interaction allowance × supplementation, the fixed effect of interaction pasture allowance × period and the residual error, respectively. The PA effect was tested using the cow effect as the residual term.

Pasture data, averaged per treatment and period (n = 18) were analyzed using the following model (PROC GLM; SAS Institute, 1999):

$$Y_{ijk} = \mu + \text{period}_i + \text{allowance}_j + \text{supplementation}_k + [\text{pasture allowance}_j \times \text{supplementation}_k] + e_{ijk};$$

A total of six orthogonal contrasts were used to determine: 1) the mean effect of silage supplementation at similar PA (LU + HU vs. LA + HA); 2) the mean effect of silage supplementation at similar post-grazing sward height (LU + HU vs. LH + HH); 3) the mean effect of supplement strategy (LA + HA vs. LH + HH); the mean effect of interaction between PA and silage supplementation at similar PA [PA × (LU + LA vs. HU + HA)]; 5) the mean effect of interaction between PA and silage supplementation at similar post-grazing sward height [PA × (LU + LH vs. HU + HH)]; 6) the mean effect of interaction between PA and supplement strategy [PA × (LA + LH vs. HA + HH)].

5.4 RESULTS

5.4.1 Pre-grazing pasture characteristics

The pre-grazing pasture mass averaged 3,080 and 4,553 kg DM/ha when measured above 3.0 cm and ground level respectively. The platemeter sward height averaged 15.0 cm (Table 9). The pre-grazing pasture mass and sward height were slightly lower on LH and HA treatments, leading to significant interactions PA × A and PA × (A vs. H). The extended tiller and sheath height did not differ between treatments, averaging 28.4 and 12.3 cm respectively. The extended lamina height averaged 16.0 cm, with small difference (-0.7 cm) between A treatments.

Pre-grazing pasture CP concentration was greater at low than high PA, averaging 165 and 154 g/kg respectively. The pasture DM, OM, NDF, ADF, ADL and OM digestibility contents were similar for all treatments.

5.4.2 Grazing management and post-grazing pasture characteristics

As planned, PA above 3.0 cm was similar between U and A silage supplementation strategies, averaging 15.4 and 31.0 kg DM at low and high PA, for a corresponding of area per/d, averaging 51 and 108 m², respectively (Table 10). Post-grazing sward height was lower at low than high PA, averaging 5.6 and 8.5 cm, respectively. Corn silage supplementation at similar PA, resulted on increase of post-grazing sward height at low PA tended to increase with corn silage supplementation at similar PA (+1.2 cm), but not differ at high PA, averaging 8.5 cm. Post-grazing extended tiller, sheath and lamina heights were lower at low than at high PA. The post-grazing extended lamina height was higher on A in than on U and H silage supplementing strategies treatments (+1.0 cm on average; $P < 0.05$), leading a 27% less of lamina-free tiller on A supplementing strategy

As planned, the post-grazing platemeter sward height was similar for U and H silage supplementation strategies, averaging 5.2 and 8.4 cm at low and high PA, respectively. To achieve this similar post-grazing sward height, it was necessary to decrease the daily area per cow by 19%.This represent a decrease of PA above 3.0 cm of 3.5 and 4.9 kg DM/d at low and high PA, for 4.7 and 4.1 kg DM of silage, respectively, i.e. -0.75 and -1.20 kg DM offered/ kg DM of silage consumed.

The chemical composition of selected pasture was similar in all treatments, with values close to offered pasture for OM and CP and lower NDF and ADF than offered pasture. The nutritive values of selected pasture was unaffected by treatments and the NE_L value averaged 6.77 MJ/kg DM, and PDIN and PDIE averaging 100 and 95 g/kg DM respectively.

Table 9 - Effect of pasture allowance (PA) and two strategies with corn silage supplementation (S) on pre-grazing pasture characteristics: pasture mass, sward height, chemical composition, and nutritive value of offered pasture.

	Treatment[2]							Contrast[3] (*P*-value)						
Pasture allowance	Low			High				S			PA × S			
Supplementation	LU	LA	LH	HU	HA	HH	r.s.d[1]	A	H	A vs. H	PA	PA × A	PA × H	PA × (A vs. H)
Pasture mass, kg DM/ha														
Above 3.0 cm	3,106	3,193	2,906	3,191	2,864	3,219	112	0.092	0.214	0.617	0.676	0.010	0.108	0.001
Above ground level	4,577	4,665	4,378	4,666	4,339	4,693	111	0.089	0.209	0.614	0.634	0.009	0.105	0.001
Pre-grazing sward height, cm														
Rising platemeter	15.1	15.4	14.4	15.3	14.1	15.4	0.44	0.102	0.231	0.623	0.830	0.011	0.124	0.001
Extended tiller	28.8	28.2	28.0	28.6	27.9	28.6	1.25	0.400	0.611	0.721	0.984	0.938	0.574	0.525
Extended sheath	12.3	12.2	12.1	12.2	12.8	12.2	1.38	0.739	0.950	0.697	0.813	0.688	0.891	0.780
Extended lamina	16.5	16.0	15.8	16.4	15.1	16.4	0.38	0.002	0.159	0.024	0.454	0.108	0.177	0.010
Chemical composition, g/kg DM														
DM, g/kg	184	181	177	178	178	185	7.97	0.672	0.918	0.741	0.926	0.767	0.150	0.239
OM	894	897	896	899	902	901	4.88	0.292	0.456	0.749	0.066	0.959	0.980	0.940
CP	165	166	165	158	149	155	7.11	0.320	0.745	0.495	0.006	0.281	0.820	0.392
NDF	519	514	521	518	512	522	8.24	0.325	0.469	0.099	0.835	0.919	0.867	0.792
ADF	253	249	255	255	252	253	5.30	0.278	0.944	0.250	0.658	0.897	0.484	0.417
ADL[4]	25	26	25	24	25	25	2.54	0.425	0.675	0.692	0.640	0.944	0.857	0.914
Nutritive value														

OM digestibility[5]	0.780	0.776	0.778	0.782	0.774	0.780	0.006	0.102	0.584	0.252	0.833	0.710	0.949	0.667

[1]Residual standard deviation
[2]Treatments: U = 0 kg of supplement DM/d; A = 5 kg of supplement DM/d with same PA of 0 cows; H = 5 kg of supplement DM/d with same residual sward height of U cows
[3]Contrasts: A = effect of supplementation at same PA (LU + HU vs. LA + HA); H = effect of supplementation at same post-grazing sward height (LU + HU vs. LH + HH); A vs. H = effect of supplementation strategy (LA + HA vs. LH + HH); PA × A = interaction effect between PA and supplementation at same PA [PA × (LU + LA vs. HU + HA)]; PA × H = interaction effect between PA and supplementation at same post-grazing sward height [PA × (LU + LH vs. HU + HH)]; PA × (A vs. H) = interaction effect between PA and supplementation strategy [PA × (LA + LH vs. HA + HH)].
[4]Acid detergent lignin.
[5]Calculed from pepsin-cellulase digestibility (Aufrère and Michalet-Doreau, 1988)

Table 10 - Effect of pasture allowance (PA) and two strategies with corn silage supplementation (S) on pasture allowance, offered area, post-grazing sward height, and nutritive value of selected pasture

	Treatment[2]							Contrast[3] (*P*-value)						
Pasture allowance	Low PA			High PA				S				S × PA		
Supplementation	LU	LA	LH	HU	HA	HH	r.s.d[1]	A	H	A vs. H	PA	PA × A	PA × H	PA × (A vs. H)
Pasture allowance, kg DM/d														
Above 3.0 cm	15.4	15.4	11.9	31.0	31.0	26.1	0.46	0.981	0.001	0.001	0.001	0.981	0.024	0.023
Above ground level	22.8	22.6	18.1	45.5	47.2	38.2	0.73	0.105	0.001	0.001	0.001	0.046	0.011	0.001
Offered area, m²/cow per day	51	50	42	103	113	85	4.79	0.139	0.001	0.001	0.001	0.061	0.122	0.004
Post-grazing sward height, cm														
Rising platemeter	5.3	6.5	5.1	8.5	8.6	8.3	0.42	0.024	0.502	0.008	0.001	0.060	0.968	0.056
Extended tiller	9.4	11.7	10.1	15.5	15.6	15.2	1.22	0.114	0.788	0.176	0.001	0.155	0.522	0.420
Extended sheath	7.3	8.4	7.7	10.6	9.7	9.9	0.79	0.825	0.745	0.599	0.001	0.061	0.286	0.372
Extended lamina	2.1	3.3	2.4	4.9	5.9	5.3	0.70	0.020	0.427	0.085	0.001	0.768	0.917	0.698
Proportion of lamina-free tillers[3]	0.59	0.42	0.57	0.23	0.17	0.23	0.04	0.001	0.609	0.002	0.001	0.050	0.698	0.099
Chemical composition, g/kg DM														
DM, g/kg	184	181	177	178	178	185	7.97	0.672	0.918	0.741	0.926	0.767	0.150	0.239
OM	895	895	894	896	905	901	3.55	0.066	0.321	0.359	0.006	0.058	0.226	0.434
CP	159	167	165	169	157	162	6.05	0.726	0.922	0.661	0.738	0.016	0.089	0.359

NDF	497	501	495	486	495	495	9.40	0.227	0.498	0.587	0.237	0.649	0.373	0.640
ADF	242	243	240	238	238	240	8.75	0.855	0.978	0.835	0.474	0.972	0.695	0.671
ADL[5]	24	26	26	26	24	25	2.49	0.992	0.610	0.613	0.952	0.388	0.461	0.880
Nutritive value														
NEL MJ/kg DM	6.76	6.72	6.75	6.80	6.78	6.81	0.07	0.478	0.957	0.510	0.142	0.746	0.701	0.948
PDIN[6], g/kg DM	97	102	101	103	96	99	3.69	0.718	0.916	0.648	0.719	0.017	0.089	0.371
PDIE[7], g/kg DM	95	94	95	95	94	95	0.46	0.024	0.703	0.048	0.463	0.136	0.247	0.711

[1]Residual standard deviation.
[2]Treatments: 0 = 0 kg of supplement DM/d; 5A = 5 kg of supplement DM/d with same PA of 0 cows; 5H = 5 kg of supplement DM/d with same residual sward height of 0 cows
[3]Contrasts: A = effect of supplementation at same PA (LU + HU vs. LA + HA); H = effect of supplementation at same post-grazing sward height (LU + HU vs. LH + HH); A vs. H = effect of supplementation strategy (LA + HA vs. LH + HH); PA × A = interaction effect between PA and supplementation at same PA [PA × (LU + LA vs. HU + HA)]; PA × H = interaction effect between PA and supplementation at same post-grazing sward height [PA × (LU + LH vs. HU + HH)]; PA × (A vs. H) = interaction effect between PA and supplementation strategy [PA × (LA + LH vs. HA + HH)].
[4]Tillers with main lamina totally defoliated.
[5]Acid detergent lignin.
[6]Protein truly digested in the intestine, with nitrogen-limiting microbial synthesis in the rumen (INRA, 2007)
[7]Protein truly digested in the intestine, with energy-limiting microbial synthesis in the rumen (INRA, 2007)

5.4.3 DM intake, substitution rate, and energy balance

Pasture intake averaged 13.0 and 15.8 kg DM/d at low and high PA, respectively, that's represent an increase in pasture intake of 0.16 and 0.13 kg DM/d per kg DM of PA above 3.0 cm and ground level respectively. At low PA, the silage DM intake (average = 4.6 kg DM/d) was similar to silage DM offered. At high PA, the silage offered was partly refused, leading to lower silage DM intake (3.7 kg DM/d). Total DM intake averaged 16.0 kg DM/d at low PA, 12% less than at high PA, averaging 18.2 kg DM/d. Similarly, NE_L and PDIE supplies were lower at low than at high PA, but the NE_L and PDIE balance were similar for all treatments, averaging 1.03 and 1.05 of NE_L and PDIE requirements respectively.

The PI decrease on average 22% with silage supplementation. This reduction was higher on H than on A treatments, with pasture DM intake averaging 12.5 and 13.8 kg DM/d, respectively. The decrease of pasture intake on A supplement strategy tended to be greater at high PA. The total DM intake increased with silage supplementation only at low PA, by 1.7 kg DM/d inducing the increase of NE_L supply for LA and LH treatments. The substitution rate was lower at low than at high PA (0.63 vs. 1.23 at low and high PA respectively). The PDIE supply increase only on A silage supplementing strategy at low PA, but decreased 11% with silage supplement at high PA.

5.4.4 Milk production, milk composition, and BW

Milk production averaged 20.8 and 23.8 kg/d at low and high PA, respectively, which is 0.20 and 0.13 kg/d per kg DM of PA above 3.0 cm and ground level respectively (Table 11). Milk protein concentration averaged 28.9 and 31.8 g/kg at low and high PA respectively, leading to lower milk protein production at low than at high PA (600 vs. 757 g/g; P<0.001). The production of 4% FCM averaged 20.8 and 21.9 kg/d at low and high PA respectively. In general, milk production was not effected by silage supplementation, averaging 22.1, 22.6, and 22.3 kg/d for U, A and H silage supplementing strategies. However, milk production response to silage supplementation differed according to PA and also partly according to silage supplementing strategy. At high PA, milk production and composition were unaffected by silage supplementation, either on A or H strategy. At low PA, milk production, 4% FCM and milk fat concentration were greater on LA treatment (+1.4 kg/d, +1.0 kg/d and +33.5 g/d respectively) in comparison to average values of treatments LU and LH. The milk protein concentration was greater on A and H supplementing strategies at low PA, consequently the milk protein production increase on average 8% on LA and LH treatments. The milk fat concentration was similar for all treatments averaging 35.6 g/kg. The BW was lower on U treatments (average = 576 kg) and increase 10 and 4 kg on A and H supplementing strategies.

Milk urea N was greater at low than high PA (2.4 vs. 1.8 mmol/L respectively). Blood urea N concentration averaged 2.0 mmol/L and was unaffected by PA. Milk and blood urea N concentration decreased on average by 31% and 36% respectively with silage.

Table 11 - Effect of pasture allowance (PA) and two strategies with corn silage supplementation (S) on DMI, energy balance, milk production, milk composition, BW, protein intake, milk and blood urea N, and grazing behavior in grazing dairy cows.

	Treatments[3]								Contrast[4] (*P*-value)						
Pasture allowance	Low			High					S				PA × S		
Supplementation	LU	LA	LH	HU	HA	HH	r.s.d[1]	r.s.d[2]	A	H	A vs. H	PA	PA × A	PA × H	PA × (A vs. H)
DMI, kg/d															
Pasture	14.9	12.7	11.3	18.8	14.8	13.7	2.07	4.70	0.001	0.001	0.016	0.004	0.069	0.114	0.807
Corn Silage	0.0	4.5	4.7	0.0	3.3	4.1	0.46	0.67	0.001	0.001	0.001	0.001	0.001	0.008	0.006
Total	14.9	17.2	16.0	18.8	18.1	17.8	1.96	4.84	0.118	0.954	0.132	0.024	0.002	0.023	0.361
Substitution Rate		0.49	0.77		1.21	1.24									
NE_L supply, MJ/d	100	113	106	127	120	118	13.0	32.2	0.290	0.700	0.151	0.017	0.002	0.024	0.370
PDIE[5] supply, kg/d	1.4	1.5	1.4	1.8	1.6	1.6	0.18	0.45	0.958	0.157	0.142	0.020	0.002	0.022	0.382
NE_L balance[6]	0.97	1.05	1.01	1.09	1.04	1.04	0.12	0.16	0.456	0.987	0.465	0.125	0.020	0.092	0.506
PDIE balance[7]	1.06	1.09	1.06	1.08	1.02	1.01	0.12	0.17	0.634	0.260	0.513	0.334	0.107	0.242	0.652
Milk Production, kg/d	20.1	21.5	20.8	24.0	23.7	23.7	1.18	6.86	0.058	0.690	0.130	0.030	0.003	0.079	0.188
Milk fat concentration g/kg	36.7	36.4	36.5	35.1	34.7	34.4	1.04	6.18	0.138	0.084	0.803	0.142	0.838	0.299	0.403
Milk protein concentration, g/kg	28.2	29.5	29.1	32.2	31.4	31.7	1.27	3.20	0.493	0.566	0.911	0.001	0.001	0.034	0.210
4% FCM production, kg/d	19.1	20.4	19.7	22.2	21.8	21.6	1.08	6.41	0.101	0.940	0.087	0.092	0.003	0.034	0.342
Milk fat production, g/d	740	783	759	837	822	809	43.5	259	0.184	0.627	0.072	0.223	0.006	0.023	0.593
Milk protein production, g/d	568	633	600	773	746	752	43.9	215	0.066	0.600	0.185	0.001	0.001	0.013	0.068

BW, kg	559	571	566	592	600	593	7.90	106	0.001	0.028	0.004	0.159	0.286	0.147	0.697
Protein intake (g/kg DM)	159	141	137	169	141	142	7.23	24.4	0.001	0.001	0.378	0.006	0.004	0.091	0.187
Milk urea N (mmol/L)	3.1	2.0	2.0	2.2	1.5	1.8	0.35	0.62	0.001	0.001	0.097	0.001	0.007	0.001	0.129
Blood urea N (mmol/L)	2.8	1.9	1.6	2.4	1.6	1.8	0.47	0.61	0.001	0.001	0.480	0.218	0.601	0.004	0.018
Grazing behavior															
Total grazing time, min/d	496	447	434	499	452	469	22.0	61.5	0.001	0.001	0.706	0.239	0.793	0.004	0.007
First grazing bout duration, min	177	170	180	115	106	130	19.3	32.2	0.099	0.062	0.001	0.001	0.824	0.211	0.143
Mean grazing bout duration, min	98	79	75	80	72	74	10.5	18.6	0.001	0.001	0.591	0.023	0.031	0.001	0.207
Number of grazing bouts	5.2	5.8	5.8	6.4	6.4	6.6	0.77	1.29	0.170	0.043	0.480	0.002	0.084	0.217	0.643
Pasture intake rate, g DM/min	30.2	28.5	26.1	37.8	32.8	29.2	4.39	9.70	0.002	0.001	0.006	0.012	0.109	0.035	0.573

[1]Residual standard deviation for model

[2]Residual standard deviation for PA

[3]Treatments: U = 0 kg of supplement DM/d; A = 5 kg of supplement DM/d with same PA of 0 cows; H = 5 kg of supplement DM/d with same residual sward height of U cows

[4]Contrasts: A = effect of supplementation at same PA (LU + HU vs. LA + HA); H = effect of supplementation at same post-grazing sward height (LU + HU vs. LH + HH); A vs. H = effect of supplementation strategy (LA + HA vs. LH + HH); PA × A = interaction effect between PA and supplementation at same PA [PA × (LU + LA vs. HU + HA)]; PA × H = interaction effect between PA and supplementation at same post-grazing sward height [PA × (LU + LH vs. HU + HH)]; PA × (A vs. H) = interaction effect between PA and supplementation strategy [PA × (LA + LH vs. HA + HH)].

[5]Protein truly digested in the intestine, with energy-limiting microbial synthesis in the rumen (INRA, 2007)

[6]Proportion of NE_L requirements satisfied by NE_L supply
[7]Proportion of PDIE requirements satisfied by PDIE supply

5.4.5 Grazing behaviour

Total grazing time averaged 466 min/d and decreased with silage supplementation on average by 47 min/d (i.e. -11 min/d per kg DM of silage). Only with H strategy, this decrease of grazing time was greater at low than at high PA (-62 vs. -30 min/d). The duration of the first grazing bout was greater at low than at high PA (176 vs. 117). The number of grazing bouts increased by 0.9 bouts/d associated to reductions by 9 min on mean grazing bout at high PA. The daily number of grazing bouts increased by 0.5 bouts/d (+9%) with A strategy. Mean grazing bout duration decreased on average by 14 min (-16%) with silage supplementation. This decrease was higher at low than at high PA (-21 vs. -7 min, respectively)

Pasture intake rate was lower at low than at high PA (28.3 vs. 33.3 g DM/min). The pasture intake rate decrease with silage supplementing both PA, but on H treatments this effect was greater, averaging -6.3 vs. -3.4 g DM/min on A and H treatments, respectively.

5.5 DISCUSSION

The aim of the present study was to evaluate the effects of corn silage supplementation strategy either at similar PA or at similar post-grazing sward height, on PI, milk production response and grazing behavior of dairy cows, under low and high PA.

The two supplementation strategies were achieved successfully, that means that similar PA was achieved between U and A strategies, and similar post-grazing height was achieved between U and H strategies. To our knowledge, this is the first study comparing these two strategies, one (A) being for scientific purpose, the second one (H) for scientific and more practical purposes. The structural, botanical and chemical characteristics of pasture offered were similar between treatments, enabling to study the supplementation strategies and their interactions with PA *per se*.

5.5.1 Overall effect of pasture allowance

The two PA levels were well contrasted, and the high range of PA studied (15 or 21 kg DM offered/d above 3.0 cm or at ground level) induced a large difference in PI (2.8 kg DM/d) and of milk production (3.0 kg/d) between low and high PA. This nutritional range is a good preliminary condition for studying the interaction between corn silage supplementation strategy and PA on PI and milk production of grazing dairy cows. The overall relationships observed between PA and PI or milk production, averaging 0.12 kg DM intake and 0.13 kg of milk per kg of PA at ground level, respectively, are similar to that obtained on a literature meta-analysis by Pérez-Prieto and Delagarde (2013), under the same ranges of PA. Moreover, the large decrease of post-grazing sward height and the large increase in the proportion of lamina-free tillers when PA was lowered are consistent with known effects of PA on these practical indicators, and clearly indicates restrictive pasture conditions at low PA (DELAGARDE et al., 2001a; 2001b).The fact that the reduction of PI with decreasing PA was related to a proportional decrease of pasture DM intake rate without affecting daily grazing time is consistent with the literature review by Pérez-Prieto and Delagarde (2013).

5.5.2 Pasture allowance × corn silage supplementation interaction

The lower substitution rate at low to high PA indicates that intake regulation when cows are supplemented differs according to pasture availability. Corn silage supplementation had positive effects on total DM intake and on milk production only under limiting PA. These results are consistent with previous silage supplementation studies under different PA (MOATE et al., 1984; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011). On these studies, the substitution rate varied, on average, from 0.14 to 0.75 from low to high PA, with an average of 5 kg DM of silage DM intake. At low PA, the PI being limited, the intake capacity of the cows is not reached, and it is easier for cows to maintain PI when they are supplemented, leading to decreased substitution rate and low reduction of total DM intake with forage supplementation (PHILLIPS, 1988). At low PA, the intake of 4.7 kg DM of corn silage can increase the total DM intake by 3.7 kg DM, with a substitution rate averaging 0.22 (STOCKDALE, 1996; STOCKDALE, 1997b; WOODWARD et al., 2006). According to the GrazeIn model (DELAGARDE et al., 2011), the substitution rate is strongly related to the PA, and substitution rates predicted by the model were 0.68 and 0.96 for low and high PA, respectively, which is close to the observed values (0.63 and 1.23).

Despite only medium level of silage supplementation, partial refusal of corn silage (approx. 1 kg DM/d) has been observed at high PA, which may indicate a partial preference of cows for grazed pasture under *ad libitum* pasture availability. On rotational grazing, this voluntary reduction of silage intake with increasing PA has not been reported in the literature. Under set-stocking management on good quality pastures, similar reduction of silage intake by 1 kg DM/d when increasing sward height has been observed (HERNANDEZ-MENDO and LEAVER, 2004; 2010). Their results seem also to indicate that this partial preference for grazed pasture occur mainly in spring, but not in autumn, when the relative palatability of pasture compared to corn silage may be lower.

The average substitution rates observed in this study may be regarded as relatively high when compared to some similar studies in the literature (STOCKDALE, 1996, PÉREZ-PRIETO et al., 2011). One hypothesis is that the supply of 5 kg DM of corn silage on a pasture containing only 160 g CP/kg DM may lead to some deficit in diet CP level, which could affect negatively intake and/or ruminal digestion, increasing substitution rate. If diet CP levels of supplemented cows *per se* (approximately 140 g CP/kg DM) may not be regarded as clearly limiting (PEYRAUD and ASTIGARRAGA, 1998) for affecting intake, milk urea or uremia lower than 2 mmol/L may be regarded as low values for grazing cows (WITTER et al., 1999; ASTIGARRAGA et al., 2002; BARGO et al., 2002). In our study, no additional protein source was provided with corn silage, because of the relative low level of corn silage supplementation and the expected high CP value of the grazed pasture. With white clover pastures containing 240 g CP/kg DM, the inclusion of protein supplements unaffected the substitution rate (STOCKDALE, 1997a; 1997b). With low CP-pastures (113 g CP/kg DM), the replacement of 40% of corn silage supplement by cottonseed meal enabled to reduce the substitution rate from 0.32 to 0.14 (STOCKDALE, 1997b). It is known that the inclusion of a protein source on a low CP-pasture can increase diet digestibility, with potential positive effects on DM intake (PEYRAUD et al., 1997; DELAGARDE et al., 1999).

The average decrease of grazing time with corn silage supplementation, namely 12 min/d per kg of corn silage DM intake, was similar to that already reported in other studies (PHILLIPS and LEAVER, 1985, GRAF et al., 2005; PÉREZ-PRIETO et al., 2011). It is however noteworthy that, when cows are supplemented, the 18% relative reduction of pasture intake is related to an only 10% relative reduction of grazing time, because of a concomitant 9% relative reduction of pasture intake rate at both PA. Similar reduction of pasture intake rate in forage-supplemented cows have already been observed (PÉREZ-RAMIREZ et al., 2008; PÉREZ-PRIETO et al., 2011), and suggest that the supply of corn silage may lower the motivation to graze or to eat fast, because of the insurance that part of the diet is easily eatable. This also suggests that the relative variation of grazing time cannot be used directly as an indicator of the relative variation of PI.

The milk production response to corn silage supplementation was in agreement with variations of substitution rate and total DM intake. In fact, milk production response to corn silage supplementation was positive only when total DM intake increased, i.e. at low PA in our study. The ability of forage supplements to increase milk production in grazing cows has been already observed to be correlated with the ability of the supplement to increase total DM intake and energy supply (PHILLIPS, 1988). Under severe grazing conditions, i.e. low PA, milk production can increase by 0.68 kg per kg of corn silage DM intake (STOCKDALE, 1996; WOODWARD et al., 2006; BURKE et al., 2008; PÉREZ-PRIETO et al., 2011). At the contrary, at high PA, milk production remains generally unaffected by corn silage supplementation (CHÉNAIS et al., 1997; STOCKDALE, 1996). This may be particularly true with high-quality pastures, where nutritive value of total diet can decrease when high-quality pasture is replaced by a forage of lower quality. As an example, with pure white clover pastures, even with low substitution rate (0.40), the supply of corn silage unaffected milk production, when PA was not limiting for pasture DM intake (STOCKDALE, 1996). The variation of milk protein concentration with corn silage supplementation was positively related to that of milk production and of energy supply, as previously observed by Coulon and Rémond (1991). At low PA, for each kg of corn silage DM intake, the milk protein concentration can increased by 0.20g/kg per kg of silage DM intake, which is in line with the average of 0.25g/kg per kg of silage DM reported by Miguel et al. (2014) and Pérez-Prieto et al. (2011).

5.5.3 Corn silage supplementation strategies

Quantifying the substitution rate between grazed pasture and a forage supplement at similar PA allows to predict animal response, all other thinks being equal. However, because of lower PI of supplemented compared to unsupplemented cows, supplemented treatment leads to higher post-grazing sward height and lower pasture use per hectare, which is not acceptable from a practical point of view. In practice, farmers always want to maximize pasture use efficiency through controlling post-grazing sward height, thus increasing stocking rate or decreasing area per cow to achieve a post-grazing sward height as low as if cows were unsupplemented. This is the H strategy compared to the A strategy in this experiment. As expected, the reduction of PA for targeting similar post-grazing sward height in the H supplementation strategy compared to unsupplemented cows affected negatively PI, and H cows consumed less pasture that A cows, resulting in greater substitution rate and lower milk production response. These effects were mostly noticeable at low PA. In the H strategy, PA had to be reduced by 0.75 and 1.0 kg DM/d above 3.0 and ground level per kg of corn silage DM intake at low PA. According to Pérez-Prieto and Delagarde (2013), the same reduction of PA resulted on a decrease of 1.5 kg of pasture DM intake, similar to that observed in our study (-1.4 kg DM) on LH treatment compared to LA treatment. This shows that, at low PA, the reduction of pasture DM intake observed between U and H strategies results from the cumulative effects of two biological phenomena: the substitution rate linked to forage supply *per se*, along with the decrease of pasture intake related to lower PA. The implication is that substitution rate estimated between two supplementation levels, where cows are grazing as a single herd within the same paddock (thus similar post-grazing sward height), will be lower than that estimated when supplemented and unsupplemented cows are separated, and compared at similar PA. This has been already included in the GrazeIn model (DELAGARDE et al., 2011), which is able to simulate this difference of substitution rate between strategies, through the calculation of an individual true pasture allowance for different cows within the same paddock. At high PA, the relative reduction of PA to achieve similar post-grazing sward height in H than in A supplementation strategy was not sufficient enough to interact with the effect of silage supplementation on PI leading to similar substitution rates. With no restriction of pasture availability, it can be assumed that PI regulation is driven by metabolic or energetic constraints rather than by feed availability (POPPI et al., 1987). Variations of PI according to PA are most evident from low to medium PA than from medium to high PA (PÉREZ-PRIETO and DELAGARDE, 2013).

5.5.4 Practical implications

On good quality pastures, corn silage supplementation can increase milk production per cow, but only at low pasture availability. To achieve a similar post-grazing sward height between an unsupplemented herd and a herd supplemeted with corn silage, it is necessary to decrease pasture allowance at ground level by approximately 1 kg DM/d for each kg DM of corn silage eaten. On commercial farms, where farmers objective should be to have low post-grazing sward height to maintain pasture quality of future rotations and to maximize pasture use efficiency per hectare, the substitution rate between pasture and corn silage will be always high (H strategy), due to the cumulative effects of substitution rate *per se* and of reduction of pasture allowance. As a consequence, milk production per cow will not be enhanced by corn silage supplementation.

5.6 CONCLUSIONS

Reducing the PA by 1 kg DM/d per 1 kg DM of supplement consumed maintained a high pasture utilization rate of corn silage supplemented cows, even with increases the substitution rate in comparison to supplemented cow at similar PA. That strategy is effective to maintain the individual milk production of cows when increase the stocking rate, especially at low PA. On general, the supplementation with corn silage unaffected the milk production of grazing cows but at low PA the total DM intake increase, affecting positively the milk production of supplemented cows at similar PA. Supplementing with corn silage was not able to maintain the milk production of grazing cows at mid-lactation when compared at unsupplemented cows at high PA, when values of substitution rate increases.

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6. ARTIGO IV

6.1 META-ANALYSIS OF THE EFFECT OF FORAGE SUPPLEMENTATION STRATEGIES ON SUBSTITUTION RATE AND MILK PRODUCTION RESPONSE IN DAIRY COWS ROTATIONALLY GRAZING TEMPERATE PASTURES

6.2 INTRODUCTION

Increase the proportion of grazed pasture on diet of dairy cows allows to reduce milk production costs. For this, it is necessary to increase the proportion of pasture harvested directly by cows, which may be associated to high stocking rates in efficient pasture-based dairy systems (BRYANT and DONNELLY, 1974). As an example, increasing stocking rate from 1.6 to 2.6 cows per hectare represents an increase of pasture harvested of about 20% (BAUDRACCO et al., 2011). That increase in stocking rate penalizes the individual milk production but increases the milk production per hectare (BAUDRACCO et al., 2011; McCARTHY et al., 2011; PEYRAUD and DELAGARDE, 2013). With the increase in the number of cows per hectare, the area available for grazing decreases, making the use of land a major challenger for dairy farmers on pasture-based dairy systems (RAMSBOTTON et al., 2015). To maintain the milk production per cow and per hectare when the grazing area is limited, it is necessary to adopt mixed feeding systems, that include pasture and conserved forages, to support the energy requirements of cows under low pasture availability.

Forage supplements are mostly on situations with pasture shortage, to limit reductions or variations of total DM intake, increasing generally the efficiency of land use (PHILLIPS, 1988). The main objective of forage supplementation is to increase the total DM intake or at least to meet the cow intake capacity when the pasture solely is not able to supply the cows requirements, affecting or not positively the milk production compared to unsupplemented cows. In a series of studies conducted by Stockdale (1994; 1996; 1997a; 1997b) at low pasture allowance (PA: averaging 20 kg DM/d above ground level), supplementing grazing dairy cows with corn silage increased the individual milk production by 0.61 kg per kg of supplement DM intake. On these studies, the total DM intake increased on average by 4.0 kg DM/d while silage intake was of 5.3 kg DM/d. The milk production response seems to vary also according to the type of forage supplement, averaging 0.17 kg of milk per kg of hay DM intake (STOCKDALE, 1981; 1999; REIS and COMBS, 2000; WALES and DELLOW, 2000) and 0.34 kg of milk per kg of pasture silage DM intake (MOATE et al., 1984; CHAVES et al., 2002; WOODWARD et al., 2006; MORRISON and PATTERSON, 2007), both provided at low pasture availability.

For Mayne (1991), the primary reason explaining low milk response to forage supplementation at pasture is the high value of substitution rate between grazed pasture and a conserved forage, providing only small increase in total DM and energy intake. When forage supplements are offered to grazing dairy cows, the pasture DM intake generally decreases because the substitutive effects of the supplement (called filling effect in the INRA feeding systems). That reduction of pasture DM intake per kg of supplement DM intake is called substitution rate (FAVERDIN et al., 1991). High substitution rate when cows are well fed at high PA can reduce the profitability of mixed feeding systems because of the low use efficiency of pasture harvested and of supplement given (STOCKDALE, 2000). In a series of 9 experiments under good grazing conditions in spring, the supplementation with 5 kg DM of corn silage unaffected the milk production of grazing dairy cows (CHÉNAIS et al., 1997).

The average value of substitution rate with forage supplements at grazing are greater than that generally observed with concentrates (MAYNE and WRIGHT, 1988; STOCKDALE, 2000). The predictive model GrazeIn (DELAGARDE et al., 2011) estimates an average value of 0.8 for substitution rate with corn silage supplement, ranging from 0.4 to 1.1 according to PA, substitution rate decreasing with decreasing PA. To increase the use efficiency of conserved forages, it would be necessary to predict the substitutive effects and to estimate total DM intake in order to choose the best supplementation strategy. However, substitutive effects have multifactorial sources, and may be related to characteristics of pasture (pasture height, pasture mass, botanical composition), grazing management (PA), animal (production level, genetic merit, stage of lactation) and supplements (amount, type) (STOCKDALE, 2000; BARGO et al., 2003; INRA, 2007). There are no predictive equations available in the literature allowing to predict pasture intake or milk production responses of dairy cows to forage supplementation at grazing, nor on the effect of grazing management (PA) on these relationships.

The objective of the present meta-analyses was to evaluate the effect of different forage supplement types (corn silage, pasture silage or hay) on substitution rate and milk production responses (milk, fat and protein) of grazing dairy cows. The meta-analysis will also differentiate two possible grazing management strategies used for comparing unsupplemented and supplemented cows: either a similar same PA for the two supplementation levels, or either a lower PA for supplemented cows than for unsupplemented cows. The first strategy enables to determine the effect of forage supplementation under similar initial conditions, while the second one enables to determine the effect of forage supplementation when pasture use efficiency (per hectare) remains high or is increased for supplemented cows.

6.3 MATERIAL AND METHODS

6.3.1 Literature search

A computerized literature search using the Agricola, CAB abstracts and Web of Science was conducted to create the database included in this study. The objective was to identify papers related to dairy cows nutrition on pasture-based diets supplemented with conserved forages. The following keywords were utilized: pasture, substitution rate, supplementation, dairy cow, grazing, forage, hay, silage and straw, using different combinations. The papers were selected if they met the following criteria: (1) lactating dairy cows, (2) measurement of pasture, forage supplement and concentrate intake (kg DM/d), (3) milk production data and (4) at least 2 levels of forage supplementation compared.

6.3.2 Data entry

The initial database included 60 papers written by 21 research groups, published from 1956 to 2016. All papers included 81 experiments and 371 comparisons between different levels of forage supplementation. The database was created so that each row represents the comparison between two levels of forage supplementation in a same experiment, for example 0 vs. 4 kg DM/d of corn silage. Columns represented the characteristics of treatments, several codes and measured variables, such as milk production (kg/d) or milk fat concentration (g/kg). The similar characteristics were filled in the database for the two levels of forage supplementation, with one block of columns per level of forage supplementation. The low level of forage supplementation was called “lowest” and the high level was called “highest”. When only two levels of forage supplementation are reported in one study, thus this comparison represents one row in the file, comparing the lowest vs. the highest levels. When more than two levels of forage supplementation are reported in the same study, the number of comparisons (rows) are proportional to the number of levels of forage supplementation, as showed in Table 12.

Table 12 - Example of comparisons (lowest vs. highest) with data from a paper with three forage supplementation levels, 0, 4, or 8 kg DM/d

Number of comparison	Lowest	Highest
1	0 kg DM/d	4 kg DM/d
2	0 kg DM/d	8 kg DM/d
3	4 kg DM/d	8 kg DM/d

To create the database, the data were computarized according to four categories:

Experimental characteristics: created to understand and define the experimental conditions of the selected studies. Research group, type of pasture, forage and concentrate, pasture allowance and pasture mass, season and date of the experiment, method for estimating pasture DM intake, and statistical design were listed. The data from pasture mass (PM – kg DM/ha) and PA were standardized above ground level (PM_0 and PA_0, respectively), according to equations described by Delagarde et al. (2011). This standardization of PM and PA above ground level allowed to avoid possible misinterpretations of results due to the large differences in PM according to the height at which it is measured (PÉREZ-PRIETO and DELAGARDE, 2012).

Pre-experimental characteristics of cows: these data were collected to be able to calculate the energy balance of cows during the experiment, when all other needed variables were available. Stage of lactation (DIM), milk production (kg/d) and

BW (kg) were reported in the database. Fat-corrected milk production (kg/d) was calculated from milk production and milk fat concentration according to INRA (2007). The net energy requirements for maintenance and milk production were calculated from BW and milk production according to INRA (2007).

Feeds characteristics: the chemical composition of pasture, concentrate and forages were reported in the database when available. The main characteristics of feeds were DM, OM, CP, NDF and ADF, standardized in g/kg DM. When the organic matter (OM) concentration of pasture or forage supplement forage was not described, average values available in INRA feed tables (INRA, 2007) were used, or fixed at 900 g/kg of DM as a standard value otherwise. Net energy values (UFL) of concentrates were obtained in INRA feed tables (INRA, 2007), or fixed at a standard value of 1.05 UFL/kg DM for commercial concentrates. Digestibility was standardized as OM digestibility. Values of DM digestibility (DMd) were converted in OMd from the following equation:

$$OMd = 1.0688 \times DMd - 2.3418$$ (RENÉ BAUMONT, pers. communication).

The UFL (Unité Fourragère Lait) values were calculated from CP, crude fiber (CF, estimated from NDF or ADF) and OMd according to INRA (2007) for pastures and conserved forages.

Animal performance: pasture DM intake (kg DM/d), milk production (kg/d), milk composition (fat and protein concentrations, in g/kg of milk) and grazing behaviour (grazing and rumination time, min/d) were reported in the database for the lowest and highest levels of forage supplementation, for each comparison. Fat-corrected milk production (kg/d) was calculated from milk production and milk fat concentration according to INRA (2007). The net energy balance (UFL/d) was calculated from the difference between the amount of UFL ingested and the cows requirements as described by INRA (2007). The pasture intake rate (g DM/min) was calculated as the ratio between pasture intake (kg of DM/d) and grazing time (min/d).

6.3.3 Data filtering

Two sub-database were created. The first sub-database contains only comparisons of forage supplementation levels at similar PA (*SPA-subset* – 61 comparisons). The second sub-database contains only comparisons of forage supplementation levels where the PA is decreased at highest level of forage supplementation compared to the lowest level (*LPA-subset* – 52 comparisons). In papers to be included in the *SPA-subset*, the difference between PAs in each comparison should not exceed 2.5 kg DM/d above ground level. In papers to be included in the *LPA-subset*, the highest level of forage supplementation needed to have a PA at least 2.5 kg DM/d above ground level less than that at the lowest level.

After that first filtering, additional filtering criteria were used on both subsets. Comparisons with a difference between lowest and highest levels of forage supplementation of less than 2 kg DM/d were excluded. In fact, that low difference may decrease the accuracy of the estimation of substitution rate and milk production response to forage supplementation. Similarly, the difference between concentrate supplementation levels within a comparison should not exceed 1 kg DM/day, to avoid possible misinterpretations of results with possible concentrate interaction on substitution rate and milk production response. Only studies where pasture DM intake was measured by external markers (*n*-alkanes, chromic oxide, ytterbium oxide) or by pasture methods (pasture mass difference) were selected. Only studies on temperate pastures were selected, mainly because of the very low number of comparisons with tropical pastures. Cows should also have access to pasture at least 16 h/d on both lowest and highest levels of forage supplementation.

This study focuses only on global substitution rate, i.e. when the lowest forage supplementation level is close to zero (control treatment without forage supplement). This choice was mainly because comparisons with three or more levels of forage supplements in a same experiment are very few, not allowing to study the marginal substitution rate between two levels of forage supplementation. For this, the lowest level of forage supplement intake within each comparison should not exceed 1 kg DM/d. Higher values were excluded from both subsets.

6.3.4 Final calculations

Intake, milk production and feeding behavior responses to forage supplementation were calculated by difference between the lowest and highest levels of forage supplementation. These values were divided by the difference in forage DM intake between the lowest and highest levels of forage supplementation, to calculate responses per kg DM forage supplement eaten.

6.3.5 Statistical analyses

As studied variables are all calculated within each experiment, it was not possible to include a study effect in the statistical analyses, because it is already included in the calculated variable (substitution rate, milk production response, etc).

Sources of variation of predicted variables were thus studied by simple or multiple linear regressions, considering one or several explicative variables. The influence of the type of supplement (corn silage, pasture silage or hay) on the relationships was also tested including the supplement type as an additional factor in the regressions (covariance analyses). Only significant relationships ($P < 0.05$) are reported in the Results section.

6.4 RESULTS

6.4.1 General databases descriptions

The *SPA-subset* included 61 comparisons between two levels of forage supplementation reported in 19 papers published from 1981 to 2016 (Table 13). The most utilized forage supplements were corn silage (34) followed by hay (15) and then pasture silage (12). The difference between highest and lowest levels of forage supplementation within each comparison varied from 2.0 to 9.5 kg DM/d, averaging 4.6 kg DM/d. On that database, the experimental designs were mainly latin squares, with an average number of cows utilized on each treatment of 5, with on average 125 DIM. The PA varied from 7 to 57 kg DM/d above ground level between comparisons, averaging 25 kg DM/d above ground level.

The *LPA-subset* was composed by 52 comparisons between two levels of forage supplementation reported in 8 papers published from 1981 to 2016 (Table 14). On average, the highest level of forage supplementation received 7.2 kg DM/d more forage and 16 kg DM/d less PA than the lowest level, featuring a PA reduction of 2.2 kg DM/d per kg DM of forage supplement intake. The levels of PA averaged 33 kg DM/d and 17 kg DM/d on the highest and lowest levels of forage supplementation, respectively. The most utilized forage supplement was hay (35) followed by corn silage (9) and pasture silages (8). On that database, the number of cows on each treatment averaged 4, the experimental designs were mainly latin squares, with 56 DMI on average.

Considering both databases, the hay was composed mainly by a mixture of perennial ryegrass and white clover (47) or alfalfa (2). Pasture silage was mainly silage of perennial ryegrass (10) followed by sulla (8) and lotus (2). The most utilized pastures on *SPA-subset* were white clover (18) and mixture of perennial ryegrass and white clover (18), followed by perennial ryegrass (12), persian clover (3) and annual ryegrass (2). On *LPA- subset*, 35 comparisons were made on mixtures of perennial ryegrass and white clover, followed by perennial ryegrass (10) and white clover (1). Fourteen pasture types have not been described. The average pasture CP, ADF, NDF, and net energy concentrations were close between databases, averaging 195, 445, 261 g/kg DM and 0.88 UFL/kg DM, respectively. Pasture intake was determined by the sward-sampling technique (94), the *n*-alkanes technique (16), or by the fecal output (from external markers) and pasture digestibility technique (3).

Table 13 - Summary of the 18 papers included in the meta-analyses to determine the effect of forage supplementation on pasture intake, substitution rate, milk production, milk composition, and grazing behavior of grazing dairy cows at similar PA (*SPA-subset*).

Reference	Country[1]	Forage Comp[2]	Forage Supplement[3]	Forage kg DM/d[4]	Concentrate kg DM/d[5]	Pasture[6]	No. of Cows[7]	DIM[8]	PA_0[9]
Moran and Stockdale (1992)	AU	2	Corn silage	2.9	0.3	Mixed	9	38	45.0
Moran and Croke (1993)	AU	1	Corn silage	2.4	0.0	Mixed	5	74	25.0
Stockdale (1994)	AU	3	Corn silage	6.2	0.0	Persian clover	4	60	16.5
Stockdale and Dellow (1995)	AU	10	Corn silage	4.3	0.0	White clover	4	157	21.2
Stockdale (1996)	AU	2	Corn silage	4.4	0.0	White clover	4	213	28.9
Stockdale (1997a)	AU	4	Corn silage	4.7	0.0	White clover	4	132	21.5
Stockdale (1997b)	AU	2	Corn silage	4.9	0.0	White clover	4	234	21.3
Burke et al. (2008)	IE	1	Corn silage	3.2	0.0	Perennial ryegrass	12	140	38.3
Pérez-Prieto et al. (2011)	FR	2	Corn silage	6.8	1.0	Perennial ryegrass	6	230	45.8
Miguel et al. (2014)	BR	2	Corn silage	3.2	0.5	Annual ryegrass	6	175	34.3
Miguel et al. (2016)	FR	2	Corn silage	3.9	0.0	Perennial ryegrass	6	175	34.5
Moate et al. (1984)	AU	4	Pasture silage	4.5	0.0	NR[10]	4	NR	11.3

Chaves et al. (2002)	NZ	4	Corn and past. Silage	5.5	0.0	NR	10	156	18.0
Woodward et al. (2006)	NZ	4	Corn and past. Silage	5.5	0.0	Perennial ryegrass	10	146	25.0
Morrison and Patterson (2007)	UK	3	Corn and past. Silage	4.3	0.0	Perennial ryegrass	6	49	28.3
Stockdale et al. (1981)	AU	8	Hay	6.7	0.0	Mixed	2	9	20.3
Stockdale (1999)	AU	1	Hay	3.9	0.0	Mixed	8	147	31.5
Wales et al. (2000)	AU	4	Hay	2.6	0.0	Mixed	6	191	26.0
Reis and Combs (2000)	US	2	Hay	3.2	-0.3	Mixed	10	91	41.9

[1]AU = Australia; IE = Ireland; FR = France; BR = Brazil; NZ = New Zealand; UK = United Kingdom; US = United States.
[2]Number of forage supplementation comparisons (comp) considered within each paper.
[3]Pasture silage = compose by one or several species. Hay = compose by one or several species.
[4]Mean difference between the lowest and highest level of forage supplementation within each paper
[5]Mean difference between the lowest and highest level of concentrate supplementation within each paper
[6]Mixed = mixture of two or several species.
[7]Number of cows on each treatment.
[8]Days in milk at the start of treatment application
[9]Pasture allowance above ground level.
[10]Not reported

Table 14 - Summary of the 8 papers included in the meta-analyses to determine the effect of forage supplementation on pasture intake, substitution rate, milk production, milk composition, and grazing behavior of grazing dairy cows at different supplementing strategy (*LPA-subset* - forage supplemented treatments receive lower PA than unsupplemented treatments)

Reference	Country[1]	Forage Comp[2]	Forage Supplement[3]	Forage kg DM/d[4]	Concentrate kg DM/d[5]	Pasture[6]	No. of Cows[7]	DIM[8]	dPA_0[9]
Stockdale (1996)	AU	1	Corn silage	4.4	0.0	White clover	4	213	20.5
Burke et al. (2008)	IE	1	Corn silage	3.2	0.0	Perennial ryegrass	12	140	11.8
Pérez-Prieto et al. (2011)	FR	1	Corn silage	6.8	1.0	Perennial ryegrass	6	230	22.4
Miguel et al. (2016)	FR	4	Corn silage	4.5	0.0	Perennial ryegrass	6	175	15.6
Moate et al. (1984)	AU	2	Pasture silage	4.5	0.0	NR[10]	4	NR	7.5
Chaves et al. (2002)	NZ	4	Corn and past. silage	5.5	0.0	NR	10	156	20.0
Woodward et al. (2006)	NZ	4	Corn and past. silage	5.5	0.0	Perennial ryegrass	10	146	25.0
Stockdale et al. (1981)	AU	35	Hay	8.4	0.0	Mixed	3	8	20.3

[1]AU = Australia; IE = Ireland; FR = France; BR = Brazil; NZ = New Zealand; UK = United Kingdom; US = United States.
[2]Number of forage supplementation comparisons (comp) considered within each paper.
[3]Pasture silage = compose by one or several species. Hay = compose by one or several species.
[4]Mean difference between the lowest and highest level of forage supplementation within each paper

[5]Mean difference between the lowest and highest level of concentrate supplementation within each paper
[6]Mixed = mixture of two or several species.
[7]Number of cows on each treatment.
[8]Days in milk at the start of treatment application
[9]Pasture allowance above ground level.
[10]Not reported

Table 15 - Summary statistics of the studies included in the meta-analyses to determine the effect of forage supplementation level on pasture DM intake, milk production, milk composition, and grazing behavior of grazing dairy cows at similar PA (*SPA- subset*)

		Forage supplementation level							
		Lowest		Highest		Difference[2]		Response[3]	
Item	n[1]	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
All forage supplements (complete database)									
PA_0, kg DM/day	61	25.2	*9.9*	25.3	*10.0*	0.1	*0.7*	-	-
DMI, kg/d									
- Pasture	61	11.5	*3.0*	9.8	*3.1*	-1.7	*1.1*	-0.39	*0.27*
- Forage	61	0.0	*0.1*	4.6	*1.7*	4.6	*1.7*	-	-
- Concentrate	61	0.7	*2.1*	0.7	*2.1*	0.1	*0.2*	-	-
- Total	61	12.2	*3.4*	15.2	*2.9*	3.0	*1.7*	0.63	*0.27*
Milk production, kg/d	61	16.2	*4.9*	18.2	*4.7*	2.0	*1.5*	0.41	*0.31*
Milk fat concentration, g/kg	57	41.7	*3.2*	41.0	*3.2*	-0.7	*2.0*	-0.15	*0.52*
Milk protein concentration, g/kg	49	31.0	*2.8*	31.5	*2.3*	0.5	*1.1*	0.07	*0.33*
Grazing time, min/d	9	477	*90.3*	412	*55.4*	-65.4	*42.7*	-17.3	*11.7*
Ruminating time, min/d	5	397	*13.9*	439	*43.1*	41.6	*53.6*	7.2	*9.5*
Pasture intake rate, g of DM/min	9	26.9	*4.6*	25.0	*5.8*	-1.88	*4.21*	-0.45	*1.17*
Corn silage subdatabase									
PA_0, kg DM/day	34	26.6	*10.1*	26.8	*10.4*	0.2	*0.5*	-	-
DMI, kg/d									
- Pasture	34	13.2	*2.2*	11.6	*2.1*	-1.6	*1.2*	-0.36	*0.28*
- Forage	34	0.0	*0.0*	4.5	*1.3*	4.5	*1.3*	-	-
- Concentrate	34	0.0	*0.0*	0.1	*0.3*	0.1	*0.3*	-	-
- Total	34	13.2	*2.2*	16.2	*2.1*	3.0	*1.4*	0.67	*0.27*
Milk production, kg/d	34	17.0	*4.3*	19.5	*4.1*	2.5	*1.4*	0.54	*0.28*
Milk fat concentration, g/kg	33	41.9	*3.2*	41.1	*3.3*	-0.8	*1.7*	-0.20	*0.47*
Milk protein concentration, g/kg	33	30.3	*2.4*	31.1	*2.2*	0.8	*0.9*	0.17	*0.21*
Pasture silage subdatabase									
PA_0, kg DM/day	12	20.9	*10.0*	20.9	*10.0*	0.0	*0.0*	-	-
DMI, kg/d									
- Pasture	12	9.9	*3.2*	8.4	*3.2*	-1.5	*1.0*	-0.31	*0.16*

- Forage	12	0.0	*0.0*	4.7	*1.3*	4.7	*1.3*	-	-
- Concentrate	12	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	-	-
- Total	12	9.9	*3.2*	13.2	*3.2*	3.2	*1.1*	0.69	*0.16*
Milk production, kg/d	12	13.7	*1.8*	15.2	*2.3*	1.5	*1.1*	0.34	*0.25*
Milk fat concentration, g/kg	9	41.9	*2.3*	40.4	*1.9*	-1.5	*2.2*	-0.37	*0.59*
Milk protein concentration, g/kg	9	31.3	*1.1*	31.5	*1.2*	0.2	*0.6*	0.04	*0.14*
Hay subdatabase									
PA_0, kg DM/day	15	25.4	*8.9*	25.5	*8.7*	0.1	*1.3*	-	-
DMI, kg/d									
- Pasture	15	8.9	*2.0*	6.9	*2.2*	-2.0	*0.7*	-0.51	*0.30*
- Forage	15	0.0	*0.1*	5.0	*2.7*	4.9	*2.8*	-	-
- Concentrate	15	2.8	*3.7*	2.8	*3.6*	0.0	*0.1*	-	-
- Total	15	11.7	*4.9*	14.6	*3.2*	2.9	*2.7*	0.48	*0.31*
Milk production, kg/d	15	16.4	*7.0*	17.6	*6.2*	1.2	*1.8*	0.17	*0.26*
Milk fat concentration, g/kg	15	41.4	*3.9*	41.4	*3.9*	-0.1	*2.4*	0.09	*0.54*
Milk protein concentration, g/kg	7	34.2	*3.9*	34.2	*3.9*	-0.9	*1.2*	-0.37	*0.55*

[1]n = number of data
[2]Difference between the highest and lowest levels of forage supplementation
[3]Effects of 1 kg of DM intake of forage supplement on DM intake, milk production, milk composition and grazing behaviour.

Table 16 - Summary statistics of the studies included in the meta-analyses to determine the effect of forage supplementation level on pasture DM intake, milk production, milk composition, and grazing behavior of grazing dairy cows under different supplementation strategies (*LPA-subset* – forage supplemented treatments receive lower PA than unsupplemented treatments).

		Forage supplementation level							
		Lowest		Highest		Difference[2]		Response[3]	
Item	n[1]	Mean	*SD*	Mean	SD	Mean	*SD*	Mean	*SD*
All forage supplements (complete database)									
PA_0, kg DM/day	52	33.5	*14.0*	17.4	*8.5*	-16.1	*9.6*	-2.7	*2.2*
DMI, kg/d									
- Pasture	52	11.2	*4.0*	6.3	*3.5*	-5.0	*1.6*	-0.79	*0.38*
- Forage	52	0.0	*0.0*	7.2	*2.6*	7.2	*2.6*	-	-
- Concentrate	52	0.0	*0.0*	0.0	*0.1*	0.0	*0.1*	-	-
- Total	52	11.2	*4.0*	13.5	*2.0*	2.3	*3.0*	0.22	*0.38*
Milk production, kg/d	52	15.3	*3.6*	14.8	*2.8*	-0.5	*2.2*	-0.13	*0.33*
Milk fat concentration, g/kg	48	40.4	*3.4*	41.5	*2.5*	1.1	*3.3*	0.18	*0.57*
Milk protein concentration, g/kg	13	32.1	*1.6*	31.6	*1.9*	-0.5	*1.3*	-0.15	*0.27*
Grazing time, min/d	5	508	*22*	445	*15*	-63.4	*28.5*	-12.4	*3.2*
Ruminating time, min/d	1	393	-	483	-	90.0	-	13.2	-
Pasture intake rate, g DM/min	5	33.4	*6.3*	25.4	*4.8*	-8.0	*2.9*	-1.7	*0.7*
Corn silage subdatabase									
PA_0, kg DM/day	9	43.2	*9.3*	25.2	*8.1*	-18.0	*8.1*	-3.8	*1.6*
DMI, kg/d	9								
- Pasture	9	16.4	*2.6*	11.0	*2.4*	-5.4	*1.5*	-1.15	*0.27*
- Forage	9	0.0	*0.0*	4.8	*1.0*	4.8	*1.0*	-	-
- Concentrate	9	0.0	*0.0*	0.1	*0.3*	0.1	*0.3*	-	-
- Total	9	16.4	*2.6*	15.9	*1.7*	-0.6	*1.6*	-0.14	*0.30*
Milk production, kg/d	9	19.7	*4.5*	18.7	*4.2*	-1.0	*2.4*	-0.22	*0.44*
Milk fat concentration, g/kg	9	38.6	*3.6*	39.1	*3.8*	0.5	*1.2*	0.09	*0.22*
Milk protein concentration, g/kg	8	32.3	*1.9*	31.5	*2.2*	-0.7	*1.6*	-0.19	*0.33*
Pasture silage subdatabase									
PA_0, kg DM/day	8	36.8	*14.5*	18.0	*7.2*	-18.8	*7.3*	-4.0	*1.8*
DMI, kg/d									
- Pasture	8	14.7	*4.5*	8.7	*3.5*	-6.0	*1.2*	-1.28	*0.28*
- Forage	8	0.0	*0.0*	0.0	*0.0*	4.8	*1.0*	-	-

- Concentrate	8	0.0	*0.0*	4.8	*1.0*	0.0	*0.0*	-	-
- Total	8	14.7	*4.5*	13.5	*3.6*	-1.2	*1.3*	-0.28	*0.28*
Milk production, kg/d	8	16.4	*1.5*	14.2	*1.4*	-2.2	*1.3*	-0.47	*0.27*
Milk fat concentration, g/kg	5	42.2	*0.8*	41.0	*2.0*	-1.2	*1.6*	-0.23	*0.32*
Milk protein concentration, g/kg	5	31.8	*1.3*	31.6	*1.5*	-0.3	*0.5*	-0.08	*0.14*
Hay subdatabase									
PA_0, kg DM/day	35	30.3	*13.9*	15.2	*7.9*	-15.0	*10.4*	-2.1	*2.2*
DMI, kg/d									
- Pasture	35	9.1	*2.0*	4.5	*2.1*	-4.6	*1.5*	-0.58	*0.22*
- Forage	35	0.0	*0.0*	8.4	*2.3*	8.4	*2.3*	-	-
- Concentrate	35	0.0	*0.0*	0.0	*0.0*	0.0	*0.0*	-	-
- Total	35	9.1	*2.0*			3.8	*2.2*	0.42	*0.26*
Milk production, kg/d	35	13.9	*2.6*	13.9	*1.4*	0.1	*2.1*	-0.03	*0.62*
Milk fat concentration, g/kg	35	40.6	*3.4*	42.1	*1.9*	1.5	*3.7*	0.26	*0.22*
Milk protein concentration, g/kg	-	-	-	-	-	-	-	-	-

[1]n = number of data

[2]Difference between the highest and lowest level of forage supplementation

[3]Effects of 1 kg of DM intake of forage supplement on DM intake, milk production, milk composition and grazing behaviour.

6.4.2 SPA-subset

The summary of statistics is described on Table 15 for the *SPA-subset*. The SR was on average 0.39, with higher values for hay than for corn silage or pasture silage (0.51 vs 0.36 vs 0.31, respectively). The milk production increased on average by 0.41 kg per kg DM of forage supplement intake. That response was however greater for corn silage, followed by pasture silage and then hay (0.54 vs 0.34 vs 0.17, respectively). The supplementation with corn silage or pasture silage decreased the milk fat concentration, with an average decrease of 0.20 and 0.37 g/kg per kg DM supplement intake, respectively. The supplementation with hay increased the milk fat concentration by 0.17 g/kg per kg DM of supplement intake, and decreased the milk protein concentration (-0.37 g/kg per kg DM of supplement intake). Corn silage and pasture silage increased the milk protein concentration, with higher milk protein concentration response with corn silage than with pasture silage supplementation (0.17 vs 0.04 g/kg per kg DM of supplement intake, respectively). The grazing time decreased on average by 17 min/d per kg DM of supplement intake, which represents a 3.5% decrease of grazing time per kg DM of supplement intake. There was also a decrease of pasture intake rate with forage supplementation, which averaged 0.45 g DM/min (-1.7%) per kg DM of supplement intake. This means that the decrease of pasture DM intake with forage supplementation is mediated through concomitant reduction of grazing time (2/3 of the effect) and pasture intake rate (1/3 of the effect).

On the *SPA-subset*, the best factor explaining the SR is the PA (Table 17 and Figure 1). The predictive models considering PA were more precise (R^2 of 0.47 and 0.40 vs. 0.19 and 0.17; standard error of 0.20 and 0.21 vs. 0.27 and 0.25) than predictive models considering either supplementation level variation or net energy balance of the cows. On average, the SR increases by 0.18 per each increase of 10 kg of PA. This may represent a difference of SR of 0.5 to 0.6 between a low and a high PA (20 vs. 50 kg DM/d of PA, respectively). At similar PA, the SR differed according the forage supplement type. According to the model 1 (Table 17), for a similar PA, the predicted value of SR was greater for hay than for corn silage and pasture silage (+ 0.17 and + 0.12, respectively). The SR was also positively related to net energy balance, with an average increase in SR of 0.08 per each increase in net energy balance of 1 UFL/day ($P < 0.01$). Cows with low net energy balance had a low SR (Figure 1 C). The level of forage supplementation had a significant and negative effect on SR ($P < 0.05$). For each additional kg DM/d of forage supplement, the predicted SR decreased by 0.05 (model 4: Table 17). However, the precision of the prediction of SR of that model is much less than when PA is taken into account (R^2 0.17 vs. 0.47 for models 4 and 1 respectively). Including supplementation level in model 1 did not improve the precision of the prediction.

The increase of substitution rate affected negatively the milk production response (Figure 2 A). On average, an increase of substitution rate of 0.1 lead to a decrease of milk production response by -0.04 kg/kg of forage DM intake (model 5: Table 17). These responses varied according to the forage type. Using the predictive model 5 with the averaged value of SR obtained on Table 15 for the *SPA-subset* (0.39), the milk production response was greater with corn silage, followed by pasture silage and then hay (0.53 vs. 0.30 vs. 0.22 kg of milk per kg DM of supplement intake, respectively). The milk fat concentration response increased with increasing SR (Table 17; Figure 2 B). Any increase in SR by 0.1 represents a decrease of 0.05g/kg per kg of forage supplement intake (model 6: Table 17). With corn silage, that response was greater when compared to the averaged effects of hay and pasture silage. At low protein pastures, the milk fat concentration response decrease by 0.06g/kg per kg of forage when the CP decrease 10 g/kg DM (model 7: table 17).

Figure 3 - Effect of **A)** pasture allowance, **B)** forage supplementation level, and **C)** energy balance on substitution rate of grazing cows supplemented with conserved forages (corn silage (□), pasture silage (Δ), hay (●)) compared at similar PA (S*PA-subset*). Effect of **D)** substitution rate, **E)** forage supplementation 1level, and **F)** total intake variation of grazing cows supplemented with conserved forages compared at similar PA (*SPA-subset*).

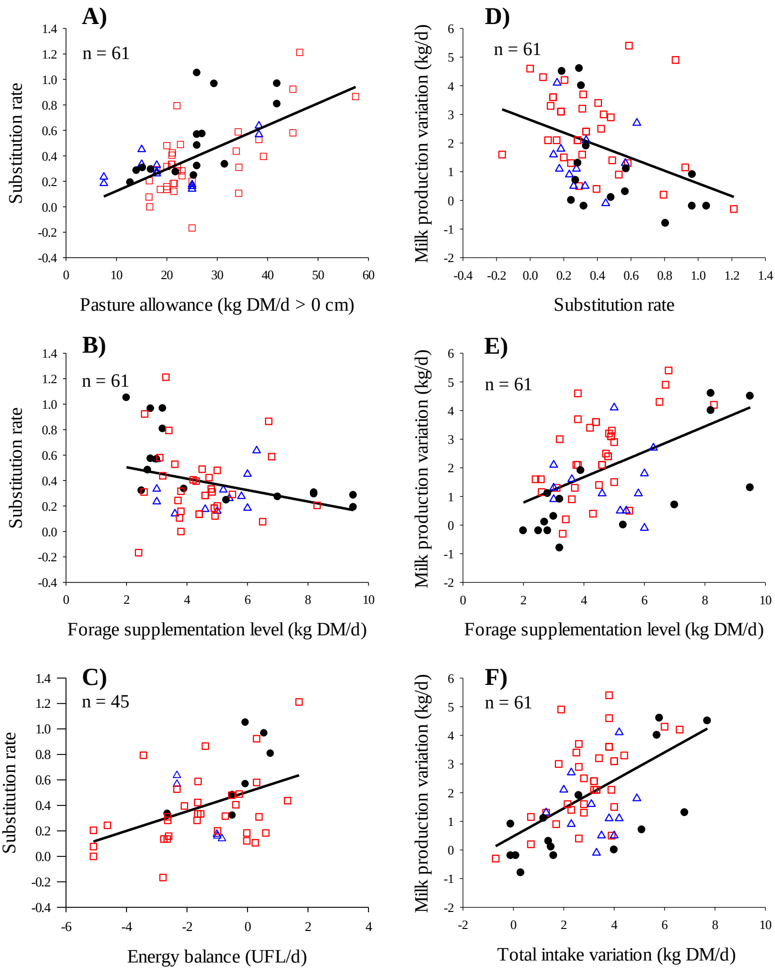


Figure 2 - Effect of substitution rate on **A)** milk production response, **B)** milk fat response, **C)** 4% FCM production response, and **D)** milk protein response of grazing cows supplemented with conserved forages (corn silage (□), pasture silage (Δ), hay (●)) compared at similar PA (*SPA-subset*).

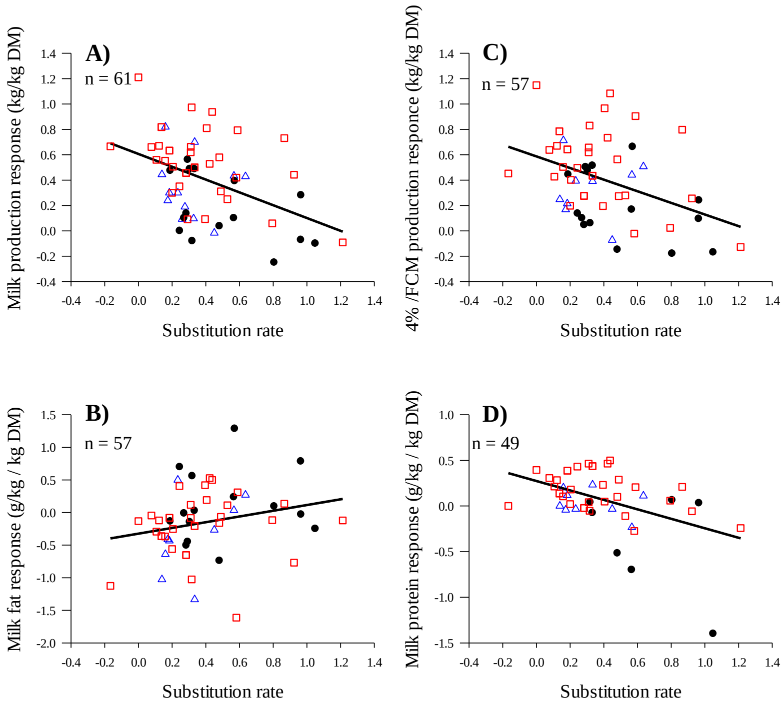


Table 17 - Multiple linear regression models for predicting the substitution rate, milk production response, milk fat response and milk protein response on grazing cows supplemented with conserved forages at similar PA (S*PA-subset*)

Predicted variable	Substitution rate				Milk production response (kg/kg DM)	Milk fat response (g/kg / kg DM)	Milk protein response (g/kg / kg DM)
Model number	1	2	3	4	5	6	7
n	61	61	45		61	53	45
R^2	0.47	0.40	0.19	0.17	0.40	0.26	0.67
SD	0.20	0.21	0.27	0.25	0.24	0.46	0.21
Origin	-0.036	-0.048	0.506	0.623	0.516	-1.454	0.906
Fixed effect of supplement type							
- Corn silage	-0.076			-0.047	0.179	-0.015	0.178
- Pasture silage	-0.021			-0.080	-0.049	-0.245	-0.070
- Hay	+0.097			0.127	-0.130	0.260	-0.108
Animal							
- Energy balance (UFL/d)			0.077				
- Substitution rate					-0.421	0.487	-0.403
Supplementation level variation (kg DM/d)				-0.049			
- Forage NDF (g/kg DM)							-0.002
Pasture							
- PA_0 (kg DM/d)	0.018	0.017					
- CP (g/kg DM)						0.006	0.002

The milk protein concentration response was different according the forage type and decrease by 0.04 when the substitution rate increase 0.1. The decrease of CP content of pasture and the increase of NDF content of forage supplement promote the decrease of milk protein concentration response.

6.4.3 LPA-subset

The summary of statistics is described on table 16 for the *LPA-subset*. The SR averaged 0.79 for all forage supplements. Higher substitutive effects were observed on pasture silage, followed by corn silage and hay (1.28 vs. 1.15 vs. 0.58). On average, the milk production was little affected by forage supplementation, averaging 15 kg/d, even with the increase of total DM intake on highest level of supplementation. The milk fat production was low effect by corn silage supplementation, but decrease with pasture silage and increase with hay supplementation (-0.23 and +0.26 g/kg per 1 kg DM of forage supplement intake respectively. The milk protein concentration decrease on average 0.15 g/kg per 1 kg of forage supplement intake. That decrease was higher with corn silage than pasture silage supplementation (-0.19 vs. -0.08 respectively). The grazing time decreased on average by 12.4 min/d (-2.4%) per kg DM of forage supplement intake. The pasture intake rate decreased by 1.7 g DM/min (-5%) per kg DM of forage supplement intake. On the SPA-subset, the decrease of pasture DM intake with forage supplementation is thus mediated through a large variation of pasture intake rate (2/3 of the effect) and of a smaller relative variation of grazing time (1/3 of the effect).

On the *SPA-subset*, the SR is correlated with the pasture allowance variation and to the forage supplementation level (Table 18 and Figure 3). According the predictive model 8 (Table 18), the SR decreased with increasing forage supplementation level (- 0.05 per kg DM of forage supplement intake) and increased with decreasing PA of highest level of supplementation (- 0.16 per each 10 kg of PA decrease at highest level of supplementation). That result differed according the forage type, lower SR being observed with hay than with corn silage and pasture silage (-0.38 on average). Cow with low energy balance showed also a low SR when they werer supplemented (Figure 3 C). The substitution rate increased by 0.13 per each increase of energy balance of 1 UFL/d (model 9: Table 18).

The milk productive responses were negatively affected by SR, except for milk fat concentration response (Figure 4). The milk production response decreased by 0.09 kg per kg DM of supplement intake for each increase of 0.1 of SR (model 10: table 18). According to that model, the milk response was greater with corn silage than with pasture silage and hay (+ 0.23 on average). The milk fat concentration response to forage supplementation varied according to the forage type and to supplementation level (model 11: table 18). For each increase of 1 kg DM of forage supplement, the milk fat concentration increased on average by 0.12 g/kg, with greater response when cows were supplemented with hay. The milk protein concentration response decreased by 0.05 g/kg per kg DM of forage supplement intake for each 0.1 of increase in SR, independently of the forage type (model 12: table 18).

Figure 3 - Effect of **A)** pasture allowance variation , **B)** forage supplementation level, and **C)** energy balance on substitution rate of grazing cows supplemented with conserved forages (corn silage (□), pasture silage (Δ), hay (•)) compared at different PA (*LPA-subset*). Effect of **D)** substitution rate, **E)** forage supplementation level, and **F)** total intake variation of grazing cows supplemented with conserved forages compared at different PA (*LPA-subset*).

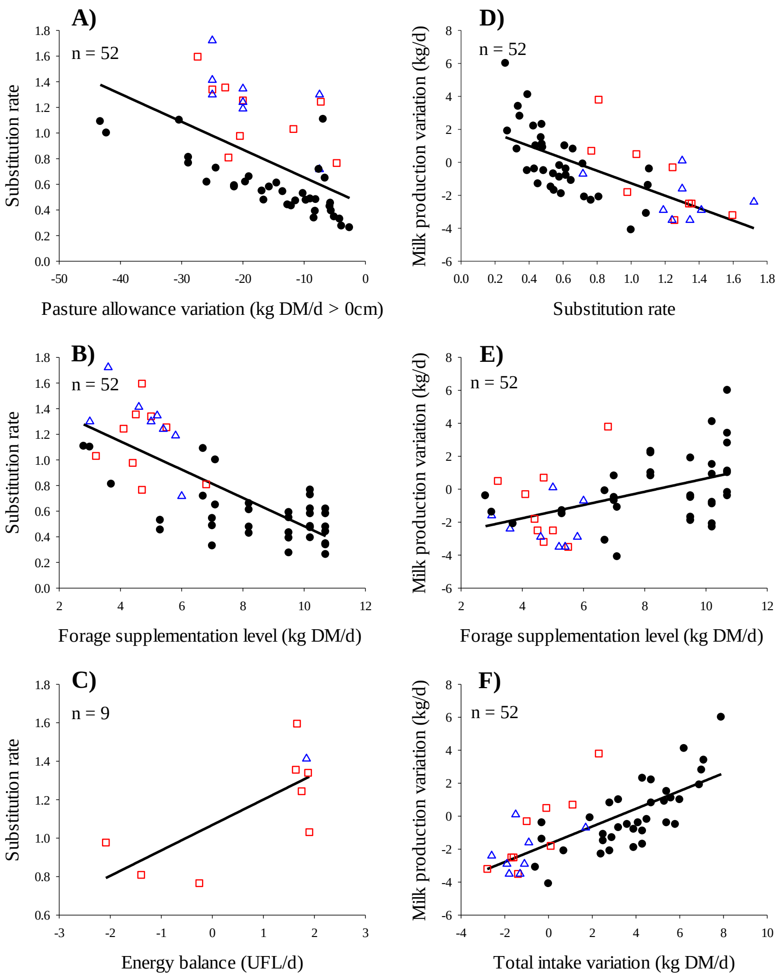


Figure 4 - Effect of substitution rate on **A)** milk production response, **B)** milk fat response, **C)** 4% FCM production response, and **D)** milk protein response of grazing cows supplemented with conserved forages (corn silage (□), pasture silage (Δ), hay (•)) compared at different PA (*LPA subset*).

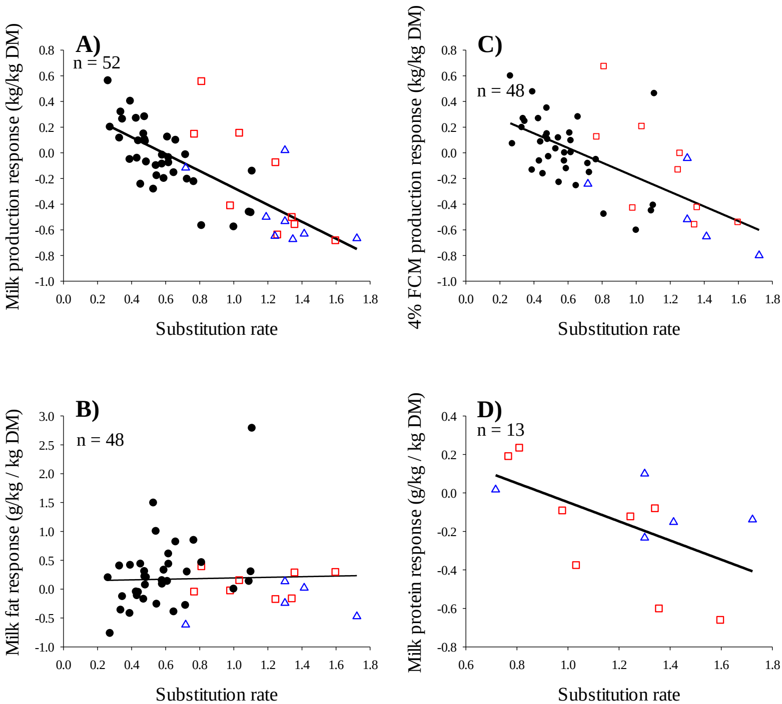


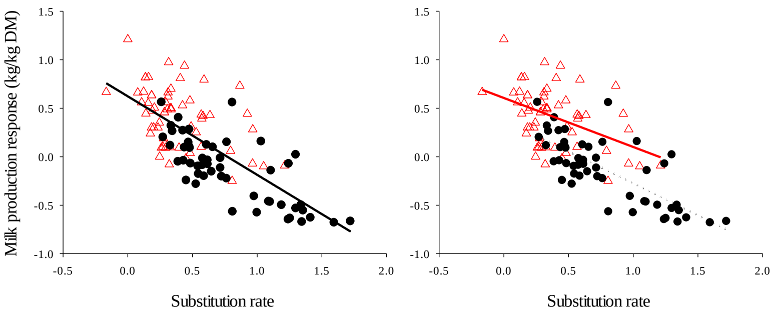
Table 18 - Multiple linear regression models for predicting the substitution rate, milk production response, milk fat response and milk protein response on grazing cows supplemented with conserved forages at different PA (*LPA-subset* – forage supplemented treatments receive lower PA than unsupplemented treatments).

Predicted variable	Substitution rate		Milk production response (kg/kg DM)	Milk fat response (g/kg / kg DM)	Milk protein response (g/kg / kg DM)
Model number	8	9	10	11	12
N	52	9	52	48	13
R^2	0.88	0.53	0.65	0.27	0.33
SD	0.14	0.21	0.20	0.50	0.23
Origin	1.059	1.068	0.675	0.761	0.448
Fixed effect of supplement type					
- Corn silage	0.069		0.156	-0.091	
- Pasture silage	0.186		0.023	-0.448	
- Hay	-0.255		-0.179	0.539	
Animal					
- Energy balance (UFL/d)		0.132			
Substitution rate			-0.912		-0.497
Supplementation level variation (kg DM/d)	-0.055			0.123	
Pasture					
- PA_0 variation (kg DM/d)	-0.016				
CP (g/kg DM)					

6.4.4 Global relationships between substitution rate and milk responses

The average values of substitution rate were lower on *SPA-subset* than on *LPA-subset* (0.39 vs. 0.79, respectively). At the reverse, the average values of milk production response to forage supplementation were higher on SPA-subset than on LPA-subset (0.41 vs. -0.13, respectively). On both databases, SR was the main factor explaining milk production response (Figure 5). The relationship (slope) between the milk production response and the substitution rate was similar on the two databases but, at similar SR, the milk production response was lower on the *LPA-subset* than on the *SPA-subset* (-0.30). On both databases, any increase in SR of 0.1 lead to a decrease of milk production response by 0.06 kg per kg DM of forage supplement intake. The effect of forage type on milk production response differed according the database. The most effective forage to increase the milk production is the corn silage on both databases.

Figure 5 - Relation between milk production response (kg of milk per kg DM of forage supplement) and substitution rate when forage supplement is given either at similar PA (*SPA-subset*, Δ) or at lower PA (*LPA-subset*, ●) than unsupplemented cows. Milk production response = 0.49 + α - 0.602 × substitution rate, with α = 0.15 for DPA-subset and -0.15 for SPA-subset (n= 113, R^2=0.62, SD=0.25).



6.5 DISCUSSION

The objective of the present meta-analyses was to evaluate the effect of different forage supplement types (corn silage, pasture silage or hay) on substitution rate and milk production responses (milk, fat and protein) of grazing dairy cows. This study showed two possible grazing management strategies used for comparing unsupplemented and supplemented cows: either a similar same PA for the two supplementation levels (*SPA-subset*), or either a lower PA for supplemented cows than for unsupplemented cows (*LPA-subset*).

6.5.1 Substitution rate

On both database, the main factors related to the substitution rate were associated to the PA. On *SPA-subset*, the variation of SR is directly related to average values of PA on both forage types. That relation between the pasture allowance and substitution rate was similar to presented on GrazeIn model (DELAGARDE et al., 2011) with increasing SR with increase the PA. At low PA, cows are underfeed on pasture and the supplementing with conserved forages has a low effect on pasture intake, leading a low substitution rates (STOCKDALE, 1996; PHILLIPS, 1998). On this meta-analysis, the SR on *SPA-subset* varied from 0.3 to 1.1 with increase the PA, similar to results obtained by Delagarde et al (2011) and a review of Phillips (1988). However, at medium PA (30-35 kg DM/d > 0 cm) the averaged value obtained in our model (0.5 for both forage types) were lower than described were lower than predicted by Delagarde et al. (2011), averaging 0.8. In a series of studies of grazing cows supplemented with conserved forages on two contrasting PA, the average value of SR was 0.5 (MOATE et al., 1984; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011; MIGUEL et al., unpublished). On these studies, the

PA ranged from 7 to 68 kg DM/d > 0 cm and the grazing cows received on average 5 kg DM of supplement, showing the coherence of our results.

On *LPA-subset*, the high values of SR, compared to obtained on *SPA-subset*, was expected and was resulted from the associative effects of PA reduction and substitutive effects of forage supplementation *per se*. To adjust the residual sward height to target a similar value to unsupplemented cows at low PA, Miguel et al. (unpublished) reduce the PA from 22.6 to 18.1 kg DM/day to corn silage supplemented cows. That strategy represent an increase of SR by 0.06 for each decrease of PA by 1 kg DM/day, when compared supplemented cows at similar PA (MIGUEL et al., unpublished). On our meta-analysis the reduction of each 1.0 kg of PA on LPA-subset, represent an increase of SR by 0.02, with similar forage supplementing level. According the model of Pérez-Prieto and Delagarde (2013) for the similar PA allowance reduction from lowest to highest level of forage supplementation on *LPA-subset*, the pasture intake decrease by 11.2 to 8.5 kg DM/d. That indicated the on half part of pasture intake reduction is associated to the PA, not to substitutive effects of forage supplementation. Generally, that strategy is not able to maintaining the total DM intake of forage supplemented cows at pasture when compared at unsupplemented cows at high PA (CHAVES et al., 2002; WOODWARD et al., 2006;). For this, Moran and Stockdale (1992) increased the level of forage supplementation from 3 to 8 kg DM/day to compensate the reductions from high to low PA. Our model indicate the increase the level of supplementation decrease the SR, with positive effect on total DM intake on *LPA-subset*.

The relationship between the energy balance and the SR on grazing cows supplemented with conserved forages at similar PA was previously reported by Pérez-Prieto et al. (2011). The increase of energy balance of unsupplemented cows with increase the PA promoted the increase of SR from 0.50 to 0.75

(PÉREZ-PRIETO et al., 2011). On this meta-analysis, that relation between the energy balance and SR has a similar slope than Faverdin et al. (1991) between conserved forages and concentrates, and Peyraud and Delaby (2001) between pasture and concentrates.

The forage supplementation has a similar effect on decrease the grazing time on both databases, however the low values of pasture intake rate on highest level of supplementation on *LPA-subset* showed the associative effect PA variation. For Pérez-Prieto et al. (2011), the increase on nutritional status of supplemented is related to strong behavioural adaptations compared to unsupplemented cows. For these authors, supplemented cows are low motivated to graze, taking to low grazing times and pasture intake rates. Reductions of grazing time and pasture intake rate were reported by a series of authors with forage supplemented cows on pasture (PHILLIPS and LEAVER, 1985; GRAF et al., 2005; PÉREZ-PRIETO et al., 2011; MIGUEL et al, unpublished). On average for each kg of silage intake the grazing time reduced and the pasture intake rate reduce on average 14 min/d and 0.9 g DM/min respectively (PHILLIPS and LEAVER, 1985; GRAF et al., 2005; PÉREZ-PRIETO et al., 2011; MIGUEL et al, unpublished). The PA reduction affected strongly the pasture intake rate, with less 2.5 g DM/min per each decrease of 10 kg DM of PA, affecting negatively the pasture intake (PÉREZ-PRIETO and DELAGARDE, 2013). Miguel et al. (unpublished) showed this associative effect of PA reduction and substitution rate on pasture intake rate. The decrease of PA by 20%, represent a decrease of 10% on pasture intake rate in comparison to cows supplemented with corn sil

age at similar PA. That large variation on pasture intake rate on *LPA-subset* may have contributed to high SR in comparison to *SPA-subset*.

6.5.2 Milk productive responses

The effect of substitution rate on milk responses were reported by Mayne (1991) for grazing cows supplemented with conserved forages. For that author the low effects of forage supplementation on milk production of cows are related to the high substitution rates. However, no equations are available on literature to quantify this relationship. On both databases, all milk productive responses increasing with decrease the SR, with similar predicted values, except the fat production response on *LPA-subset*. The milk production response are similar slope on both bases, and with increase 0.06 kg/d per kg DM of forage intake with increase the SR by 0.1. At low SR, the total DM intake increase with forage supplementation, affecting positively the milk production of cows. This efficacy to forage supplements to increase the milk production of grazing cows was obtained at underfeed situations, especially at low PA (Stockdale 1996; 2000). At low PA, grazing cows supplemented with 5 kg DM of corn silage increasing the milk production on average by 3 kg/d compared to unsupplemented cows when the substitution rate averaged 0.2 (Stockdale 1994; Stockdale and Dellow, 1995; Miguel et al, 2014). However, with increase the PA from 19 to 39 kg DM/d the milk production response decrease from 0.8 to 0.1 kg/kg of forage supplement intake (Stockdale 1996).

Although the substitution rate better explain the variation on milk fat and protein concentration responses, characteristics of pastures and forage were related to the milk fat and protein production responses on *SPA-subset*. For each increase of 10g/kg DM of pasture CP content, the milk fat and protein production response increase 0.06 and 0.02 g/kg per kg DM of forage intake respectively. That increase of fat and protein content of milk are positively correlated with energy of diet (Coulon and Rémond, 1991), however even with energy supply of corn silage, at pasture with low CP contents the milk protein content can decrease on pastures with low CP content (Stockdale 1997b). Normally, when the cows are supplemented with conserved forages without concentrates, the total protein of diet depressed with increase the substitution rate (Kolver et al., 2001; Miguel et al., unpublished). However on white clover pastures at low PA, even with depressed of CP content of diet, the milk protein concentration was unaffected with intake of 4 kg DM of corn silage (Stockdale 1997b).

6.6 CONCLUSIONS

The present meta-analyses showed that substitution rate and milk response to dairy cow supplemented with conserved forages are strongly related to PA allowance variations. At similar initial grazing condition, the forage supplementation can increase the total DM intake and the energy supply of grazing cows specially at low PA, when the substitution rate is lower. To increase the use of pasture per hectare, reduce the PA of supplemented cows can be a strategy of dairy farmers, however on that condition, the substitution rate is greater and to compensate the low available pasture the increase the level of supplementation is necessary. On both strategies, the increase of the substitution rate promote the decrease of milk productions response with forage supplementation.

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7. DISCUSSÃO GERAL

Objetivou-se com esta tese identificar e compreender os principais fatores que afetam o consumo do pasto de vacas em lactação quando suplementadas com forragens conservadas. Foram estudados também os efeitos desta suplementação no consumo total de MS de vacas em pastejo e consequentemente na resposta leiteira.

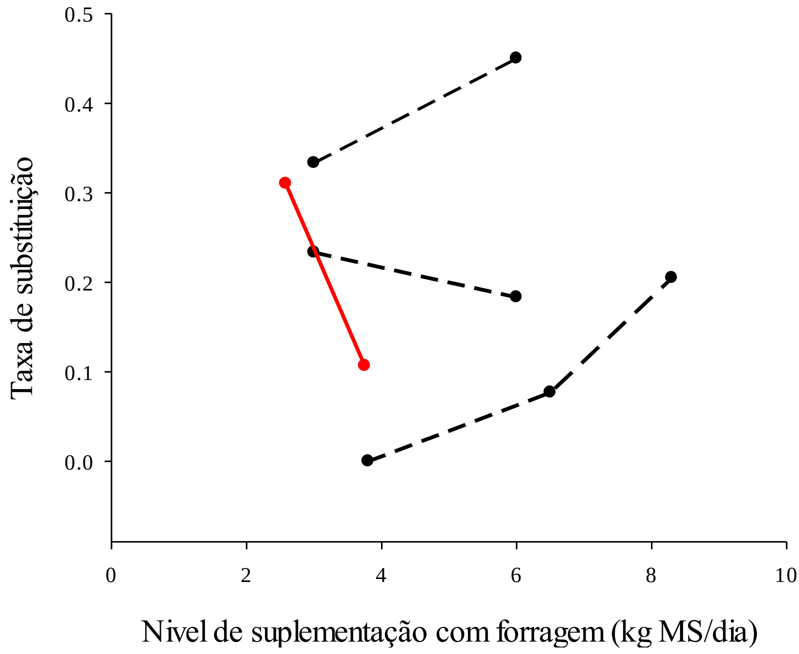
Nos dois primeiros experimentos, realizados em Lages, SC, fatores ligados a estrutura do pasto afetaram negativamente o seu consumo, impossibilitando os autores de responder algumas das perguntas formuladas em nossa estratégia de pesquisa. Tanto o efeito do nível da suplementação quanto da oferta do pasto no primeiro e segundo experimentos, respectivamente, não foram observados. O processo de ressemeadura natural, prejudicou o crescimento dos pastos que foram semeados logo após a colheita do milho nos nossos experimentos realizados em Lages. Neste caso a presença dos perfilhos mais velhos prejudicou o estabelecimento dos novos perfilhos, diminuindo a densidade do pasto. Em boas condições de manejo encontramos comumente uma densidade de 201 kg de MS/ha/cm (RIBEIRO-FILHO et al., 2009), porém em nossos experimentos estes valores foram cerca de 25% inferiores, prejudicando o consumo do pasto dos animais em decorrência dos baixos valores da biomassa pré-pastejo. Dessa forma, a discussão teve como foco os efeitos gerais da suplementação com silagem de milho sobre o consumo e a resposta produtiva de vacas leiteiras em pastejo.

Esta discussão baseia-se nas respostas das questões elaboradas em nossa estratégia da pesquisa. Para isto, nos servimos de nossos resultados experimentais, da comparação dos nossos resultados com resultados de experimentos com protocolos semelhantes e também das equações geradas na meta-análise (Tabelas 17 e 18).

Quais são os efeitos do nível de suplementação com silagem de milho sobre a taxa de substituição e de que maneira esta variável afeta a resposta produtiva de vacas leiteiras em pastejo?

Os efeitos do nível de ingestão de silagem de milho sobre a taxa de substituição e a resposta produtiva de vacas em pastejo seguem inconclusivos e necessitam de mais pesquisas. Embora nosso resultado experimental e os resultados obtidos na meta-análise indiquem uma queda na taxa de substituição com o aumento do nível de suplementação com forragens conservadas, estes, são incoerentes com os dados disponíveis na literatura. O modelo proposto em nossa meta-análise indica uma queda na taxa de substituição de 0,05 por kg de MS consumida de silagem de milho. Entretanto, o modelo GrazeIn (DELAGARDE et al., 2011) demonstra um aumento na taxa de substituição com o aumento da dose do suplemento, o que corrobora com os dados obtido por Moate et al. (1984) e Stockdale (1994a) em experimentos com vacas em pastejo e níveis crescentes de suplementação com forragens. Nestes experimentos, a taxa de substituição aumentou cerca de 0,05 para cada aumento 1 kg de MS de silagem consumida (MOATE et al., 1984; STOCKDALE 1994a). Quando comparamos nossos resultados experimentais com os demais obtidos na literatura (Figura 6) podemos observar a incoerência dos dados, sendo um dos motivos que nos levou a não discuti-los. No nosso primeiro experimento, além dos limites impostos pela biomassa pré-pastejo no consumo do pasto, o consumo de silagem de milho foi similar entre os dois níveis de suplementação, sendo em média 3,6 kg de MS/dia. A grande quantidade de refugo não era esperada, sendo que os animais com o nível mais alto de suplementação (8 kg de MS/dia) ingeriram somente cerca de 54% da quantidade do suplemento ofertada. Uma alternativa seria a realização de experimentos com níveis mais baixos de suplementação com silagem, como 2 e 4 kg de MS/dia, ou 3 e 6 kg de MS de silagem por dia por exemplo.

Figura 6 - Relação entre o nível de suplementação com foragem e a taxa de substituição global (nível de suplementação do tratamento testemunha igual a 0) de vacas em pastejo com base em nosso resultado experimental (linha sólida) e demais resultados experimentais obtidos na literatura por Moate et al. (1984) e Stockdale (1994) (linha tracejada).



Alguns experimentos demonstram que a resposta produtiva diminui com a elevação da oferta do suplemento (Figura 7), o que seria explicado pelo aumento da taxa de substituição com o aumento do nível de ingestão de silagem de milho (STOCKDALE, 1994a). Entretanto, em ensaios com animais estabulados, recebendo forragem verde e suplementação com silagem de milho, o aumento do nível de suplementação reduziu a resposta produtiva em todas as comparações (Figura 8; STOCKDALE, 1994b; 1995). Estes resultados são explicados, ao menos parcialmente, porque a elevação do nível de suplementação com silagem de milho pode afetar negativamente a digestibilidade total de dieta, principalmente quando os pastos possuem baixos teores de proteína. Este efeito não foi observado em nossa meta-análise, o que evidencia a importância do maior detalhamento das características do pasto e do suplemento na predição de respostas produtivas quando vacas leiteiras em pastejo são suplementadas com diferentes níveis de forragem conservada.

Figura 7 - Relação entre o nível de suplementação com forragem e a resposta na produção leiteira de vacas em pastejo com base em resultados experimentais (linha sólida) e os obtidos por Moate et al. (1984) e Stockdale (1994a) (linha tracejada).

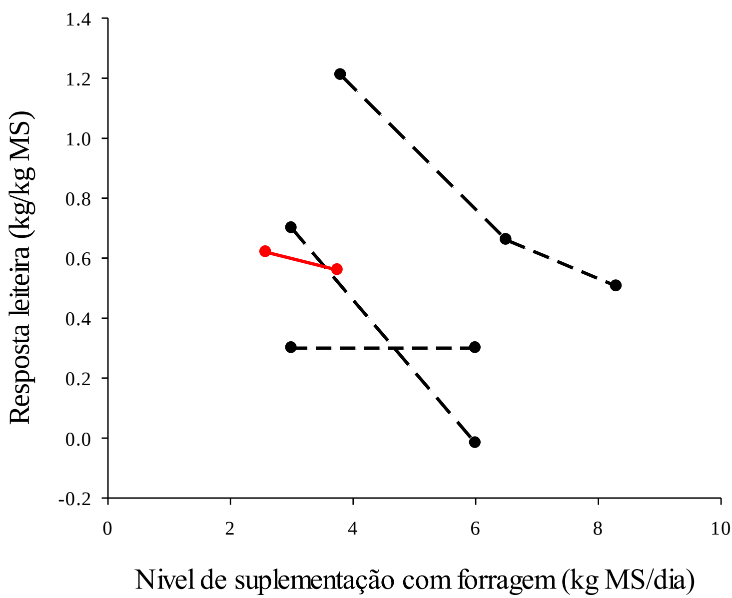
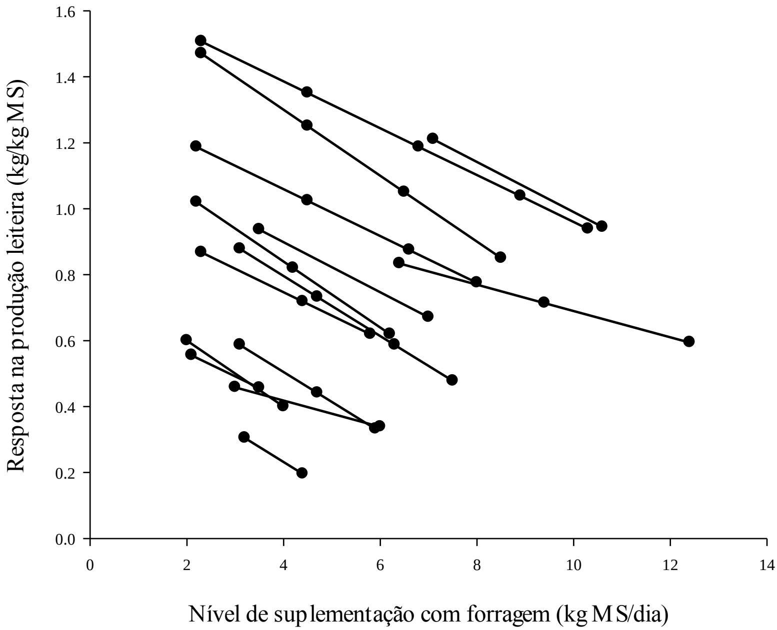


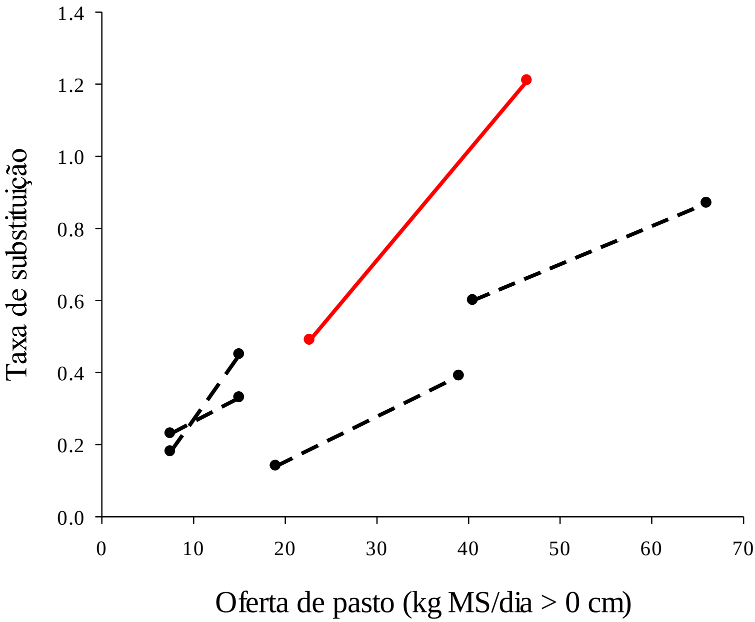
Figura 8 - Relação entre o nível de suplementação com foragem e a resposta na produção leiteira de vacas estabuladas recebendo forragem verde e suplementadas com silagem de milho. Adaptado de Stockdale (1994b; 1995).



A oferta diária do pasto afeta a taxa de substituição e a resposta produtiva de vacas leiteiras quando suplementadas com silagem de milho?

A oferta diária do pasto pode ser considerada o principal fator relacionado a taxa de substituição de vacas suplementadas com silagem de milho, atuando diretamente na resposta produtiva destes animais. Nossos resultados experimentais vão ao encontro de outros observados na literatura (MOATE et al., 1984; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011) quanto ao efeito da oferta do pasto na taxa de substituição de vacas suplementadas com forragens conservadas (Figura 9). Animais em baixa oferta tem seu consumo de pasto limitado por fatores não-nutricionais (POPPI et al., 1987), sendo possível manter esta ingestão quando recebem suplementação. Isto resulta em maior consumo de MS total, devido às menores taxas de substituição (PHILLIPS, 1988). Segundo Pérez-Prieto et al. (2011), a elevação na taxa de substituição com o aumento da oferta do pasto estaria ligada ao balanço energético das vacas não suplementadas. O maior aporte energético com elevação da oferta do pasto de 42 para 68 kg de MS acima do nível do solo em vacas não suplementadas, está relacionado com a elevação na taxa de substituição de 0,51 para 0,75 (PÉREZ-PRIETO et al., 2011). No modelo proposto em nossa meta-análise, à medida que o balanço energético de vacas não suplementadas aumenta em 1,0 UFL/dia (7,12 MJ EL_l), a taxa de substituição aumenta 0,08 quando comparada à taxa de substituição observada nos animais com menor balanço energético. Esta resposta é similar a observada por Faverdin et al. (1991) quanto aos efeitos substitutivos da suplementação com concentrado em vacas consumindo forragens conservadas.

Figura 9 - Relação entre a oferta do pasto e a taxa de substituição de vacas em pastejo com base em resultados experimentais (linha sólida) e nos resultados obtidos por Moate et al. (1984), Stockdale, (1996) e Pérez-Prieto et al. (2011), (linha tracejada).



Com base no modelo proposto em nossa meta-análise, ao compararmos duas situações contrastantes de oferta de pasto, por exemplo 25 vs. 60 kg de MS/dia acima do nível do solo, as taxas de substituição previstas varia de 0,3 a 1,0 em baixa e alta oferta, respectivamente. Esta variação é similar a observada por Delagarde et al. (2011) e Phillips (1988). Entretanto, considerando-se uma oferta de pasto usual entre 30 e 35 kg de MS/dia acima do nível do solo, nossos modelos indicam uma taxa de substituição média de 0,5 para vacas em pastejo suplementadas com silagem de milho, inferior a proposta por Delagarde et al. (2011), que é em média 0,8. Considerando dados experimentais com uma gama de variação na oferta de pasto de 7,5 a 68 kg de MS/dia acima do nível do solo, a taxa de substituição média foi de 0,5 para vacas que receberam em média 5 kg de MS de silagem por dia (MOATE et al., 1984; STOCKDALE, 1996; PÉREZ-PRIETO et al., 2011). Nesta comparação foram incluídos também os resultados do nosso terceiro experimento, mostrando a coerência dos nossos resultados com os dados da literatura.

A oferta de pasto possui forte correlação com a resposta leiteira dos animais suplementados com silagem de milho. O modelo GrazeIn (DELAGARDE et al., 2011) prevê uma variação de 0,8 a -0,4 kg de leite/kg de MS de silagem consumida à medida que a quantidade de pasto ofertada aumenta. Este efeito da oferta também foi demonstrado em vacas estabuladas recebendo pasto e suplementação com silagem de milho (STOCKDALE, 1994b; 1995). A resposta produtiva diminui de 1,5 para 0,2 kg de leite/kg MS ingerida de suplemento com o aumento da oferta do pasto (STOCKDALE, 1994b; 1995). Nosso modelo leva em consideração a taxa de substituição, a qual é estimada levando em conta a oferta do pasto. Segundo o modelo, a elevação de 0,1 na taxa de substituição promove queda na resposta leiteira de 0,04 kg/kg de MS de silagem de milho consumida. Isto corresponde a uma redução na resposta à suplementação de 0,57 para 0,27 kg de leite/kg de suplemento à medida que a oferta do pasto acima do nível do solo aumenta de 25 para 60 kg de MS/dia. É bem conhecido que altas taxa de substituição inibem o aumento do consumo total de MS de vacas suplementadas com forragens conservadas, diminuindo a sua resposta produtiva (MAYNE, 1991). Além disso, a substituição de pastos de boa qualidade por suplementos com médio a baixo valor nutritivo também provoca a diminuição da produção de leite (MOATE et al., 1984). Dessa forma, mesmo com aumento do consumo de MS total promovido pela suplementação, Stockdale (1996) observou que a suplementação com 4 kg de MS de silagem de milho, diminuiu a resposta leiteira de 0,8 para 0,1 kg de leite/kg de suplemento. Isso ocorreu devido à queda na qualidade da dieta, a qual foi provocada pelo aumento da taxa de substituição (de 0,14 para 0,40 kg de forragem ingerido a menos/kg de suplemento ingerido).

Será que forma como é medida a taxa de substituição em pesquisas, permite prever a taxa de substituição e as respostas produtivas com a suplementação com silagem de milho em propriedades comerciais, onde o produtor normalmente busca bem valorizar o uso do pasto?

A utilização de dados de pesquisas sobre os efeitos da suplementação com silagem de milho em animais em pastejo, podem geram ao produtor problemas na gestão da pasto. Segundo Reid et al. (2015), somente em situações de oferta de pasto semelhante obtemos o real efeito do suplementação sobre o consumo do pasto, o que de acordo com os nossos resultados experimentais é correto, sendo que em média observamos taxas de substituição menores do que apresentadas na literatura. Porém dificilmente vamos observar produtores que ao suplementarem seu animais em pastejo, retiram estes da pastagem com alturas residuais maiores, neste caso “aceitando” os efeitos substitutivos. Na prática, ocorre um ajuste da oferta a fim de compensar estes efeitos substitutivos. Entretanto este critério de manejo nunca foi abordado de maneira científica.

Observamos que ao reduzir a oferta do pasto em 1 kg de MS/dia ao nível do solo para cada quilograma de silagem de milho consumida, obtemos uma altura de saída do pasto similar aos animais não suplementados. Neste caso a taxa de substituição aumenta, devido a ação concomitante da queda na oferta do pasto e dos efeitos substitutivos do suplemento no consumo do pasto. Vimos no terceiro experimento que em baixa oferta, a taxa de substituição elevou-se de 0,49 para 0,77 ao adotarmos a este critério de manejo. Segundo Pérez-Prieto e

Delagarde (2013), a redução da oferta do pasto em 1 kg de MS/dia, resulta na queda do consumo em 0,13 kg de MS do pasto, atuando principalmente na redução da velocidade da ingestão. Em nosso experimento, a redução na oferta diária do pasto em 4,5 kg de MS promoveu a queda de 2,4 g de MS/min na velocidade de ingestão da vacas suplementadas com altura de saída similar a não suplementadas em baixa oferta de pasto. Esta redução é maior do que a prevista pelo modelo proposto por Pérez-Prieto e Delagarde (2013), o qual prediz redução na velocidade de ingestão de 0,23 g de MS/min para cada kg de MS de pasto oferecido a menos. Ao controlar a altura residual do pasto das vacas suplementadas em baixa oferta, não obtivemos o efeito esperado da suplementação, que seria o aumento do consumo total de MS, consequentemente da produção de leite. Neste caso, o ganho produtivo ao adotar esta estratégia de manejo em baixa oferta é o aumento da capacidade de carga do pasto, sem afetar a produção de leite individual do animais.

A adoção deste método de manejo não foi eficaz no controle da altura residual nem na resposta produtiva dos animais em alta oferta de pasto. A redução do consumo promovido pela queda da oferta diária do pasto é mais evidente quando se parte de uma oferta considerada média para uma oferta considerada baixa (PÉREZ-PRIETO e DELAGARDE, 2013). Isto explica a ausência do efeito da redução da oferta no controle da altura residual das vacas suplementadas em alta oferta observado no nosso experimento.

Como vimos anteriormente, a resposta produtiva a suplementação é ligada a oferta do pasto, sendo que em situações de restrição na oferta diária, a suplementação com forragens conservadas é capaz de aumentar o consumo total de MS, consequentemente a produção diária de leite. Quando o consumo do pasto aumenta devido à alta oferta diária, o retorno com a suplementação com silagem diminui, devido principalmente a elevação da taxa de substituição (STOCKDALE, 1996). O consumo de 5 kg de MS de silagem não foi capaz de manter o nível de produção de vacas no terço médio de lactação quando a oferta do pasto diminuiu de 50 para 25 kg de MS/dia (CHAVES et al., 2002; WOODWARD et al., 2006). No experimento 3 desta tese, os animais em baixa oferta suplementados com silagem (21,5 kg leite/dia), não alcançaram níveis produtivos obtidos em alta oferta sem suplementação (24,0 kg leite/dia). Isto mostra que o pasto *per se*, quando manejado em alta oferta, foi capaz de atender as demandas nutricionais de vacas no terço médio de lactação, provendo ao animais, mesmo que não suplementados, energia suficiente para a manutenção e produção de leite.

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8. CONSIDERAÇÕES FINAIS

De acordo com os resultados obtidos em nossos experimentos e também na meta-análise, pode-se afirmar que a quantidade de pasto disponível, bem como suas características estruturais são determinantes da resposta à suplementação o com silagem de milho para vacas leiteiras em pastejo. Em situações onde a oferta e a estrutura do pasto não são limitantes, a produção individual de leite não é afetada pela suplementação com forragens conservadas. Nestes casos, a colheita do pasto diminui devido aos efeitos substitutivos da suplementação, diminuindo assim a eficiência produtiva de todo o sistema. Pastos de bom valor nutritivo (≥ 6.4 MJ EL_L/kg MS; ≥ 190 g PB/kg MS), sem restrições de ordem estrutural e manejados em alta oferta são capazes de atender as demandas nutricionais de vacas no terço médio de lactação. Nesta situação, os animais suplementados tendem a dar preferência ao consumo do pasto, com aumento a quantidade de silagem refugada, como vimos no nosso terceiro experimento.

De outra forma, a suplementação com forragens conservadas promove o aumento no consumo total de MS e da produção de leite de vacas em patejo quando os animais tem acesso a pastos com restrições de ordem quantitativa e/ou estrutural. Conforme observado nos experimentos conduzidos em pastos de azévem anual, a estrutura do pasto influenciou a resposta a suplementação. Quando a biomassa pré-pastejo foi inferior a 2000 kg MS/ha, a suplementação mostrou-se eficaz em aumentar o consumo total de MS mesmo em uma oferta de pasto mediana (primeiro experimento).

Mesmo com a queda da taxa de substituição em situações de baixa oferta diária de pasto quando comparadas a alta oferta, este fator ainda é um empecilho para os produtores que suplementam seus animais em pastagens, uma vez que diminui a proporção de forragem colhida. A estratégia proposta em nosso trabalho (diminuir a altura residual do pasto por meio de redução na oferta dos animais suplementados) se mostrou eficaz. Entretanto, foram observados efeitos acumulativos da redução da oferta e da suplementação no consumo do pasto, o que resultou em consumo total de MS similar às vacas não suplementadas. Isto explica o porquê na prática não observamos respostas produtivas quanto a suplementação com silagem. Esta estratégia, portanto, seria eficaz em situações de baixa oferta diária de pasto, decorrentes de épocas do ano com baixas taxas de acúmulo de forragem e/ou da intensificação do sistema, com aumento do número de animais por hectare, uma vez que é possível manter a produção individual de leite.

Seguem as dúvidas quanto ao efeito do nível de suplementação com forragens na taxa de substituição e na resposta produtiva de vacas em pastejo. Nossos resultados experimentais e os obtidos na meta-análise vão de encontro aos apresentados na literatura. Contudo, o número de experimentos testando estes efeitos ainda é pequeno e as características do pasto e do suplemento parecem ter grande influência nesta resposta. Durante todo o processo de pesquisa bibliográfica observamos também o pequeno número de experimentos com pastos de clima tropical, o que seria de extremo interesse para as condições encontradas no Brasil e em outras regiões de clima subtropical ou tropical.