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Ana Paula Burin Fruet

EFEITO DE PASTAGEM CONSORCIADA E DIFERENTES NÍVEIS DE CONCENTRADO NO DESEMPENHO PRODUTIVO E QUALIDADE DA CARNE DE NOVILHOS

Santa Maria, RS, Brasil
2018

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciência e Tecnologia dos Alimentos.**

Orientador: Prof. Dr. José Laerte Nörnberg
Coorientador: Prof. Dr. Alexandre Nunes Motta de Souza

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Aprovada em 20 de agosto de 2018:

_____

José Laerte Nörnberg, Dr.
(Presidente/Orientador)

_____

Adriano Garcia Rosado Júnior, Dr. (IFF)

_____

Diego Zeni, Dr. (IFF)

_____

Luciana Potter, Dra. (UFSM)

_____

Roger Wagner, Dr. (UFSM)

Santa Maria, RS
2018

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RESUMO

EFEITO DE PASTAGEM CONSORCIADA E DIFERENTES NÍVEIS DE CONCENTRADO NO DESEMPENHO PRODUTIVO E QUALIDADE DA CARNE DE NOVILHOS

AUTORA: Ana Paula Burin Fruet
ORIENTADOR: José Laerte Nörnberg
COORIENTADOR: Alexandre Nunes Motta de Souza

O objetivo do estudo foi avaliar o efeito de três diferentes sistemas alimentares de terminação de novilhos compostos por concentrado e pastagem consorciada de leguminosas e gramíneas no desempenho produtivo da pastagem e dos novilhos, características da carcaça, presença de ruminite, abomasite e abscessos hepáticos, assim como qualidade da carne. Novilhos foram terminados por 91 dias com dieta composta exclusivamente grão inteiro de milho (GRAIN), em pastagem consorciada de gramíneas e leguminosas e suplementação de 1,4% do peso vivo (SUPP), ou somente em pastagem consorciada de leguminosas e gramíneas (PAST). A pastagem consorciada proporcionou alto nível proteico (mais de 19% MS) e digestibilidade (mais de 88% MS) durante todo experimento. Piquetes utilizados para terminação de novilhos com dietas SUPP e PAST apresentaram similar qualidade nutricional. Quando comparado com PAST, a terminação SUPP aumentou o ganho de peso total por hectare, taxa de lotação, ganho de peso diário e total. O ganho de peso diário foi superior na dieta GRAIN do que SUPP e PAST (1,81, 1,51 e 1,20 kg/animal/dia respectivamente, $P < 0,001$). Novilhos terminados no tratamento GRAIN obtiveram aumento de espessura de gordura subcutânea e marmoreio quando comparado com PAST. Não houve diferença no peso de carcaça quente de bovinos terminados com dietas GRAIN e SUPP. Lesões abomasais foram mais prevalentes em novilhos terminados no GRAIN do que PAST. Dietas composta por pastagem (PAST e SUPP) reduziram a relação n-6/n-3 ($P < 0,001$) e elevaram a deposição muscular de C18:2 *cis*-9 *trans*-11 ($P < 0,001$). Carne de novilhos terminados com GRAIN apresentaram maiores valores de compostos voláteis associados com oxidação lipídica e a intensidade de off-flavor foi maior em carne de novilhos alimentados com GRAIN quando comparado com PAST. A carne de novilhos alimentados com GRAIN apresentou inferior coloração vermelha ($P < 0,001$) e maior oxidação lipídica ($P < 0,001$) durante a o período de estocagem, assim como maior formação de metamioglobina ($P < 0,001$) do dia 7 ao 13 quando comparado com PAST. Os níveis musculares de α-tocoferol foram superiores em novilhos terminados em pastagem consorciada ($P < 0,001$). A inclusão de concentrado na dieta de terminação de novilhos eleva o desempenho produtivo, no entanto, carcaças de novilhos terminados em pastagem consorciada de gramíneas e leguminosas apresentam desejável acabamento. A presença de forragem, independentemente do nível utilizado, é essencial para melhorar o perfil lipídico, diminuir compostos voláteis relacionados com oxidação lipídica, minimizar off-flavor, manter a estabilidade de cor durante estocagem aeróbica e prevenir a oxidação lipídica e da mioglobina.

Palavras-chave: Estabilidade oxidativa. Gramíneas. Grão inteiro. Leguminosas. *Longissimus thoracis*.

ABSTRACT

EFFECT OF LEGUME-GRASS PASTURE AND DIFERENT CONCENTRATE LEVELS ON GROWTH PERFORMANCE AND BEEF QUALITY

AUTHOR: Ana Paula Burin Fruet
ADVISOR: José Laerte Nörnberg
CO-ADVISOR: Alexandre Nunes Motta de Souza

The objective of this study was to evaluate the effects of three finishing systems based on concentrate and legume-grass pasture on pasture and growth performance, carcass traits, presence of rumenitis, abomasitis, and liver abscesses, and beef quality. Steers were finished for 91 days with an exclusively whole corn grain-based (GRAIN) diet, grazed on legume-grass pasture plus 1.4% of body weight of whole corn grain supplementation (SUPP), or grazed on legume-grass pasture (PAST) only. The legume-grass pasture provided high levels of protein (more than 19% DM) and improved digestibility (more than 88% DM) over all three periods. Pasture of paddocks where steers were assigned to SUPP and PAST treatments showed similar nutritional quality. When compared to PAST, SUPP diet increased total weight gain per hectare, stocking rate, daily and total weight gains. The increase of weight gains was high to GRAIN than SUPP and PAST (1.81, 1.51, and 1.20 kg/animal/day respectively). Steers finished on GRAIN had higher fat thickness and marbling score when compared to PAST. There were no differences on hot carcass weight for cattle finished on GRAIN and SUPP. Abomasum lesions were more prevalent in steers finished on GRAIN when compared to PAST. Pasture diets (PAST and SUPP) led to lower n-6/n-3 ratio ($P < 0.001$), and highest deposition of C18:2 *cis*-9 *trans*-11 ($P < 0.001$) in the lean. Beef from steers fed GRAIN had the highest values of volatile compounds associated with lipid oxidation. Off-flavor intensity was significantly greater on beef from steers fed GRAIN when compared to PAST. Steaks from steers fed GRAIN were less red ($P < 0.001$), showed higher lipid oxidation ($P < 0.001$) during retail display, and higher metmyoglobin formation ($P < 0.001$) from day 7 to 13 when compared to PAST. Levels of α-tocopherol were higher in steaks from steers fed diets containing legume and grass ($P < 0.001$). Inclusion of concentrate on finishing diet of steers increase growth performance, however, carcass from steers finished on legume-grass pasture has desirable traits. The roughage, independently of what level was offered, is essential to improve fatty acid profile, decrease volatile compounds associated with lipid oxidation, minimize off-flavor, maintain retail color and prevent lipid and myoglobin oxidation.

Keywords: Oxidative Stability. Grass. Whole grain. Legume. *Longissimus thoracis*.

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SUMÁRIO

1. INTRODUÇÃO

Pesquisadores têm demonstrado a possibilidade de produzir carne visando seus atributos qualitativos como coloração, aroma, textura, estabilidade lipídica e proteica. Assim como, visando a saúde do consumidor por meio da modificação do perfil de ácidos graxos, já que os lipídios são considerados os principais constituintes maléficos da carne. Entre as maneiras de imprimir as características desejáveis, utiliza-se a dieta de terminação como um modificador do produto final.

A dieta de terminação de bovinos é dependente do sistema de terminação adotado, sendo que, de forma objetiva pode-se dividir em três sistemas distintos: sistema de terminação a pasto, caracterizado pelo regime exclusivo de pastagem e apenas suplementação mineral; suplementação energética, que preconiza a utilização de suplementação energética a pasto; confinamento, que inseri o método de confinamento para terminação de bovinos.

O sistema extensivo de terminação, com uso exclusivo de pastagem, é mais implementado no Brasil, tal regime a pasto caracteriza-se por produzir carne com maior estabilidade oxidativa e perfil lipídico favorável à saúde do consumidor (HUMADA et al., 2014; LUCIANO et al., 2013; LUCIANO et al., 2009; SANTÉ-LHOUTELLIER et al., 2008; TANSAWAT et al., 2013) quando comparado à terminação com suplementação ou alto nível de concentrado. Em contrapartida, sistemas que utilizam dietas energéticas intensificam a produção e permitem maior desfrute do rebanho, rendimento econômico e produção de carne (COUTINHO FILHO et al., 2006).

Pesquisas demonstraram o efeito da relação volumoso:concentrado no desempenho produtivo (ROBERTS et al., 2009) ou desempenho e qualidade da carne de bovinos (DUCKETT et al., 2013; KERTH et al., 2007), sendo que nestes estudos havia uma fonte de volumoso mesmo no nível máximo de concentrado na dieta. Foram identificados dois estudos que avaliaram o uso exclusivo de concentrado em comparação com terminação em pastagem utilizando ovinos como modelo animal: Fruet et al. (2016) avaliaram o efeito da terminação a pasto, suplementação em pastagem e terminação exclusivamente com concentrado no desempenho produtivo e qualidade da carne. Enquanto, Luciano et al. (2012) analisaram a estabilidade oxidativa durante o armazenamento da carne de ovinos terminados com uso exclusivo de concentrado e a pasto.

Com base nos estudos supracitados, sabendo que a proporção de volumoso e concentrado da dieta é responsável por modificações no perfil lipídico e processos oxidativos

da carne, pesquisas que utilizam níveis extremos desta relação e um regime intermediário, caracterizado pela suplementação, tornam-se relevantes no que diz respeito ao desempenho produtivo, características da carcaça, qualidade da carne e na estabilidade oxidativa durante o armazenamento da carne bovina.

2 OBJETIVOS

2.1 OBJETIVOS GERAIS

Avaliar o desempenho produtivo, características de carcaça e qualidade da carne de bovinos terminados em três sistemas alimentares: pastagem consorciada de aveia, azevém e trevo, suplementação com grão inteiro de milho em pastagem consorciada e, exclusivamente concentrado em confinamento.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar o efeito da inclusão do grão inteiro de milho no ganho de peso de novilhos;
- Avaliar a produtividade e qualidade nutricional do consórcio de gramíneas e leguminosas no período de terminação de novilhos de corte;
- Determinar o rendimento, as características de carcaça e possíveis lesões no rúmen, retículo, omaso, abomaso e fígado em diferentes sistemas de terminação;
- Avaliar a influência dos sistemas alimentares nos atributos qualitativos da carne;
- Avaliar o efeito de diferentes sistemas de terminação de novilhos de corte na estabilidade da mioglobina, lipídica e proteica da carne durante o armazenamento sob refrigeração em ambiente aeróbico;
- Comparar o nível de deposição de α-tocoferol na carne de novilhos submetidos a distintos sistemas alimentares;
- Identificar compostos voláteis que contribuem para a caracterização do sabor da carne bovina e efetuar análise sensorial da carne de novilhos submetidos a diferentes sistemas alimentares a fim de avaliar a aceitação dos consumidores.

3. REVISÃO DE LITERATURA

3.1 SISTEMAS ALIMENTARES DE TERMINAÇÃO

Os sistemas de terminação de bovinos de corte no Sul do Brasil preconizam a utilização de pastagens, sendo que nas estações de primavera e verão existe abundância de

massa forrageira e, em contrapartida, no período de outono e inverno ocorre uma diminuição na produção de pastagens (PRADO et al., 2003). Neste período crítico para a produção de bovinos de corte no Sul do país, além da baixa disponibilidade, há inferior qualidade das pastagens naturais o que resulta em baixos índices de produtividade do rebanho bovino (LOPES et al., 2008). Assim, com intuito de incrementar o desempenho animal em períodos de baixa disponibilidade de forragens, buscam-se alternativas que contribuem para a rentabilidade do sistema de produção como a utilização de pastagens cultivadas de inverno associadas ou não à suplementação energética e a terminação em confinamentos.

3.1.1 Pastagens cultivadas

Dentre as pastagens cultivadas de estação fria utilizadas no sul do Brasil, destaca-se a aveia preta (*Avena strigosa* Schreb.) por apresentar alta produção de matéria seca, qualidade forrageira, resistência ao pisoteio, além de baixo custo de produção (MACARI et al., 2006). Tal gramínea é empregada de forma isolada ou consorciada com outras espécies de clima temperado, como o azevém anual (*Lolium multiflorum* Lam.) que é caracterizado pela facilidade de ressemeadura natural, resistência às doenças, bom potencial de produção de sementes e pela possibilidade de associação com outras espécies (SANTOS et al., 2002).

As misturas de espécies forrageiras anuais de inverno visam elevar a produção de matéria seca e o período de utilização de pastagens por meio de picos de produção em épocas distintas. A aveia caracteriza-se por ser uma fonte forrageira logo no início do inverno e apresentar um ciclo mais curto quando comparado ao azevém, enquanto que o azevém possui desenvolvimento inicial lento, com reconhecido pico de produção hiberno-primaveril, e grande rendimento de forragem por hectare durante o ciclo de produção. Sendo assim, o consórcio de espécies precoces (aveia) e tardias (azevém) permitem um equilíbrio de oferta de forragem ao longo do período de produção (NORO et al., 2003), entre maio e novembro, totalizando até 180 dias de pastejo (ROSO et al., 1999).

Macari et al. (2006) relataram a utilização destas forragens consorciadas em um período de 116 dias, assim como, Bremm et al. (2008) mantiveram novilhas em pastejo durante 112 dias com níveis médios de proteína bruta (28,4%), nutrientes digestíveis totais (56,1%) e fibra em detergente neutro (37,1%). Salienta-se que Hellbrugge et al. (2008) verificaram perda nutricional da pastagem de azevém ao decorrer dos períodos experimentais, devido ao aumento nos níveis de fibra em detergente neutro e um decréscimo nos níveis de proteína bruta. Assim, o emprego de leguminosas no consórcio, como o trevo branco

(*Trifolium repens*) e vermelho (*Trifolium pratense*), pode ser uma alternativa para manter elevada qualidade nutricional da pastagem ao longo do período de utilização devido a sua contribuição proteica e elevada digestibilidade (BUXTON et al., 1985). A manutenção da qualidade nutricional da pastagem pode resultar em incremento de desempenho animal e, como consequência, melhores ganhos por hectare. A relevância em buscar um satisfatório ganho de peso por área se dá pelo fato desta variável definir a renda alcançada no sistema pecuário, já que é calculada pela multiplicação dos ganhos individuais pela taxa de lotação e número de dias de utilização da pastagem (PÖTTER, 2008).

3.1.2 Suplementação energética em pastagens cultivadas

A suplementação energética em pastagens também é um meio de elevar o desempenho animal, pois colabora com o acréscimo da velocidade de crescimento dos animais através de um melhor balanceamento dos nutrientes, além de possibilitar o aumento da produção animal por área sem redução do ganho individual devido à substituição do consumo de forragem pelo suplemento (HELLBRUGGE et al., 2008; RESTLE et al., 2001). Nas situações em que a disponibilidade e/ou a qualidade do pasto são inadequadas, a suplementação contribui para a mantença do desempenho produtivo do rebanho (DIXON; STOCKDALE, 1999). O sistema de terminação com suplementação permite a redução da oferta de forragem. Bargo et al. (2003) destacam que quando bovinos são alimentados exclusivamente com pastagem é necessário haver uma oferta de forragem entre 3 a 5 vezes maior que o consumo de MS, enquanto que utilizando suplementação a oferta de forragem pode ser reduzida a duas vezes maior que o consumo.

A utilização da suplementação energética aliada à pastagem de inverno contribui com desempenho individual e taxa de lotação. O incremento da produtividade animal e da área através da suplementação se deve a ingestão de pasto diminuída em decorrência do efeito de substituição da pastagem pelos grãos, mas com consumo de matéria seca e de energia normalmente mais elevados (REARTE; PIERONI, 2001). A taxa de substituição da pastagem pelo concentrado oscila devido às mudanças estruturais e químicas do pasto, porém, dependendo do propósito de utilização do suplemento, são indesejados elevados valores de substituição para a rentabilidade do sistema, já que o custo do suplemento geralmente é superior ao custo da pastagem (BREMM et al., 2008).

Para escolher o melhor nível de suplementação energética deve-se avaliar qual o objetivo da inclusão. Quando se busca maximizar o desempenho individual, os níveis de

suplementação devem ser minimizados, possibilitando apenas elevar o ganho de peso dos animais sem almejar acréscimo na taxa de lotação, pois a base forrageira é um alimento de menor custo, e uma boa resposta em desempenho individual depende de suplementos de alta qualidade. Em contrapartida, quando o objetivo é manter elevada a taxa de lotação na pastagem, níveis de suplementação médios a altos podem ser utilizados (LOBATO; PILAU, 2004).

Em estudo realizado por Medeiros et al. (2010), em que se avaliou o efeito do nível da suplementação energética (0; 0,4; 0,8 e 1,2% PV de milho moído) no desempenho de novilhos em pastagem de azevém anual e aveia preta, foi observado que a suplementação produziu incremento no rendimento de carcaça, entretanto, o nível de suplementação não influenciou o ganho de peso dos animais (média de 1,55 kg/dia). Os autores atribuíram estes resultados a fatores como: o consumo dos suplementos inferior ao planejado devido a elevada disponibilidade média de forragem (1.527 kg/ha), oferta (14,75% PV) e qualidade nutricional da pastagem (PB - 18,32%; FDN - 56,34%). Além disso, apontaram que houve efeito de substituição no consumo da forragem pelo suplemento, sem alterações no consumo de energia pelos animais. Roberts et al. (2009) avaliaram níveis de suplementação com grão inteiro de milho (0, 0,5%, 1%, 1,5%, 2%) em pastagem de azevém e demonstraram que, ao elevar a quantidade de grãos na dieta de terminação de bovinos, houve redução linear do período de terminação e aumento linear no ganho de peso diário dos animais e rendimento de carcaça.

3.1.3 Dieta alto grão em sistema de confinamento

Com intuito de intensificar a produção para permitir maior desfrute do rebanho, rendimento econômico e produção de carne, o confinamento de animais se torna um sistema de terminação relevante. Este sistema alimentar contribui para a redução da idade de abate, gera maior rendimento de carcaça, além de permitir a terminação de animais em períodos de escassez onde os preços de comercialização estarão elevados (COUTINHO FILHO et al., 2006). Destaca-se ainda que a terminação em confinamento proporciona a liberação de pastagens para outras categorias beneficiando todo ciclo de produção (WEDEKIN et al., 1994).

Uma vez que durante processo de terminação em confinamento o maior custo de produção é a alimentação, com destaque para o concentrado (PACHECO et al., 2006), o valor da dieta deve ser considerado para obter lucratividade. Desta forma, em situações de disponibilidade de grãos a preços acessíveis, associado à dificuldade de obtenção ou

manipulação de volumosos, dietas com inferior utilização de pastagem é uma alternativa tecnológica possível de ser adotada (PAULINO et al., 2013).

Como demonstrado por Paulino et al. (2013), estudos têm descrito a viabilidade produtiva na utilização de dietas alto grão através de um inferior consumo de matéria seca e consequente menor conversão alimentar em confinamentos comerciais, sendo que o grão de milho inteiro é o principal ingrediente de dietas alto grão. Apesar do grão inteiro de milho apresentar menor digestibilidade do amido quando comparado aos grãos moídos (SILVA et al., 2012), estes são extensivamente danificados durante a mastigação, o que pode tornar o processamento físico desnecessário, resultando em substancial diminuição dos custos de alimentação (BEAUCHEMIN et al., 1994).

Em concordância, Vargas Júnior et al. (2008) demonstraram que o fornecimento de grão inteiro de milho não afetou o desempenho de bovinos jovens, sendo portanto, uma alternativa econômica e prática. Entre os motivos para a utilização de grãos com maior tamanho de partículas, evidencia-se a contribuição para a estabilidade ruminal. Gimeno et al. (2015) observaram que grãos processados com tamanho de partículas maiores geram uma fermentação ruminal menos ácida, caracterizada por menores concentrações de ácidos graxos voláteis totais e ácido láctico, além de pH ruminal mais elevado. Os autores atribuíram o achado à diminuição na disponibilidade de amido combinado ao padrão de ingestão mais homogêneo.

Como o produto final da terminação de bovinos é a carne, a busca pelo aumento de produtividade deve resultar em carne de qualidade que satisfaça as exigências dos frigoríficos, com peso e acabamento adequados, assim como, ofereça características sensoriais desejáveis pelo consumidor (AGUINAGA et al., 2006). Além do desempenho produtivo, o sistema alimentar de terminação influência diversas características físico-químicas da carne, como o perfil de ácidos graxos (SCOLLAN et al., 2014), estabilidade da mioglobina, lipídica (FAUSTMAN et al., 2010; TANSAWAT et al., 2013) e proteica (PETRON et al., 2007). Tais influências podem conferir efeitos positivos e negativos aos atributos qualitativos do produto final, sendo assim, estes efeitos devem ser considerados ao avaliar um sistema de produção.

3.2 INFLUENCIA DA DIETA DE TERMINAÇÃO NA QUALIDADE DA CARNE

A carne é composta por aproximadamente 75% de água, 20% de proteína, 3% de gordura e 2% de carboidratos e compostos inorgânicos (HUFF-LONERGAN; LONERGAN, 2005; TORNBERG, 2005). Ainda, é reconhecida por ser importante fonte de proteína,

vitamina B12, vitamina D, ácidos graxos essenciais e minerais biodisponíveis, tais como ferro, zinco e selênio (SCHÖNFELDT; GIBSON, 2008). Além de contribuírem para a nutrição humana, os principais constituintes da carne (proteínas e lipídios) podem influenciar as características sensoriais desta por meio da modificação da dieta de terminação.

3.2.1 Perfil de ácidos graxos

O perfil lipídico da carne de ruminantes é predominantemente composto por ácidos graxos saturados (AGS) como o ácido palmítico (C16:0) e esteárico (C18:0), e ácidos graxos monoinsaturados, sendo majoritário o ácido oleico (C18:1n9) (ENSER et al., 1998; VALSTA et al., 2005; WOOD et al., 2008). Apesar da carne de ruminantes normalmente ter uma relação baixa entre ácidos graxos poli-insaturados (AGPI) e AGS, o músculo contém uma série de AGPI n-3 e n-6 importantes para a nutrição humana (ENSER et al., 1998), já que alguns ácidos graxos da família n-3 e n-6 são denominados de ácidos graxos essenciais, portanto, não sintetizados por monogástricos (WEBB; O'NEILL, 2008). Destaca-se a participação do ácido linoleico (C18:2n6) seguido do ácido α-linolênico (C18:3n3) que são precursores de ácidos graxos de cadeia longa (WOOD et al., 2008).

Segundo Wood et al. (2008), mesmo utilizando uma dieta rica em AGPI, uma pequena porção destes são incorporados no tecido quando comparada com outras espécies, o ocorrido deve-se à biohidrogenação ruminal. Ao atingir o rúmen, lipídios esterificados passam por um processo de hidrólise por lipases, presentes na membrana celular bacteriana, liberando glicerol e ácidos graxos livres que, posteriormente, serão biohidrogenados (é um mecanismo de defesa dos micro-organismos contra a toxicidade dos AGI, e tal mecanismo trata da redução do número de ligações duplas de ácidos graxos insaturados) (BUCCIONI et al., 2012; KOZLOSKI, 2011).

Enquanto que em monogástricos quase a totalidade dos AGPI ingeridos é absorvida no intestino, o mesmo não ocorre em bovinos devido à biohidrogenação ruminal. Dietas constituídas de concentrado, compostas por níveis elevados de ácido linoleico, disponibilizam apenas 10% deste AGPI para a circulação sanguínea de ruminantes, o restante é transformado em AGMI e AGS. Da mesma forma, o ácido α-linolênico, um dos principais ácidos graxos na dieta dos ruminantes, uma vez que constitui mais de 50% do total de ácidos graxos de pastagens, sofre hidrogenação no rúmen e apenas uma pequena porção é depositada no músculo (WOOD et al., 2008).

A biohidrogenação ruminal resulta em numerosos isômeros do ácido linoleico e α-linolênico, assim como outros intermediários de tal processo (BUCCIONI et al., 2012; DOREAU et al., 2010; KOZLOSKI, 2011). Dentre os isômeros, alguns são amplamente estudados devido à importância para a saúde do consumidor, sendo o principal o ácido linoleico conjugado (CLA) que é um conjunto de isômeros geométricos e posicionais do ácido linoleico (C18:2cis-9cis-12). Cada isômero de posição tem quatro isômeros geométricos que são: cis, trans; trans, cis; cis, cis; trans, trans. As posições de duplas ligações de isômeros de CLA identificadas no rúmen estão contidas no intervalo de 6, 8 a 13, 15- C18:2 em diversas configurações geométricas gerando um total de 32 isômeros (SHINGFIELD et al., 2008).

O C18:2 cis-9 trans-11 (CLA ou ácido rumênico) é o maior contribuinte entre os isômeros formados (DE LA TORRE et al., 2006), representando aproximadamente 90% dos isômeros conjugados do ácido linoleico (ROY et al., 2007). Este é sintetizado no rúmen a partir do ácido linoleico e nos tecidos através de uma enzima redutase (Δ9-dessaturase) que converte o ácido vacênico (C18:1 trans-11) em CLA. Destaca-se que, em concordância com Scollan et al. (2006), estudos como o conduzido por Palmquist et al. (2004) demonstram que a via de formação de CLA a partir do ácido vacênico nos tecidos periféricos é a maior contribuinte para o valor final de CLA no músculo de ruminantes. O ácido vacênico também é formado no processo intermediário de biohidrogenação e é produzido tanto pela biohidrogenação do ácido linoleico quanto do ácido linolênico (BUCCIONI et al., 2012; CHILLIARD et al., 2007; KOZLOSKI, 2011; WOOD et al., 2008). Segundo Buccioni et al. (2012), estudos indicam ainda a formação de ácido vacênico via ação de enzimas isomerazes sobre o ácido oleico (C18:1cis-9).

A carne bovina tem sido considerada prejudicial à saúde devido a grande quantidade de AGS que constituem a fração lipídica da carne (VASTA et al., 2005), pois estão relacionados com doenças cardiovasculares (SCOLLAN et al., 2014). Destaca-se que não são todos os AGS prejudiciais a saúde, por exemplo, sabe-se que o ácido esteárico não apresenta efeito sobre a concentração plasmática de lipoproteínas de baixa densidade (SALTER, 2013). Também, a carne é constituída por ácidos graxos poli-insaturados (AGPI), principalmente ácido α-linolênico, eicosapentaenoico (EPA), docosahexaenoico (DHA) e ácido linoleico conjugado (CLA) que reduzem o risco de câncer (FERGUSON et al., 2010), doenças cardiovasculares, diabetes tipo 2, além de colaborar com a formação e funcionamento cerebral (BARCELÓ-COBLIJN; MURPHY, 2009).

Salter (2013) aponta que o ácido graxo vacênico, resultante da biohidrogenação em ruminantes, apresenta potencial de proteção ao desenvolvimento de doenças coronárias o que distingue de ácidos graxos trans industrializados. Em concordância, estudo conduzido por Roy et al. (2007), mostrou que coelhos submetidos a dieta com manteiga enriquecida com ácido vacênico e CLA tenderam a reduzir a deposição de lipídios na aorta quando comparado com o grupo controle e animais que receberam manteiga enriquecida com C18:1 trans-10. Por outro lado, coelhos alimentados com manteiga enriquecida com C18:1 trans-10 apresentaram efeitos prejudiciais sobre lipídios plasmáticos e metabolismo das lipoproteínas.

O C18:1trans-10 é um ácido graxo resultante da biohidrogenação do ácido linoleico e linolênico que está presente em maior quantidade quando ruminantes são alimentados com dieta rica em concentrado rapidamente fermentáveis ou dieta com baixa proporção de fibras. Nestas circunstâncias, as condições ruminais sofrerão alterações, o que influencia a flora bacteriana e a rota de isomerização. Por exemplo, o ácido linoleico será isomerizado em C18:2 trans-10 cis-12 (CLA) e posteriormente por ação de enzimas redutases formará C18:1 trans-10 (BUCCIONI et al., 2012; CHILLIARD et al., 2007; GRIINARI et al., 1998), processo de isomerização semelhante ocorrem para a formação de C18:1 trans-10 a partir de ácido linolênico (BUCCIONI et al., 2012).

O interesse na composição de ácidos graxos resulta principalmente da necessidade de encontrar formas para produzir carne mais saudável, isto é, elevar ácidos graxos insaturados para alcançar níveis satisfatórios à saúde (relação AGPI:AGS > 0,4). Naturalmente a carne apresenta aproximadamente uma razão AGPI:AGS de 0,1 (WOOD et al., 2003; WOOD et al., 2008). Evidencia-se ainda a importância do equilíbrio entre a relação n-6:n-3 cujo valor sugerido é inferior a quatro a fim de buscar benefícios à saúde do consumidor (WOOD et al., 2003), já que segundo Emken et al. (1994) existe uma competição entre as enzimas que participam nas reações de dessaturação e alongamento da cadeia de ácidos graxos n-6 e n-3.

Sabendo da influência da biohidrogenação ruminal na incorporação de ácidos graxos e que esta é influenciada amplamente pela dieta dos ruminantes (DOREAU et al., 2010), pesquisas têm demonstrado a relação dos níveis de consumo de concentrados e volumosos para o perfil lipídico da carne. Ácidos graxos poli-insaturados n-3 e ácidos graxos resultantes da biohidrogenação ruminal como vacênico (C18:1trans-11) e ácido linoleico conjugado (principalmente cis-9, trans-11 CLA) estão relacionados com dietas compostas predominantemente por pastagem (SCOLLAN et al., 2014).

Entre os motivos para haver elevação de n-3 na carne de animais terminados em pastagem, destaca-se a composição do alimento. Forragens contêm uma elevada proporção (50-75%) de ácido α-linolênico (DEWHURST et al., 2006) que é precursor de ácidos graxos de cadeia longa n-3, principalmente EPA e DHA (SCOLLAN et al., 2014). Desta forma, a carne de ruminantes terminados em pastagem apresenta maior absorção dos ácidos graxos de cadeia longa supracitados (WOOD et al., 2008). Além disso, os mesmos autores destacam que o maior período de permanência ruminal de forragens fibrosas favorece a elevação de ácidos graxos intermediários ao processo de biohidrogenação, como o CLA e o vacênico, por viabilizar a atividade microbiana. Este também é o motivo pelo qual a eficiência de deposição do ácido linolênico é inferior quando comparado com o ácido linoleico. Dietas com baixo teor de fibra modificam a flora ruminal o que favorece a passagem de ácidos graxos pelo rúmen sem sofrerem biohidrogenação, especialmente os ácidos oleico e linoleico (CHILLIARD et al., 2007), presentes em grande concentração na dieta de ruminantes (BUCCIONI et al., 2012). Martin e Jenkins (2002) sugerem ainda que a modificação do pH ruminal devido a diferentes dietas influencia a produção de vacênico e CLA. Os autores relatam que a síntese de vacênico e CLA é maximizada se o pH for mantido acima de 6,0, pois as bactérias celulolíticas são sensíveis à ambiente ruminal ácido.

Nuernberg et al. (2008) ao compararem duas dietas, pastagem *versus* concentrado, observaram que animais mantidos em pastagem apresentaram maior deposição de ácido α-linolênico, CLA, de ácido vacênico e inferior relação n-6:n-3 no músculo e gordura subcutânea de cordeiros. Em concordância, outros estudos sugerem semelhantes achados quando comparadas dietas utilizando pastagem e níveis de suplementação com concentrado (DUCKETT et al., 2003; MAJDOUB-MATHLOUTHI et al., 2013; SANTÉ-LHOUTELLIER et al., 2008; TANSAWAT et al., 2013).

3.2.2 Estabilidade oxidativa

Processos oxidativos são importantes fatores responsáveis pela qualidade e deterioração da carne (PETRON et al., 2007). Desta forma, a dieta de terminação animal pode afetar significativamente a suscetibilidade frente à deterioração oxidativa da carne devido a presença de fatores antioxidantes e pró-oxidantes (LUCIANO et al., 2012), sendo que a concentração de AGPI é um potencial desencadeante da oxidação e por isso o incremento de n-3, desejável nutricionalmente, gera maior predisposição a oxidação (FAUSTMAN et al., 2010).

A produção de radicais livres ou espécies reativas é parte integrante do metabolismo humano, estas substâncias podem reagir e causar dano celular (VASCONCELOS et al., 2007). Todos os componentes celulares são suscetíveis à ação de espécies reativas, porém a membrana é um dos mais atingidos em decorrência da reação entre radicais livres e ácidos graxos insaturados presentes na estrutura (FAUSTMAN et al., 2010). Tal mecanismo desencadeia uma série de reações resultando na peroxidação, alterações na integridade da membrana e morte celular. Ao longo das reações, ocorre a liberação de produtos de degradação de ácidos graxos, como os aldeídos (FERREIRA; MATSUBARA, 1997), e por isso diversos estudos utilizam a técnica de TBARS (substâncias reativas ao ácido tiobarbitúrico) que mensura o malonaldeído para avaliar o grau de oxidação lipídica da carne (LUCIANO et al., 2009; SANTÉ-LHOUTELLIER et al., 2008; TANSAWAT et al., 2013).

Faustman et al. (2010) destacam que a associação entre a oxidação lipídica e oxidação da mioglobina pode ser evidenciada em duas situações: relatos na literatura de concomitante oxidação de lipídios e mioglobina na carne durante algum período de armazenamento; e a observação que antioxidantes inibem a oxidação lipídica e colaboram com a estabilidade da cor. Os mecanismos pelos quais a oxidação lipídica poderia aumentar a oxidação da mioglobina são explicados principalmente da reatividade dos produtos derivados da oxidação de ácidos graxos insaturados, como aldeídos e hidroxinonenal. Tais metabólitos podem inibir a enzima metamioglobina redutase gerando acúmulo de metamioglobina na carne. Já a oxidação da mioglobina influencia na oxidação lipídica devido à formação de substâncias reativas que apresentam capacidade de colaborar com a continuidade da oxidação tanto da mioglobina quanto de ácidos graxos insaturados. Em concordância, Ferreira e Matsubara (1997) sugerem que o excesso de Fe^{3+} (formado durante reação de Fenton) catalisa a reação de Haber-Weiss provocando acúmulo de espécies reativas como o radical hidroxila (OH^{-}) que participa da peroxidação lipídica.

Além disso, estudos demonstram que a oxidação proteica, mensurada através da estimativa dos grupos carbonila, pode estar relacionada com valores de substâncias reativas ao ácido tiobarbitúrico (TBARS) (GRAVADOR et al., 2014; PETRON et al., 2007; SANTÉ-LHOUTELLIER et al., 2008), pois proteínas são o alvo do ataque dos radicais livres, bem como os lipídios, sendo que a ocorrência das duas reações promove modificações biológicas que afetam a qualidade da carne (LUND et al., 2011; SANTÉ-LHOUTELLIER et al., 2008).

Luciano et al. (2012) avaliaram o efeito da terminação de ovinos utilizando exclusivamente concentrado ou pastagem de azévem na estabilidade oxidativa da carne. Os

autores concluíram que animais terminados em pastagem apresentaram maior quantidade de ácidos graxos altamente peroxidáveis (AGPI com três ou mais insaturações), menor oxidação lipídica e superior estabilidade da cor. Ao avaliar o efeito de dietas compostas por pastagem ou grão na estabilidade oxidativa na carne bovina, Insani et al. (2008) verificaram que a oxidação lipídica e proteica foram maiores para animais confinados, além disso, os resultados demonstraram que o nível elevado e ação sinérgica de α-tocoferol e β-caroteno encontrados em animais terminados em pastagem contribuíram para a estabilidade oxidativa e cor da carne.

Os antioxidantes, com destaque a vitamina E, são encontrados em concentrações mais elevadas nos músculos de animais alimentados com pastagem (DESCALZO; SANCHO, 2008; SANTÉ-LHOUTELLIER et al., 2008; TANSAWAT et al., 2013). A presença ou ausência de vitamina E em tecidos animais afeta a estabilidade durante o armazenamento da carne, pois tal antioxidante é capaz de eliminar os radicais livres e, consequentemente, proteger fosfolipídios e colesterol contra a oxidação (DESCALZO; SANCHO, 2008).

3.2.3 Compostos voláteis na carne e aceitação sensorial

Atribui-se a uma grande quantidade de compostos voláteis um papel importante para determinar o flavor da carne (PRIOLO et al., 2001). O flavor é definido como o conjunto de impressões olfativas e gustativas provocadas no momento do consumo da carne, portanto, esta sensação inicia antes da introdução do alimento na boca e se propaga durante a mastigação e depois da deglutição (OSÓRIO et al., 2009).

O flavor é estimulado por componentes voláteis (aroma) e não voláteis (gosto) que interagem com receptores sensoriais gerando uma resposta sensorial referente ao produto. As liberações destes compostos de aroma da carne cozida estão envolvidas principalmente com reações de oxidação lipídica, reação de Maillard, reação de Strecker e degradação de tiamina. Desta forma, a distinção de flavor entre as espécies ocorre devido aos componentes musculares como o perfil de ácidos graxos, presença de substâncias antioxidantes e estrutura da carne (RESCONI et al., 2013).

Vários fatores estão envolvidos no acúmulo de voláteis em tecidos animais, entre estes, a dieta na terminação desempenha um papel fundamental. Dietas à base de grãos estão relacionadas com maiores quantidades de ácidos graxos de cadeia ramificada (principalmente em ovinos), lactonas (derivam de moléculas formadas no rúmen a partir da oxidação do ácido oleico e linoleico), e alguns aldeídos (relacionados com oxidação lipídica sendo que: o

hexanal, 2-heptanal, e 2,4-decadienal são originados a partir do ácido linoleico enquanto que, 4-heptanal, 2,4-heptadienal e 2,6-nonadienal são derivados da decomposição do ácido linolênico e mais relacionados com consumo de pastagem). Já a gordura da carne de animais terminados em pastagem contém altos níveis de compostos fenólicos (metabólitos secundários de plantas que podem ser transferidos ao músculo de forma direta ou sintetizados no rúmen, 4-metilfenol é o principal exemplo relacionado com pastejo), terpenos (representam uma pequena percentagem dos compostos voláteis), indóis (mais conhecido é o escatol, originado por desaminação e descarboxilação do aminoácido triptofano por microrganismos do rúmen), 2,3-octanodiona e compostos de enxofre (estão presentes em baixas concentrações, porém apresentam grande potencial de imprimir flavor) (VASTA; PRIOLO, 2006).

Consumidores consideram a carne vermelha proveniente de animais criados em pastagem distinta do produto obtido a partir de animais terminados com concentrado, porém o flavor desejável pelo consumidor está relacionado com a cultura da região onde são criados os animais. Por exemplo, consumidores da União Europeia apresentam maior aceitação por carnes provenientes de animais terminados em pastagens, e o oposto ocorre para a percepção dos consumidores norte-americanos (PRIOLO et al., 2001).

4. DESENVOLVIMENTO

O desenvolvimento desta Tese foi dividido em três manuscritos, apresentados na forma de artigos científicos.

4.1 MANUSCRITO 1

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Growth performance and carcass traits of steers finished on three different systems including legume-grass pasture and grain diets

A.P.B. Fruet[1], F.S. Stefanello[1], F. Trombetta[1], A.N.M. De Souza[2], A. Rosado Júnior[3], C.J. Tonetto[3], J.L.C. Flores[3], R.B. Scheibler[4], R.M. Bianchi[5], P.S. Pacheco[6], A. De Mello[7*] and J.L. Nörnberg[1]

[1] Department of Science and Food Technology, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.

[2] UFSM School Polytechnic, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.

[3] Farroupilha Federal Institute ,São Vicente do Sul, s/n 20 de Setembro St., São Vicente do Sul, RS 97420000, Brazil.

[4] Department of Animal Science, Federal University of Pelotas, Capão do Leão, s/n University campus, Capão do Leão, RS 96160000, Brazil.

[5] Veterinary Pathology Laboratory, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.

[6] Department of Animal Science, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.

[7] Department of Agriculture, Nutrition, and Veterinary Sciences, University of Nevada, Reno, 1664 N. Virginia St. mail stop 202, Reno, NV 89557, United States.

*Corresponding author: Amilton de Mello. Email: amilton@cabnr.unr.edu.

Short title: Finishing steers on legume-grass and grain diets

Abstract

Inclusion of legume in grass pastures optimizes protein values of the forage and promotes improved digestibility. Therefore, we hypothesized that finishing steers on a novel combination of legumes and grass pasture would produce carcasses with acceptable traits when compared to carcasses from steers finished in feedlot systems. In this study, we evaluated the effects of finishing steers on three systems including: grazing legume-grass pasture containing oats, ryegrass, white, and red clover (PAST), grazing PAST plus supplementation with whole corn grain (14 g/kg body weight [BW]) (SUPP), and on a feedlot-confined system with concentrate only (28 g/kg BW, consisting of 850 g/kg of whole corn grain and 150 g/kg of protein-mineral-vitamin supplement [GRAIN]) on growth performance of steers, carcass traits, and digestive disorders. Eighteen steers were randomly assigned to one of three dietary treatments and finished for 91 days. Data regarding pasture and growth performance were collected during three different periods (0-28, 29-56, and 57-91 days). Subsequently, steers were harvested to evaluate carcass traits, presence of rumenitis, abomasitis, and liver abscesses. The legume-grass pasture provided more than 19% DM of protein. Additionally, pasture of paddocks where steers were assigned to SUPP and PAST treatments showed similar nutritional quality. When compared to PAST, finishing on SUPP increased total weight gain per hectare, stocking rate, daily and total weight gains. The increase of weight gain was high to GRAIN than SUPP and PAST. Steers finished on GRAIN had high hot carcass weight, fat thickness, and marbling score when compared to PAST. However, these attributes did not differ between GRAIN and SUPP. Abomasum lesions were more prevalent in steers finished on GRAIN when compared to PAST. Results of this research showed that it is possible to produce carcasses with desirable market weight and fat thickness by finishing steers on legume-grass pasture containing oats, ryegrass, white and red clover. Also, supplementing steers with corn

when grazing on legume-grass pasture produced similar carcass traits when compared to beef fed corn only.

Keywords: Legume, grass, beef, whole corn grain, finishing system.

Implications

Previous research reported that finishing steers on forage decreases growth performance when compared to finishing on grains. In this study, we demonstrated that finishing diets based on legume-grass pasture plus grain supplementation provided sufficient nutrients to produce carcasses with similar weight when compared to a corn-based diet. In Brazil, carcass weight is the most important attribute for grid pricing due to the absence of a required quality and yield grading system. In addition, all finishing systems evaluated in this research generated carcasses with desirable traits, carcass weight with approximately 500 kg, and fat thickness between 3 and 6 mm.

Introduction

Pasture is the main feed source for cattle in the world, and pasture finishing is considered to be less costly than finishing cattle on grain. The economic viability of producing livestock using grazing systems depends on consistent supply and quality of forage (Redfearn *et al*., 2002). This can be achieved by using a mixture of grasses and legumes grown in the cold season (from late fall through spring). Using a mixture of temperate grasses such as oats (*Avena strigosa* Schreb.) and ryegrass (*Lolium multiflorum* Lam.) increases forage production and extends grazing season. Oats and ryegrass mixtures are widely used in southern Brazil (Patino *et al*., 2015). Oat is a cool-season annual forage that is cultivated in the autumn to be utilized in the beginning of the winter. Oat grows faster than ryegrass, which has slow initial development and

reaches maximum forage production at the end of the winter and beginning of spring (Noro *et al*., 2003).

The combination of legumes such as white clover (*Trifolium repens*) and red clover (*Trifolium pratense*) in mixtures of grasses increases the nutritional quality of pastures due to nutrient digestibility and protein content when compared to monoculture grasses (Buxton *et al*., 1985; Sleugh *et al*., 2000). Additionally, legumes fix atmospheric nitrogen (Vinther, 2006) resulting in increased forage production (Gierus *et al*., 2012). Finishing steers on temperate pastures plus grain supplementation is a common system in the southern hemisphere (Aguerre *et al*., 2013). The utilization of whole corn grain (**WCG**) as an energy source improves growth performance and carcass traits (Roberts *et al*., 2009). Currently, utilization of WCG has also been found to be as efficient as ground corn regarding weight gain when added in concentrate-based diets for finishing cattle (Carvalho *et al*., 2016).

Although feedlot finishing systems substantially grew over the last 10 years in Brazil, beef cattle has been predominantly finished on pasture (Oliveira and Miller, 2014). Beef from steers finished on pasture has a healthier fatty acid profile and improved oxidative stability (Fruet *et al*., 2016). These benefits, allied to marketing strategies that link product to local producers and feeding systems are important factors that support grazing and allows producers to explore niche markets. Recently, a governmental Uruguayan campaign to promote its beef as “Uruguayan grass-fed beef” was developed to establish a premium brand based on country-of-origin labeling and beef characteristics of production systems based on pasture (Realini *et al*., 2013).

Previous research reported that limitations of forage-finished beef include lower carcass weight (Duckett *et al*., 2013) and longer periods of time to achieve similar weight when compared to cattle finished on grains (Roberts *et al*., 2009). However, Chail *et al*. (2016) demonstrated that it is possible to obtain similar hot carcass weights

by finishing steers on legume pastures or with high grain diets. Dierking *et al*. (2010) observed that steers finished on grass-only had lower weight gain when compared to steers finished on legume-grass pasture. Based on both studies, we hypothesized that finishing steers on pastures including black oats, ryegrass, white clover, and red clover would provide similar carcass traits when compared to carcasses finished on pasture with WCG supplementation or with WCG only. In our study, we evaluated growth performance, carcass traits, and incidence of digestive disorders of steers finished on three systems including legume-grass pasture and a high grain diet based on WCG.

Material and methods

Animals, diet and finishing management

The study was conducted at the Instituto Federal Farroupilha (IFF), RS, Brazil. Handling and care of steers were approved by the IFF Ethics Committee on the Use of Animals (protocol 003/2015). Eighteen British and Zebu crossbred steers (18 to 20 months of age, initial body weight [BW] 333 ± 27.87 kg) were randomly allocated to three finishing systems based on legume-grass pasture **(PAST)**, legume-grass pasture supplemented with whole corn grain (WCG) (14 g WCG/kg BW, **SUPP**), and a grain-only diet consisting of 85% WCG and 15% protein-mineral-vitamin supplement **(GRAIN)**.

The pasture area of six hectares (ha) was conventionally prepared by plowing and disking with a density of 80 kg/ha of black oat seeds, 30 kg/ha of ryegrass seeds, 3 kg/ha of white clover seeds and 3 kg/ha of red clover. For seeding, the following concentrations were used: 300 kg/ha of N-P_2O_5-K_2O (10/30/20) and 90 kg/ha of nitrogen fertilizer in the form of urea, divided into three applications: June 29, July 24, and August 03 of 2015. The pasture was divided in 12 paddocks of 0.6 ha for the PAST treatment (n=6) and 0.4 ha for SUPP (n=6). A feeding trough containing mineral salt

and/or WCG was available in all paddocks. Steers were individually allocated into paddocks and had *ad libitum* access to mineral supplementation. Throughout the trial, forage mass availability was maintained at desirable levels by using put-and-take stocking animals (n=4). The put-and-take method consists of using additional animals during a specific period with a periodic adjustment in animal number to attempt maintaining desired grazing pressure on each treatment (Allen *et al.,* 2011).

For the PAST treatment, steers were kept on pastures of black oat (*Avena strigosa*), ryegrass (*Lolium multiflorum*), white clover (*Trifolium repens*) and red clover (*Trifolium pratense*). The average composition of the PAST diet in dry matter was: 209 g/kg crude protein (CP), 495 g/kg insoluble neutral detergent fiber corrected for ash (NDF), 291 g/kg insoluble acid detergent fiber corrected for ash (ADF),144 g/kg non-fibrous carbohydrates (NFC), 10.54 MJ/kg of metabolizable energy (ME). For the SUPP treatment, steers were allocated to a treatment identical to PAST, but supplemented with 14 g/kg of WCG of BW. The average composition of the SUPP diet considering a supply of 50:50 in dry matter was: 159 g/kg CP, 309 g/kg NDF, 169 g/kg ADF, 435 g/kg of NFC, 11.51 MJ/kg ME.

Steers fed GRAIN were kept in feedlot regime and were fed exclusively with 28 g/kg BW of concentrate. Diet consisted of 850 g/kg of WCG and 150 g/kg of protein-vitamin-mineral pellet supplement. The composition of the GRAIN diet was: 149 g/kg CP, 168 g/kg NDF, 65 g/kg ADF, 622 g/kg of NFC and 12.09 MJ/kg de ME. Steers were confined in a covered facility, in individual pens of 13.5 m^2 (n=6), with concrete floor and a trough for feeding. Steers from all treatments had *ad libitum* access to water. Prior entering the experiment, steers went through an adaptation period of 21 days (gradual supply of concentrate for WCG adaptation). Thus, a total of 18 experimental units were randomly assigned to PAST, SUPP, and GRAIN (six replications per treatment).

Steers and grazing performance evaluations were carried out according to Roberts *et al.* (2009) in three experimental periods (0-28, 29-56, and 57-91 days), totaling 91 days of finishing. Confined steers were fed twice daily, whereas grazing steers were fed dry matter adjusted by estimated forage mass. Steers from treatment SUPP received WCG once daily. In order to calculate concentrate intake and feed conversion, amounts of WCG were measured before and after steers had access to the trough. Total weight gain (**TWG**) was determined by weighing steers on the first experimental day (initial body weight, **IBW**) and at the end of third period after a 14-hour fasting period (body weight at slaughter, **BWS**). Steers were slaughtered at the end of the third period by exsanguination preceded by mechanical stunning (captive bolt).

Pasture assessment

A continuous grazing system with variable stocking rate was adopted by following the methodology described by Mott and Lucas (1952). Desired forage mass was maintained between 1200 and 1500 kg DM/ha. Forage mass was evaluated based on the visual estimation technique described by Campbell and Arnold (1973) at intervals of 14 days. Briefly, 15 estimated evaluations were performed followed by four standard evaluations, assisted by squares of 0.25 m^2 area. These evaluations also assessed the height of grazing and two sub-samples were collected from each paddock for determination of dry matter (DM). Additionally, two exclusion cages per paddock were used during grazing to estimate herbage accumulation rate (kg DM per ha/day). These values were determined by the difference between the initial and final DM present in the exclusion cages, divided by days of period.

Stocking rate adjustment calculation was estimated according to Heringer and Carvalho (2002). The stocking rate (kg of BW per ha) was calculated as the sum of the weight of the steers (test and put-and-take stocking steers) multiplied by the days spent

in the pasture, divided by the total days of the experimental periods. Forage allowance (kg DM/100 kg of BW) was calculated by the ratio between the availability of forage and the stocking rate. In order to obtain production per hectare per day, stocking rate was divided by the average weight of steers and that value was multiplied by the average daily weight gain (**DWG**). This value multiplied by pasture time (days) indicated the total weight gain per area.

Chemical composition of forage was evaluated by the hand plucked method. Briefly, the method consisted of manually collecting forage material for visual evaluation of the area, forage height, and remains of consumed forage (Campos *et al.,* 2016). Subsequently, samples were dried in a forced air circulation oven at 55° C for 72 hours. Then, samples were ground in a Wiley mill and packed in sealed plastic containers. Dry matter (DM), crude protein (CP), and mineral matter (MM) were determined according to the methodology described by the AOAC (1995). Insoluble neutral detergent fiber corrected for ash (NDF) and insoluble acid detergent fiber corrected for ash (ADF) were quantified according to Van Soest *et al*. (1991), a method that uses thermostable alpha-amylase to proper estimate NDF by removing starch interference. The *in situ* digestibility of DM was measured using four cannulated steers, which received an adaptation diet containing pasture and 1% DM of WCG for 15 days. This diet provided to steers development of ruminal bacteria able to digest the dietary treatments performed in this trail. All samples were incubated for 48 hours prior to analysis (Ørskov *et al*., 1980).

Carcass traits

Hot carcass weight (**HCW**) was recorded after slaughter. Carcasses were split in halves that were chilled for 24 h at 2 ºC. After 24 h, values of pH were assessed in the *longissimus thoracis* muscle at the 12th rib. Dressing percentage was calculated as

(chilled carcass weight X 100)/ body weight at slaughter. Chilling loss was calculated by:

$$\text{Chilling loss \%} = 100 - \left(\frac{\text{chilled carcass weight x 100}}{\text{hot carcass weight}}\right)$$

In this experiment kidney, pelvic, and heart (**KPH**) fat weight was not included in the carcass weight analysis. Ribeye area and subcutaneous fat thickness were measured at the 12-13th rib interface following the method described by the AMSA (2001). Marbling score was assessed subjectively by using a 10-point scale (1 = devoid to 10 = abundant) (Mader *et al.,* 2009). Carcass traits were evaluated by the Federal University of Santa Maria research personnel.

To evaluate carcass composition, 9th and 11th rib sections from right sides were removed and weighed. In this section, the physical separation of the lean trim fraction (*Longissimus thoracis* and muscle mixed with fat), fat (subcutaneous and intermuscular) and bone was carried out for calculation of yield of each portion, as described by Lunt *et al*. (1985). Samples of *Longissimus thoracis* and mixture of meat and fat were collected to determine the lipid fraction by the technique of Hara and Radin (1978). The value of total lipids was subtracted from the weight of the lean trim fraction and added to weight of intramuscular / intermuscular fat. The total fat included subcutaneous and intramuscular / intermuscular fat.

Macroscopic and microscopic evaluation of the gastric tract and liver

The rumen, omasum, abomasum, reticulum, and liver from all steers were collected after evisceration. Gastric tract was opened, cleaned, and a macroscopic evaluation of all gastric tract was performed to identify any presence of lesions or abnormalities. Macroscopic pictures were obtained with a Canon™ T2i camera. The

ruminal epithelium was classified according to the incidence of macroscopic lesions that characterize ruminitis on a scale from zero (no injury) to 10 (severe disseminated ulcerations) (Bigham and Mcmanus, 1975). The severity of hepatic abscesses was also scored from zero (no abscess) to three (one or more abscesses larger than 2.5 cm or more than four small abscesses) (Brink *et al*., 1990). After macroscopic evaluation, samples of evaluated tissues, regardless presence of injury or not, were placed in 10% buffered formalin and sent to the Veterinary Pathology Laboratory. Samples were stained with hematoxylin-eosin (HE) for subsequent histological analysis. Histological pictures were obtained by using a Leica DM500 light microscope equipped with a Leica ICC50 HD camera, and it was processed through the software Leica Application Suite LAS EZ.

Statistical Analysis

Data were analyzed by using SAS® 9.3 package (SAS Institute, Inc., USA). For nutritional quality and parameters of pasture, concentrate intake, and DWG, a completely randomized design with repeated measures (along experimental periods) design was used. The model was: $Y_{ijk}= \mu + P_i + T_j + PT_{ij} + a_{(i)k} + e_{ijk}$. where: Yijkl = mean value obtained for each observation; μ = overall mean of the variable in the experiment; P_i = effect of the experimental period (EP), where i = 1, 2, and 3 EP; T_j = effect of treatment j, with j = three finishing system (FS) (the GRAIN diet was not included in the analysis for pasture nutritional quality and production); PT_{ij}= interaction between EP and FS; $a_{(i)k}$ = effect of repetition within FS; e_{ijl}. = experimental error, l = six repetitions. The data were analyzed using PROC MIXED. Best covariance structure was selected by using the lower values for Akaike (AIC) and Bayesian (BIC) information criteria. Statistical differences were identified by Tukey test ($P < 0.05$).

A completely randomized design was used for analysis of total weight gain per hectare, feed conversion, TWG, and carcass traits. The model applied was: $Y_{ij}= \mu + T_i+ e_{ij}$, where: Yij = mean value obtained for each observation; μ = general mean of the variable in the experiment; T_i = effect of treatment i, with i = three finishing system (FS); e_{ij}. = experimental error, j = six repetition. Initial body weight was used as a covariate for TWG, BWS, and HCW. Therefore, adjusted LS means were presented for these traits. When treatment effect was significant in the analysis of variance ($P \leq 0.05$), means were compared by the Tukey test. The nonparametric data including the score of ruminal lesions and liver abscesses were analyzed by the NPAR1WAY procedure, using the Kruskal-Wallis test.

Results

Nutritional quality and pasture production

Pasture of paddocks where steers were assigned to SUPP and PAST treatments showed similar nutritional quality ($P > 0.05$) (Table 1). During the experiment, there was an increase in levels of DM (second and third periods were the highest, $P < 0.001$) and NDF (third was the highest, $P < 0.001$), while the ADF values ($P = 0.062$) and DM digestibility ($P = 0.205$) remained constant during the experiment. Crude protein content was higher in the second experimental period when compared to the first and third ($P < 0.001$). Crude protein values were statistically similar for first and third periods (Table 1).

There was no effect of feeding system on forage mass ($P = 0.677$) and on forage height ($P = 0.181$). However, these traits were affected ($P < 0.001$) by experimental period (Table 2). In the third period, forage mass was higher ($P < 0.001$) than the first and second periods. Forage allowance values remained statistically similar during all periods ($P = 0.291$) and differed only between treatments ($P = 0.003$). The rate of

herbage accumulation was not affected by experimental period (P = 0.308) and finishing diet (P = 0.115).

The SUPP treatment led to increased weight gain per hectare when compared to PAST, 292 kg of BW/ha versus 167 kg of BW/ha (P < 0.001, SEM = 12.33). Higher stocking rate (Table 2) and higher DWG of steers (Table 3) led to increased weight gain per area. There was an interaction effect between period and finishing system for stocking rate (P = 0.009). Stocking rate values were higher in the third experimental period for SUPP steers due to intensified forage production caused by access of steers to supplementation and the need of maintaining desirable forage mass.

Animal performance

Steers finished on GRAIN led to greater DWG, followed by steers from SUPP and PAST (P < 0.001) (Table 3). Consequently, TWG also differed among the three treatments (P = 0.005) (Table 4). Effect of experimental period was observed for DWG (P=0.004). Daily weight gain on the first experimental period was significant lower when compared to the second, whereas values observed on the third period were similar when compared to the first and second. This is in agreement with data observed when evaluating concentrate intake. Feed conversion of steers fed GRAIN was 3.8^{B}, 4.2^{B}, and 5.9^{A} kg/kg BW, for period 1, 2, and 3, respectively (P < 0.001, SEM = 0.29).

Carcass traits

Body weight at slaughter (P < 0.001), hot carcass weight (P = 0.014), fat thickness (P = 0.023) and marbling (P = 0.023) were higher in carcass from steers fed GRAIN when compared to PAST (Table 4). Rib sections from steers fed GRAIN showed higher values of total fat when compared to PAST (P = 0.034) and lower total lean trim fraction when compared to carcass from steers fed SUPP (P = 0.022) (Table 4).

Subcutaneous fat from GRAIN carcasses were higher than SUPP and PAST carcasses (P = 0.006). However, finishing system treatments did not alter intramuscular / intermuscular fat ratio (P = 0.262). Feeding systems altered carcass composition by shifting between lean tissue development to fat deposition. Steers finished on GRAIN had lower carcass lean values (%) when compared to SUPP (P = 0.022) and higher fat (%) when compared carcass from steers fed the PAST diet (P = 0.033) (Table 4).

Macroscopic and microscopic evaluation of the gastric tract and liver

No macroscopic and microscopic lesions were observed in the reticulum, omasum, and liver abscesses of cattle. The prevalence of rumenitis in finished steers from GRAIN and SUPP treatments was 33.3% (P = 0.296) (Figure 1). Ruminal macroscopic lesions, characterized by multifocal areas of scar retraction in the mucous membrane with loss of ruminal papillae were observed in the rumens from two steers finished on GRAIN and one finished on SUPP (Figure 2C). Rumen from one SUPP steer presented a focally extensive hyperemic area, with loss of papillae and detachment of the ruminal mucous membrane (Figure 2A). Microscopic evaluations showed lesions on the rumens from two GRAIN and from two SUPP steers. Lesions consisted of multifocal areas of loss of epithelium coating replaced by fibrous connective tissue. Rumens from steers fed GRAIN (n=1) and SUPP (n=1) showed multifocal areas of ulceration of the rumen epithelium, covered by fibrin, intact and degenerated neutrophils, erythrocytes, and cellular debris (Figure 2B). This suggests a mild rumenitis with a score of 0.33 for those steers whereas no signs of lesions (score=0) was observed in rumens from steers fed PAST (P = 0.362).

Macroscopically, abomasitis were observed in all steers finished on GRAIN, in 66.6% in steers finished on SUPP and in 33.3% of steers finished on PAST (P = 0.049) (Figure 1). Lesions were characterized by circular (Figure 2D) and linear erosions

ranging from 0.2 to 1.5 cm in diameter and 1 x 0.5 cm to 4 x 0.5 cm, respectively microscopic lesions were also observed on abomasum and were characterized by multifocal areas of mucosal erosion, covered by fibrin, intact and degenerated neutrophils and cellular debris. These lesions indicated the presence of mild multifocal erosive abomasitis.

Discussion

Nutritional quality and pasture production

Values for DM, CP, NDF, and MM in the pasture significantly differed among experimental periods. Values of NDF increased along experimental periods due to improved forage maturity (Redfearn *et al*., 2002). Aguerre *et al*. (2013) and Roberts *et al*. (2009) reported that higher values for NDF may limit feed intake. In this experiment, increased values of NDF did not affect digestibility of DM. This effect is due to the increase of hemicellulose values, which can be digested by ruminants, since ADF values were constant along the experimental periods.

The utilization of mixed pastures containing grasses and legumes provides animals a feed with high digestibility of DM (Sleugh *et al*., 2000) and protein concentration above to what is recommended by the National Research Council (NRC, 2000). Differently than a monoculture pasture, legume-grass mixtures increase yield and uniformity of forage quality over long periods of grazing (Gierus *et al*., 2012). Grasses benefit from nitrogen fixation performed by species of legume forage, resulting in increased production and elevated levels of CP in pasture (Gierus *et al*., 2012). At the end of the grass cycle, when a decline in nutritional quality of the pasture was expected, quality remained high due to the presence of legumes. In this study, the mix of black oats, ryegrass, white and red clover provided at least 194 g/kg of DM of CP, and 880

g/kg of digestible DM during the experiment. These values were directly affected growth performance of steers.

According to Moot (1984), forage mass values must be between 1200 to 1600 kg DM / ha to avoid limiting feed intake and poor growth performance. In this study, forage mass values were maintained in the appropriate range and a significant increase was observed in period 3 due to the pasture growth stage. These results are in agreement with Redfearn *et al.* (2002), who reported that ryegrass grew 60% at the end of its production cycle and 30% at the beginning of the reproductive stage. The ryegrass growth resulted in an increased stocking rate in the last period and constant forage allowance during the whole experiment. The difference of forage allowance between treatments (P = 0.003) is a desirable factor that maximizes the performance of the SUPP system. The SUPP diet contained WCG as an additional energy source which reduces the forage offer requirement. Bargo *et al*. (2003) recommended forage allowance twice greater than the expected intake when finishing steers with supplementation. When finishing cattle on pasture only, forage allowance should be three to five times greater than the expected DM intake. In this study forage allowance was provided based on Bargo *et al*. (2003) recommendations. In addition, steers fed SUPP were supplemented with high levels of energy (WCG). This combination led to a 40% increase in pasture productivity per area when compared to the pasture used for PAST (where steers did not receive supplementation). Supplementing energy during grazing is a determining factor that contributes to increased growth performance per ha (Roberts *et al*., 2009).

Animal performance

The highest daily and total gains observed on steers finished on GRAIN, followed by steers finished on SUPP and PAST are directly related to WCG inclusion. The use of

concentrate is a strategy to increase weight gain since it contributes to a better balance between protein and energy (Poppi and McLennan, 1995). If energy is available, amino acids will be transaminated or used directly for microbial protein synthesis. Also, carbohydrates can be used as carbon skeletons for protein synthesis in combination with ammonia (Bach *et al.,* 2005). Feeding higher levels of starch raises ruminal production of volatile fatty acids, which are used as a primary source for hepatic gluconeogenesis (Aguerre *et al*., 2013; Carvalho *et al*., 2016). When animals are fed medium to high levels of concentrate, 30% of glucose needed for basal metabolism comes from starch that is not fermented in the rumen and is available in the intestines (Huntington, 1997). Therefore, concentrate diets contribute to high glucose availability, and the remaining energy generated from hepatic gluconeogenesis can be directly used for growth and finishing.

According to NRC, in order to gain 1.36 kg/day, steers weighing 425 kg require 70% of dietary TDN (NRC, 2000). In this study, the combination of black oat, ryegrass, white and red clover produced enough TDN to meet NRC requirements. This led steers finished on PAST to show higher DWG when compared to forage-fed steers from other studies (Duckett *et al*., 2013; Roberts *et al*., 2009).

Although digestibility remained constant (Table 1), DWG was smaller in the first experimental period when compared to second (P = 0.004). This may be related to the pasture development stage and increased availability of white and red clover throughout the experiment. These legumes have great palatability, which contributes to stimulate the intake of DM in the mixed pastures with grasses (Graves *et al*., 2012). Clover and ryegrass have similar development during growth stage. However, clover has higher yields during the end of the grass stage (Carrère *et al*., 2001). The presence of legumes in mixture pastures may have contributed to possible increased intake of DM and DWG throughout the experiment. In the third period, steers fed the GRAIN diet had similar

concentrate intake and increase of feed conversion when compared to the second period. This is due to increased fat deposition in the carcass, resulting in significant increase in feed conversion during the final stages of finishing. Overall fat accretion efficiency is approximately 1.7 times that of protein (Owens *et al*., 1995), which increases feed conversion.

Carcass traits

In Brazil, the optimal slaughter weight for steers is approximately 500 kg, whereas subcutaneous fat thickness must range from 3 to 6 mm (Carvalho *et al*., 2016). Carcasses with those characteristics were observed in steers from all three finishing systems (PAST, SUPP, and GRAIN) and attained the best Brazilian quality grades. The BWS and HCW are expected to be greater in carcasses from steers receiving high concentrate diets when compared to grass-fed if both are finished on similar time endpoints (Duckett *et al*., 2013). In our study, steers finished on GRAIN had high BWS and HCW than steers fed PAST, however, steers fed SUPP showed similar HCW when compared to steers fed GRAIN. Possibly, this happened due to improved nutritional values of the legume-grass pasture when compared to grass pastures. Combinations of legume-grass pastures and concentrate sources may provide sufficient nutrient balance to promote similar HCW when compared to concentrate diets (Poppi and McLennan, 1995). Therefore, using a mixture of black oat, ryegrass, white and red clover plus WCG supplementation as finishing diets is an alternative that provides similar HCW when compared to finishing on grain diets. In countries that do not have beef carcass grading systems based on quality and yield grades, HCW is the most important grid pricing parameter related to producer revenue.

Regarding fat deposition, our findings agree with Duckett *et al*. (2013), who reported higher fat content on carcasses and greater marbling in grain-fed beef when

compared to grass-fed. In this study, we evaluated the effects of diets on the *longissimus thoracis* muscle, however, deposition of fat in the lean may vary since muscle my show different composition and morphology. Li *et al*. (2018) demonstrated that adipogenic gene expression is higher in muscles from the loin compared to chuck or round muscles.

The increased fat deposition in GRAIN carcasses supports the notion that feed conversion increased in the third period of this treatment due to the energetic value of lipids (Owens *et al*., 1995). In addition, changes in carcass composition, which shifted between lean tissue and fat, can be attributed to the degree of maturity of animals. When animals approach maturity, there is a significant decrease in lean tissue development, which can decline to zero when the animal reaches adult body size. In this situation, as long as there is no energy limitation, cattle can continue depositing fat, raising the proportion of adipose tissue in relation to lean (Owens *et al*., 1995).

Macroscopic and microscopic evaluation of the gastric tract and liver

Diets with high amounts of concentrate increase production of volatile fatty acids, decrease ruminal pH values, increase acidosis, and lead to digestive epithelium damage (rumenitis) (Penner *et al*., 2011). In this study a similar prevalence of rumenitis was observed in rumens from steers fed SUPP and GRAIN. Our findings suggest that these injuries are not only related to feeding concentrate (14 g/kg or 28 g/kg of BW), but also to adaptation of the ruminal epithelium (Penner *et al*., 2011) and rapid stabilization of rumen pH of animals adapted to the level of concentrate (Schwaiger *et al*., 2013). Supplementing grains once a day during grazing to finish cattle is a common practice in the southern hemisphere. This leads to greater variation of ruminal pH when compared to feeding total mixed diets (forage plus concentrate) (Aguerre *et al*., 2013). In our

study, supplementing concentrate once during the day contributed to equal prevalence of rumenitis on steers fed SUPP and GRAIN.

Erosions were observed on abomasa of steers from all three finishing systems, however increased prevalence of erosions were observed on abomasa from steers fed SUPP and GRAIN. Abomasitis is commonly found in cattle from all production systems, whereas adult animals develop this type of injury due to an imbalance between aggression (volatile fatty acids concentration) and defense mechanisms (protective mucus layer), compromising the integrity of the abomasal mucous (Hund *et al*., 2016). The inclusion of WCG increased the prevalence of injuries on the abomasum, possibly due to higher concentrations of volatile fatty acids.

The use of legume-grass pasture provided adequate nutritional requirements to obtain carcasses with yield targets based on Brazilian standards. Finishing diets containing WCG only promoted greater weight gain when compared to mixed pasture and pasture plus WCG. The exclusive concentrate diet increased fat deposition on carcasses, but provide similar HCW to pasture plus WCG. Even though differences in some carcass traits were observed, all three finishing systems produced carcasses that met Brazilian industry requirements. Results of this research showed that it is possible to produce carcasses with desirable traits by finishing steers on legume and grass pasture including oat, ryegrass, white and red clover.

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Table 1 *Pasture attributes of legume-grass paddocks grazed by steers finished with (SUPP) or without (PAST) supplementation during three experimental periods (EP)*

	Finishing system (FS)			*P*-values			
	SUPP	PAST	Mean[1]	FS	EP	FS*EP	SEM
Dry matter - DM (g/kg)							
1°EP	156.1	153.4	154.8^{B}	0.716	<0.001	0.544	2.7
2°EP	177.0	166.8	171.9^{A}				
3°EP	180.6	186.9	183.8^{A}				
Mean[2]	171.2	169.1					
Crude protein – CP (g/kg DM)							
1°EP	197.1	191.8	194.5^{B}	0.203	<0.001	0.054	3.2
2°EP	243.5	252.1	247.8^{A}				
3°EP	217.1	184.8	201.0^{B}				
Mean[2]	219.2	209.6					
Neutral detergent fiber - NDF (g/kg DM)							
1°EP	460.4	464.7	462.5^{B}	0.608	<0.001	0.660	5.8
2°EP	462.9	459.0	460.9^{B}				
3°EP	543.4	563.0	553.2^{A}				
Mean[2]	488.9	495.6					
Acid detergent fiber – ADF (g/kg DM)							
1°EP	290.4	301.1	295.8	0.423	0.062	0.791	3.5
2°EP	273.8	272.2	273.0				
3°EP	289.1	298.9	294.0				
Mean[2]	284.4	290.8					
Mineral matter – MM (g/kg DM)							
1°EP	129.9	122.7	126.3^{AB}	0.949	<0.001	0.710	3.6
2°EP	146.0	151.6	148.8^{A}				
3°EP	102.9	102.8	102.8^{B}				
Mean[2]	126.2	125.7					
Digestibility of DM (g/kg DM)							
1°EP	883.4	911.9	897.6	0.548	0.205	0.301	0.4
2°EP	893.8	898.6	895.8				
3°EP	887.8	872.5	880.2				
Mean[2]	888.1	894.3					

[1]Means of experimental periods (EP);

[2]Means of finishing systems (FS);

[A,B] Values within a column with different superscripts differ significantly at $P < 0.05$.

651 **Table 2** *Parameters of legume-grass mixture pasture with (PAST) or without*

652 *supplementation (SUPP) over the three periods (EP)*

	Finishing system (FS)			*P*-values			
	SUPP	PAST	Mean[1]	FS	EP	FS*EP	SEM
Forage mass (kg of DM/ ha)							
1°EP	1206	1328	1267^{B}	0.677	<0.001	0.442	28.7
2°EP	1211	1240	1226^{B}				
3°EP	1451	1421	1436^{A}				
Mean[2]	1290	1330					
Forage height (cm)							
1°EP	21.60	24.60	23.10^{A}	0.181	<0.001	0.341	0.39
2°EP	19.55	21.60	20.57^{B}				
3°EP	23.55	24.08	23.81^{A}				
Mean[2]	23.42	21.56					
Rate of herbage accumulation (kg DM/ha per day)							
1°EP	53.33	49.66	51.50	0.115	0.308	0.721	1.34
2°EP	55.25	53.48	54.36				
3°EP	60.90	53.15	57.02				
Mean[2]	56.49	52.10					
Stocking rate (kg BW/ha)							
1°EP	848^{B}	748^{A}	798	<0.001	0.287	0.009	18.9
2°EP	936^{ABa}	620^{Bb}	778				
3°EP	1054^{Aa}	638^{ABb}	846				
Mean[2]	946	669					
Forage allowance (kg DM/100 kg BW)							
1°EP	11.40	13.23	12.31	0.003	0.290	0.081	0.43
2°EP	10.66	16.20	13.43				
3°EP	10.75	17.23	13.99				
Mean[2]	10.93^{b}	15.55^{a}					

653 [1]Means of experimental periods (EP);

654 [2]Means of finishing systems (FS);

655 ^{A,B,C} Values within a column with different superscripts differ significantly at $P < 0.05$;

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

657

Table 3 *Intake of concentrate and daily weight gain during the experimental periods (EP) of finishing in feedlot with exclusively concentrate (GRAIN), legume-grass mixture pasture with (SUPP) or without supplementation (PAST)*

	Finishing system (FS)				*P*-values			
	GRAIN	SUPP	PAST	Mean[1]	FS	EP	FS*EP	SEM
Concentrate intake (% of BW)								
1°EP	2.28	1.14	-	1.71^{B}	<0.001	0.022	0.312	0.09
2°EP	2.44	1.39	-	1.91^{A}				
3°EP	2.31	1.40	-	1.85^{A}				
Mean[2]	2.35^{a}	1.31^{b}	-					
Daily weight gain (kg/animal per day)								
1°EP	1.90	1.27	0.82	1.33^{B}	<0.001	0.004	0.084	0.06
2°EP	1.90	1.66	1.35	1.64^{A}				
3°EP	1.65	1.60	1.43	1.56^{AB}				
Mean[2]	1.81^{a}	1.51^{b}	1.20^{c}					
Feed conversion (kg/kg BW)								
1°EP	3.8^{B}	-	-	-	-	<0.001	-	0.29
2°EP	4.2^{B}	-	-	-				
3°EP	5.9^{A}	-	-	-				

[1]Means of experimental periods (EP);

[2]Means of finishing systems (FS);

^{A,B,C} Values within a column with different superscripts differ significantly at $P < 0.05$;

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

Table 4 *Carcass characteristics, on ninth to 11th rib section and carcass composition of steers finished in feedlot with exclusively concentrate (GRAIN), legume-grass mixture pasture with (SUPP) or without supplementation (PAST)*

Characteristics	Finishing system (FS)				
	GRAIN	SUPP	PAST	*P*-value	SEM
Total weight gain (TWG, kg)	165.8[a]	137.2[b]	110.9[c]	0.005	2.96
Body weight at slaughter (BWS, kg)	498.6[a]	470.0[b]	443.7[c]	<0.001	7.63
Hot carcass weight (HCW, kg)	286.4[a]	270.9[a]	253.4[b]	0.014	4.92
Chilling loss (%)	2.03	1.68	2.01	0.329	0.10
Dressing percentage (%)	56.36	56.56	55.97	0.730	0.29
KPH (%)	2.36	1.76	1.53	0.058	0.14
Fat thickness (mm)	5.95[a]	4.33[ab]	4.11[b]	0.023	0.31
Ribeye area (cm^2)	55.33	59.68	57.46	0.630	1.76
Marbling score[1]	4.50[a]	4.50[a]	3.33[b]	0.023	0.21
pH	5.62	5.63	5.61	0.965	0.04
9-10-11th rib section composition					
9-10-11th rib section (kg)	4.72	4.33	4.20	0.339	0.14
Total lean trim fraction (%)	48.82[b]	55.42[a]	54.45[ab]	0.022	1.11
Longissimus thoracis (%)	21.63	24.85	24.22	0.085	0.63
Lean (%)	27.18	30.56	30.23	0.122	0.74
Total fat (%)	33.66[a]	28.40[ab]	27.79[b]	0.034	1.05
Subcutaneous fat (%)	11.40[a]	8.57[b]	8.18[b]	0.006	0.49
Inter/intramuscular fat (%)	22.26	19.83	16.61	0.262	0.72
Total bone (%)	17.27	16.23	17.70	0.454	0.47
Carcass composition					
Carcass lean (%)	61.20[b]	64.83[a]	64.30[ab]	0.022	0.61
Carcass fat (%)	22.99[a]	19.73[ab]	19.35[b]	0.033	0.65
Carcass bone (%)	16.06	15.46	16.31	0.456	0.27

[1]Marbling was assessed subjectively, using a 10-point scale (1 = devoid, 2 = practically devoid, 3 = traces 4 = slight 5 = small 6 = modest 7 = moderate, 8 = slightly abundant, 9 = moderately abundant and 10 = abundant).

[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$

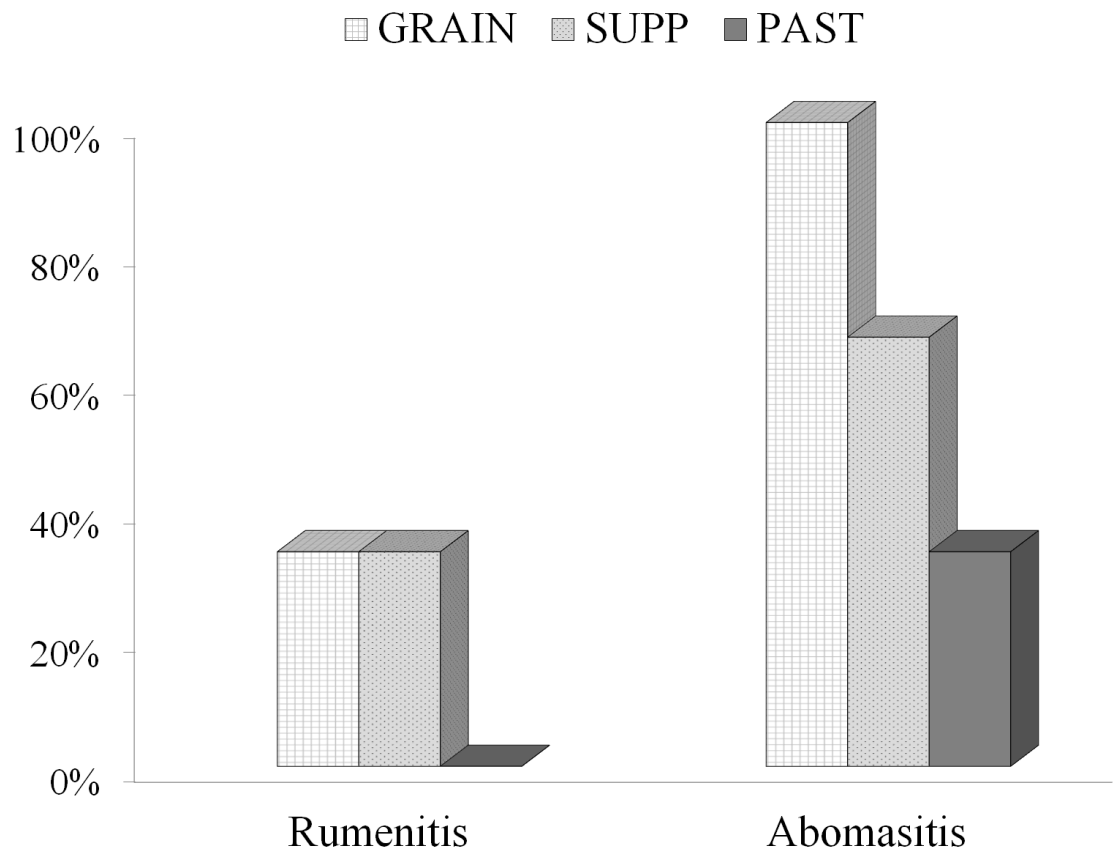

Figure 1. Prevalence of ruminal lesions (rumenitis) and abomasites (abomasitis) in steers finished in feedlot with exclusively concentrate (GRAIN), legume-grass mixture pasture with (SUPP) or without supplementation (PAST).

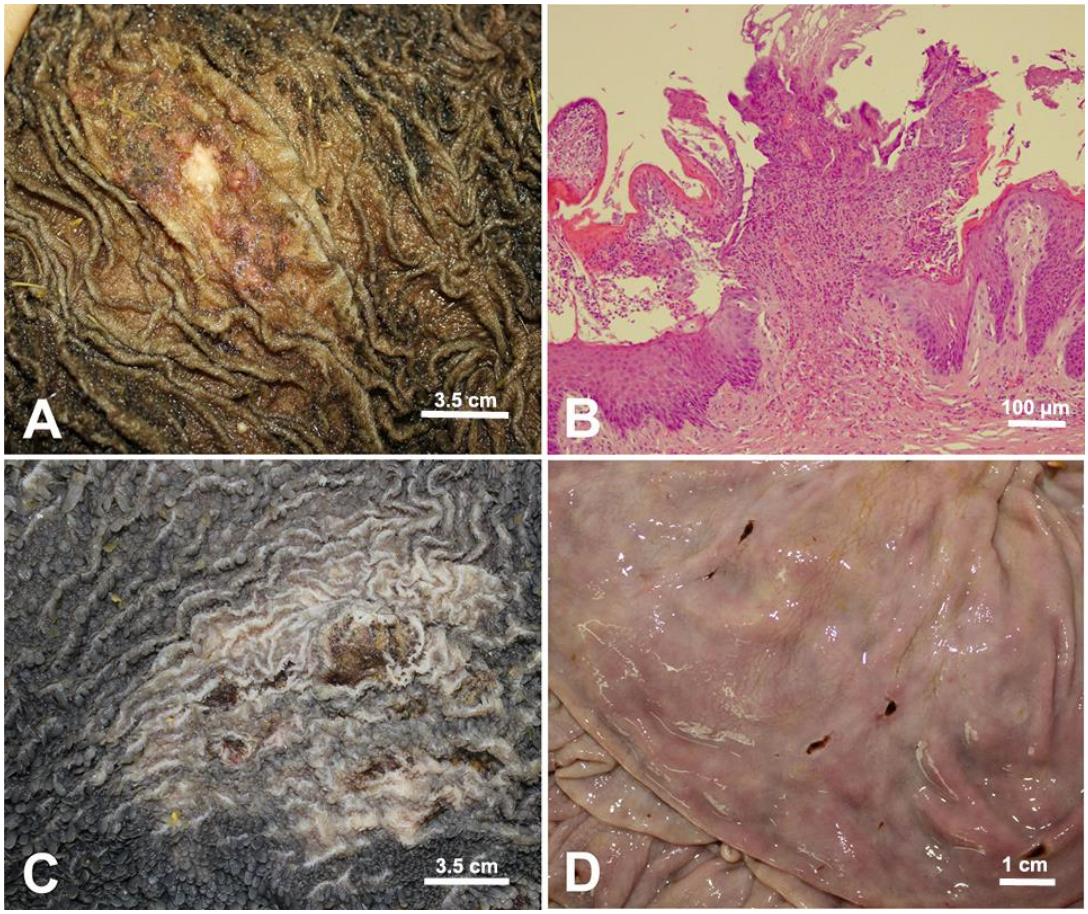

Figure 2. (A) Rumen. Reactive area with loss of papillae and mucosal detachment, characterizing subacute rumenitis injury in SUPP. (B) Rumen Area of ulceration of ruminal mucous, with replacement by neutrophils, fibrin and cellular debris observed in GRAIN and SUPP. H&E, obj.10x. (C) Rumen. Multifocal areas of scar retraction of the mucous and loss of papillae, characterizing chronic rumenitis injury observed in GRAIN and SUPP. (D) Abomasum. Multifocal areas of mucosal erosion observed in all finishing systems.

4.2 MANUSCRITO 2

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Effects of feeding legume-grass pasture and different concentrate levels on fatty acid profile, volatile compounds, and off-flavor of the M. longissimus thoracis

A.P.B. Fruet[ac*], F. Trombetta[a], F.S. Stefanello[a], C.S. Speroni[a], J.Z. Donadel[a], A.N.M. De Souza[b], A. Rosado Júnior[b], C.J. Tonetto[b], R. Wagner[a], A. De Mello[c], J.L. Nörnberg[a]

[a] Department of Science and Food Technology, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.
[b] Farroupilha Federal Institute, São Vicente do Sul, s/n 20 de Setembro St., São Vicente do Sul, RS 97420000, Brazil.
[c] Department of Agriculture, Nutrition, and Veterinary Sciences, University of Nevada, Reno, 1664 N. Virginia St. mail stop 202, Reno, NV 89557, United States.

*Corresponding author: Ana Paula Burin Fruet, Email: ap_burin@hotmail.com. Phone: +55 55 99756760;

Abstract

Pasture-finished beef is becoming more popular among consumers due to concerns related to fatty acid content and sustainable practices. The effects of finishing crossbred steers on legume-grass pasture comprised of oats, ryegrass, and clover (PAST), legume-grass pasture plus whole corn grain (WCG) supplementation (SUPP), and only with WCG (GRAIN) on fatty acids profile, volatile compounds, sensory, and texture attributes were studied. Pasture diets (PAST and SUPP) led to lower n-6/n-3 ratio ($P < 0.001$), and highest deposition of C18:2 *cis*-9 *trans*-11 ($P < 0.001$) in the lean. Beef from steers fed GRAIN had the highest values of volatile compounds associated with lipid oxidation. Off-flavor intensity was significantly greater on beef from steers fed GRAIN when compared to PAST. Overall, muscles from steers finished on PAST and SUPP showed similar attributes but differ when compared to GRAIN. The presence of forage is essential to improve fatty acid profile, decrease volatile compounds associated with lipid oxidation, and minimize off-flavor.

Keywords: Beef; Fatty acid; Grain-fed; Volatile compounds; Forage.

1. Introduction

Grazing pastures and freshly cut herbage are predominantly the most important feed sources for cattle in the world (Fanchone, Archimede, Baumont, & Boval, 2010; Freitas et al., 2014; Oliveira, & Millen, 2014; Schlegel, Wyss, Arrigo, & Hess, 2016). The utilization of pastures and fresh forage with high nutritional quality such as legume-grass mixtures can improve animal performance and maintain the meat quality standard of steers finishing on pasture (Dierking, Kallenbanch, & Grün, 2010; Chail et al., 2016).

Pasture finishing leads to greater deposition of *n*-3, CLA fatty acids, and lower n-6/n-3 ratio in the lean when compared with grain-finishing (Aldai et al., 2011; Duckett, Nell, Lewis, Fontenot, & Clapham, 2013; Patino, Medeiros, Pereira, Swanson, & McManus, 2015). From a nutritional standpoint, this change may be beneficial to consumers due to increased levels of desirable fatty acids (Ferguson, 2010; Scollan et al., 2014). The incorporation of *n*-3 PUFA in the muscle is facilitated by higher concentration of linolenic acid which is often found in roughages. Although higher levels of PUFA are associated with higher lipid oxidation (De Mello et al., 2012), roughage is a natural source of antioxidants (Lindqvist, Nadeau, & Jensen, 2011), which lowers lipid oxidation rates that are usually observed in grass-finished beef. Therefore, pasture finishing improves color and lipid stability of beef and also alters concentration of volatile compounds formed during cooking due to changes in fatty acid profile (Duckett, Nell, Lewis, Fontenot, & Clapham, 2013; Humada, Sañudo, & Serrano, 2014; Kerth, Braden, Cox, Kerth, & Rankins, 2007; Luciano et al., 2013; Ponnampalam et al., 2017; Tansawat, Maughan, Ward, Martini, & Cornforth, 2013).

When comparing meat from steers finished on grain, pasture, and pasture with supplementation, previous work suggested that concentrate levels and forage ratios are factors that may affect meat attributes, however, some research also reported minimal or

no effects on meat quality based on different levels and ratios (Duckett, Nell, Lewis, Fontenot, & Clapham, 2013; Kerth, Braden, Cox, Kerth, & Rankins, 2007; Patino, Medeiros, Pereira, Swanson, & McManus, 2015; Ponnampalam et al., 2017). Reasons of this inconsistency in results may be related to the presence of preserved roughage in some dietary treatments tested by those authors. Roughage conservation methods such as haying and silage fabrication reduce the initial concentration of antioxidants and PUFA. However, Lindqvist, Nadeau, & Jensen (2011) and Stefanello et al. (2018) demonstrated that conserved forage is still a significant source of antioxidants, whereas Boufaïed et al. (2003) and Eriksson & Pickova (2007) showed that this type of feedstuff has significant levels of linolenic acid.

Currently, there is few research that studied the effects of finishing ruminants with levels of 100% concentrate and legume-grass pasture including ryegrass, oat, red and white clover. Luciano et al. (2012) showed expressive difference in oxidative stability between grass and concentrate fed, however, comparison based on supplementation level was not performed. Fruet et al. (2016) observed that there were differences in total lipids, fatty acid profile, and lipid oxidation in muscle from ewes finished on grass and only concentrate. Both studies used ovine as experimental units, which metabolize glucose and lipids from feedstuff differently than bovine (Smith & Prior, 1986). Therefore, in order to accurately understand the effects of feeding grain and forage on beef quality attributes, studying distinct diets based only on grain or roughage may provide a better overview of those effects since most of grain-fed beef is finished with diets containing different levels of roughage. The objective of this study was to evaluate texture profile, fatty acid composition, volatile compounds profile, sensory attributes, and instrumental color of beef finished on legume-grass pasture, supplemented with whole corn grain, and only with whole corn grain.

105 **2. Material and methods**

106 *2.1 Animals, dietary treatments, and sample collection*

107 Eighteen crossbred steers (Hereford, Angus, and Nelore) [18 to 20 months of age,
108 initial body weight (BW) 333 ± 27.87 kg], previously reared on pasture, were randomly
109 assigned to one of three finishing diet treatments based on legume-grass pastures
110 comprised of black oats (*Avena strigosa*), ryegrass (*Lolium multiflorum*), white clover
111 (*Trifolium repens*) and red clover (*Trifolium pretense*) (PAST), PAST with whole corn grain
112 (WCG) supplementation of 1.4% of BW (SUPP), and WCG only (GRAIN). The pasture was
113 divided in 12 paddocks whereas six were used to individually house steers assigned to PAST
114 (0.6 ha each) and six for steers assigned to SUPP (0.4 ha each). A continuous grazing system
115 with variable stocking rate was adopted by following the methodology described by Mott
116 and Lucas (1952). In order to keep desired forage mass between 1200 and 1500 kg DM/ha
117 during 91 experiment days, forage mass was evaluated based on the visual estimation
118 technique described by Campbell and Arnold (1973) at intervals of 14 days. Steers allocated
119 to treatment GRAIN were confined in a feedlot-covered facility in individual pens of 13.5 m^2
120 (n=6). Steers assigned to treatment GRAIN were individually fed with 2.8% of BW of
121 concentrate and the diet consisted of 85% of WCG and 15% of protein-vitamin-mineral pellet
122 supplement. The removal of all roughage sources from the GRAIN diet was possible due to
123 the higher particle size of WCG and the addition of 150 mg of ionophore (inside of the pellet
124 supplement) in order to avoid acidosis. All animal handling and care procedures were
125 approved by the IFF Ethics Committee on the Use of Animals (protocol 003/2015).

126 Composition of finishing diets is shown in Table 1. Average daily gain (kg) of steers
127 fed GRAIN, SUPP, and PAST was 1.81, 1.51, and 1.33, respectively (SEM =0.06 and $P < 0.001$).

Steers were backgrounded on natural pasture consisting of grasses including *Paspalum notatum*, *P. dilatatum* and *Coelorachis selloana*, *Stipa setigera, S. hyalina*, *Piptochaetium bicolor* and *P. stipoides* as well as legumes including *Trifolium polymorphum* and *Adesmia bicolor* (Freitas et al., 2014). Prior entering the experiment, steers were acclimatized to facilities and respective diets during a period of 21 days. Steers assigned to SUPP and GRAIN received a gradual supply of concentrate until levels of WCG achieved 1.4 and 2.8% of BW, respectively. After the adaptation period, steers were fed for 91 days and slaughtered at the Farroupilha Federal Institute abattoir. Carcasses were chilled for 24 h at 4 °C and left rib sections from the 5^{th} to 13^{th} rib were fabricated. The outer fat cover of the bone-in section between the 9^{th} and 11^{th} rib was removed for subcutaneous fat color analysis. Subsequently, the M. *longissimus thoracis* (LT) was excised from the rib and 2.54 cm steaks were fabricated, individually vacuum packaged, and stored at -80°C until analysis could be performed.

2.2 Chemical composition and fatty acids profile

Thirty grams of one LT steak was lyophilised (Terroni, LS3000B, Brazil) under optimal conditions (Carpentier et al., 2007) for chemical composition analysis. Moisture, crude protein, and ash were quantified according to AOAC (1995). The remaining portion of the steak was used to analyse total lipid values (Hara & Radin, 1978) and transesterification of fatty acid profile (Christie, 1989). Fatty acid methyl esters (FAME, %) were quantified using a gas chromatograph (GC) (Agilent, 45813-01, CA, USA) equipped with a flame ionisation detector (FID). Separations were accomplished using a fused silica capillary column (0.25 mm × 60 m, Supelco SP^{TM}-2362, PA, USA). Oven temperature was programmed from 100 °C to 240 °C, whereas injector and detector temperature were 250 °C and 280 °C, respectively.

The carrier gas was nitrogen at a flow rate of 0.6 mL per min. Individual fatty acids were identified by comparison of retention times with known standards (Supelco Mix 37 components FAME; trans-11-vaccenic acid methyl ester; conjugated linoleic acid methyl ester; cis -7,10,13,16,19 - Docosapentaenoic methyl ester). Fatty acids were quantified by incorporating an internal standard, methyl tricosanoic (C23:0) acid, into each sample during methylation.

2.3 Volatile compounds

Volatile compounds were evaluated following the methodology described by Donadel et al. (2013) with some modifications. Thirty grams of a 2.54 cm of LT steak was autoclaved at 121 °C for 10 min. Subsequently, samples were homogenized using a hand blender and aliquots of 5 g were placed into 20 mL glass vials, capped with a polytetrafluoroethylene rubber septum, and placed in a 60°C water bath and allowed to equilibrate for 15 min. Volatiles were extracted by headspace solid phase micro extraction (HS-SPME) using a 75 µm × 10 mm carboxen/polidimetilsiloxane fiber (Supelco, PA, USA) that was exposed into the headspace of the vial for 50 min. A Shimadzu QP2010 *Plus* GC-Mass Spectrometer (MS) (Shimadzu Co., Japan) was used to detect and separate volatile compounds. The MS was equipped with a single quadrupole analyzer to detect ions within 35–350 m/z in the electron impact at 70 eV. After adsorption, extracted compounds were desorbed from SPME fibers at the GC-MS inlet (0.75 mm) at 260 °C in splitless mode for 10 min. Compounds were separated using a polyethylene glycol capillary column (Chrompack WAX 52-CB, 60 m × 0.25 mm × 25 µm, The Netherlands) and Helium was used as carrier gas at a flow rate of 1.6 mL per min. Volatile compounds were identified by comparison of mass spectra of the NIST 7 Mass Spectral Library (2000) and linear retention indices (LRI) with literature (Acree &

176 Heinrinch, 2017; El-Sayed, 2017). The LRI were calculated by previous injection of standards
177 of n-alkanes (C_6-C_{24}). Results were expressed in arbitrary area units (peak area) x10^5. Total
178 ion chromatograms were used for the quantitation of volatile compounds.
179
180 *2.4 Meat and subcutaneous fat color, cooking losses, shear force and texture profile analysis*
181 Instrumental color measurement was recorded for lightness (L*), redness (a*), and
182 yellowness (b*) using a Minolta Chromameter (CM-700D, Minolta Inc., Japan) with a 8 mm-
183 diameter measurement area, a A illuminant and with the 10° standard observer.
184 Subcutaneous fat color was measured on the area from 9-10-11th rib section, whereas lean
185 color was measured on the LT muscle. Color measures were obtained 24 h postmortem by
186 averaging 6 readings from different areas of the subcutaneous fat and steak surfaces.
187 For cooking loss, WBSF, and sensory evaluation, steaks were thawed for 24 h at 4°C and
188 grilled on an electric grill. Steaks were flipped at 35°C and cooked until the final temperature
189 reached 71°C at the geometric center. Cooking loss was calculated from steaks used for
190 WBSF by using the following formula:
191 $$\text{Cooking loss \%} = 100 - \left(\frac{\text{grilled weight of the steak x 100}}{\text{raw weight of the steak}}\right)$$
192
193 After being removed from the grill, steaks rested for 30 min prior to final weighing.
194 For WBSF, steaks were cooled overnight at 4°C and six 1.27-cm-diameter cores were
195 removed parallel to the muscle fiber orientation. Warner-Bratzler Shear Force and texture
196 profile were analyzed using a texture analyzer (Stable Micro Systems, TA.XTplus Texture
197 Analyzer, United Kingdom). A Warner Bratzler vee-shaped (60°) blade was used to shear
198 individual cores. The crosshead speed was set at 200 mm per min. Texture profile was

199 assessed by compressing six cubes of 1.5 cm^3, using a cylindrical 36 mm-diameter probe at
200 60 mm per min. Cubes were compressed twice to 80% of their original height. Texture
201 parameters included hardness, adhesiveness, cohesiveness, springiness, and chewiness.
202
203 *2.5 Sensorial analysis*
204 A total of 11 panelists with age between 18 and 30 years old was screened and
205 trained by following the AMSA (2015) guidelines. For each session a total of 12 LT steaks
206 were served to panelists (2 steaks per steer, 2 steers per treatment, 3 treatments per
207 session). Steaks were cooked until internal temperature reached 71 °C, trimmed of
208 subcutaneous fat cover, and 22 cubed samples (11 from each steak, dimensions: 2.54 cm ×
209 1.27 cm × 1.27 cm) were served to the eleven-member panel. Each panelist received 2
210 cubes, one from steak 1 and one from steak 2, totaling six samples (two cubes per sample,
211 12 cubes total) per session. Therefore, cubes from two steaks from the same steer
212 comprised one sample. Samples were served to panelists individually, under a red light to
213 avoid visual differences, and unsalted crackers and water were available to the panelists to
214 cleanse their palates between samples. Panelists evaluated juiciness from 1=extremely dry
215 to 7= extremely juicy, initial and overall tenderness from 1=extremely tough to 7=extremely
216 tender, beef flavor intensity from 1= extremely mild to 7= extremely intense. Off-flavor was
217 also rated by using an 8-point scale (0=none, 1=extremely slight to 8=extremely intense). A
218 total of 3 sessions were conducted.
219
220 *2.6 Statistical analysis*
221 Data were analyzed as a completely randomized design whereas for sensory analysis,
222 panelist was used as block to improve precision (Gacula, 1993). Diet was considered the

main effect with three levels including PAST, SUPP, and GRAIN. Analysis was conducted using GLM procedure of SAS (Version 9.3, Cary, NC). Residual normality and homogeneity were assessed by Shapiro-Wilk and Levene's tests ($P > 0.05$). When significance ($P < 0.05$) was indicated by ANOVA, means separations were performed using the LSMEANS function and individual differences were determined by the Tukey's test ($P < 0.05$).

3. Results

3.1 Chemical composition, fatty acids profile, and volatile compounds

Dietary effects on chemical composition and fatty acids profile for the LT muscle are shown in Table 2. Beef from steers finished on GRAIN had higher content of total lipids ($P = 0.002$) and lower moisture values ($P < 0.001$) than beef from steers finished on PAST. Values of crude protein, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and PUFA/SFA ratio were not affected by finishing system ($P > 0.05$). GRAIN-fed beef showed lower values of C18:0 ($P = 0.003$), and higher concentration of C18:1n7 ($P = 0.002$), C18:1*trans* ($P < 0.001$), and monounsaturated fatty acids (MUFA) ($P = 0.002$) when compared to beef from steers finished on PAST. Beef from steers fed diets with roughage (PAST and SUPP) had lower n-6/n-3 ratio ($P < 0.001$), and higher values of C18:2*cis*-9 *trans*-11 (CLA) ($P < 0.001$), C18:3n3 ($P < 0.001$), and total n-3 ($P < 0.001$).

Dietary treatments affected the volatile compounds profile of beef (Table 3). Inclusion of WCG (SUPP diet) increased Hexanoic acid at similar levels as observed in beef from steers fed GRAIN ($P = 0.024$). Overall, GRAIN-fed beef showed significant higher values of some alcohols including 1-Pentanol, 1-Hexanol, and 1-Octen-3-ol. Lower values of aldehydes including Hexanal, 5-Methyl hexanal and Octanal were observed in beef from steers fed diets with roughage (SUPP and PAST) *(*$P = 0.008$). PAST-fed beef had higher

concentrations of 2-Methyl propanal when compared to GRAIN-fed. Beef finished on PAST showed highest values of the ester Ethylacetate and the Ketone 2-Butanone when compared to beef finished with GRAIN. GRAIN-fed beef showed the highest values of the heterocyclic compound 2-Pentyl furan ($P < 0.001$). No dietary effects were observed on hidrocarbons and sulphur compounds.

3.2 Meat and subcutaneous fat color, cooking losses, shear force, texture profile analysis, and trained sensory panel evaluation

No significant effect of dietary treatments was observed for cooking loss and WBSF. The majority of texture profile parameters were similar across treatments. However, beef from steers fed PAST and SUPP had more cohesive texture when compared to GRAIN-fed beef ($P = 0.004$) (Table 4). Diets did not affect lean color, juiciness, subjective tenderness, and beef flavor intensity. Subcutaneous fat was significant yellower on rib sections from carcasses from steers fed SUPP and PAST when compared to GRAIN, whereas GRAIN-fed beef showed higher off-flavor intensity when compared to PAST-fed ($P = 0.037$).

4. Discussion

Feeding grain increases the availability of net energy and glucose for fat synthesis and further deposition of lipid in the muscle (Scollan et al., 2006). Due to higher fat content, moisture values for GRAIN-finished beef were the lowest when compared to PAST samples. Similar results were showed by Fruet et al. (2016) and Moran et al. (2017). Regarding ash, Acheson et al. (2015) reported that ash values increase as USDA quality grade increases. The USDA quality grading system is basically based on lean color, maturity, and marbling of the

ribeye, whereas better grades are correlated with higher marbling scores. In our study higher ash values were observed in GRAIN samples, which also had higher values of total lipids.

In this study, dietary treatments altered the fatty acid profile beef. Daley et al. (2010) reported that grass-fed beef has higher concentration of C18:0 when compared to grain-fed. Lower concentrations of C18:0 in GRAIN when compared to PAST-finished beef were also observed in our study. The increase of C18:0 in beef finished on PAST is possibly associated to high concentrations of C18:3n3 (linolenic) in the PAST diet. Previous research showed that linolenic acid is rapidly hydrogenated in the rumen producing C18:0 (Ward, Scott, & Dawson, 1964). Although values of C17:0 were the lowest in beef from SUPP diets, overall concentrations of saturated fatty acids including C14:0 and C16:0 were similar in beef from all treatments.

In this study, higher proportions of C18:1*trans* were observed for GRAIN-finished when compared to beef finished on SUPP and PAST. The C18:1*trans* fatty acids included C18:1*trans*-9, C18:1*trans*-10, and C18:1*trans*-11 (*trans*-vaccenic acid). Possibly, feeding GRAIN affected the biohydrogenation pathway, shifting *trans*-vaccenic acid production to C18:1*trans*-10 in the rumen (Buccioni, Decandia, Minieri, Molle, & Cabiddu, 2012; Chilliard et al., 2007) and leading to higher deposition of C18:1*trans* fatty acids in the lean. Therefore, higher concentrations of C18:1*trans* in GRAIN-finished samples are due to increased levels of C18:1*trans*-10 in the lean. This hypothesis agrees with Duckett, Neel, Lewis, Fontenot, & Clapham (2013) findings, who showed similar effects on fatty acids profile of steers finished with concentrate or forage diets. From a health standpoint, *trans*-vaccenic acid is beneficial for human metabolism (Salter, 2013; Scollan et al., 2014). However, Roy et al. (2007) demonstrated that C18:1*trans*-10 leads to detrimental effects on plasma lipid and lipoprotein metabolism in rabbits.

294 Besides providing higher levels of C18:3n3 (Table 1), feeding forage also increases
295 rumen transit time. Contrastingly, due to the smaller particle size, GRAIN diets lead to
296 shorter rumen transit time and consequently limited microbial biohydrogenation (Wood et
297 al., 2008). This increased the deposition of 18:2n6 in the lean of GRAIN-finished samples
298 whereas beef finished on SUPP and PAST showed similar values for this fatty acid. Higher
299 concentrations of C18:3n3 in PAST diets were also responsible for higher proportions of
300 C18:2cis-9 trans-11 (CLA) in the lean when compared to GRAIN-finished beef. Although
301 C18:2cis-9 trans-11 is a conjugated fatty acid of linoleic acid, Palmquist, St-Pierre, & McClure
302 (2004) showed that higher offer of C18:3n3 promotes endogenous synthesis of C18:2cis-9
303 trans-11. Duynisveld, Charmley, and Mir (2006) reported lower contents of this fatty acid in
304 pasture-fed beef when compared to pasture supplemented with soybeans. This suggests
305 that different concentrate sources may lead to different fatty acid deposition in the lean.
306 Grass and clover contain high proportions of C18:3n3, which is the building block of the n-3
307 series of essential fatty acids (Dewhurst, Shingfield, Lee, & Scollan, 2006). In our study,
308 increasing forage proportion in dietary treatments (SUPP and PAST, respectively) gradually
309 increased levels of C20:5n3 and C22:5n3 in the lean. However, no distinct pattern was
310 observed for 22:6n3 across treatments. The highest concentration of this fatty acid was
311 detected in SUPP-finished samples.
312 As discussed before, PAST diet was the richest in C18:3n3 and directly influenced the
313 highest deposition of this fatty acid in beef (Duckett, Neel, Lewis, Fontenot, & Clapham,
314 2013; Fruet et al., 2016; Nuernberg, Fischer, Nuernberg, Ender, & Dannenberger, 2008).
315 Although the magnitude of C18:3n3 deposition in the lean is limited by the greater
316 biohydrogenation of this fatty acid during the long rumen transit (Wood et al., 2008),
317 finishing steers on PAST increased C18:3n3 in the lean at significant levels to decrease n-6/n3

318 ratio. Increased concentrations of C18:3n3 in the PAST diet can also be related to lower
319 values of total lipids since this fatty acid decreases stearoyl co-A desaturase (SCD) expression
320 and consequently inhibits adipogenesis in ruminant tissues (Turner et al., 2015).
321 Altering fatty acid composition of meat directly affects formation of volatile
322 compounds during cooking (Elmore et al., 2005; Mottram, 1998). Higher levels of C18:2n6 in
323 beef from GRAIN-finished steers led to increased formation of 1-pentanol, 1-hexanol, and 1-
324 octen-3-ol. Similar results were reported by Elmore et al. (2005) when evaluating volatiles of
325 lamb. Formation of aldehydes, furans, and some organic acids such as the hexanoic acid is
326 positively correlated with fatty acids autoxidation and negatively correlated with high
327 concentrations of α-tocopherol in the lean and lower lipid concentrations (Mottram, 1998;
328 Stetzer, Cadwallader, Singh, Mckeith, & Brewer, 2008; Vasta et al., 2011). In this study, high
329 concentration of α-tocopherol in diets containing legume and grass led to lower formation of
330 2-pentylfuran in beef from PAST and SUPP treatments, whereas higher lipid content of
331 GRAIN-finished samples may be associated with increased oxidation during cooking and
332 consequently higher values of Hexanal, 5-Methyl hexanal, and Octanal. However, a gradual
333 increase in formation of hydrophilic compounds including 2-methylpropanal and 2-butanone
334 was observed as roughage levels increased in the diets. The formation of 2-methylpropanal
335 is more associated with the Strecker degradation of amino acid precursors (valine) rather
336 than lipid oxidation (Frank, Kaczmarska, Paterson, Piyasiri, & Warner, 2017). Similar trend
337 was observed for ethylacetate. Overall, finishing steers on legume-grass pastures with or
338 without supplementation decreased the formation of volatiles associated with oxidation.
339 Although our results (Table 1) showed that α-tocopherol concentrations were numerically
340 similar in SUPP and GRAIN diets, the lower formation of hexanal, 5-methyl hexanal, and
341 octanal in SUPP when compared to GRAIN-finished beef is due to the α-tocopherol source.

342 Most of α-tocopherol in the SUPP treatment was natural, whereas the major proportion of
343 α-tocopherol used in the GRAIN diet was derived from synthetic sources. This is in
344 agreement with Descalzo et al. (2005), who demonstrated that pasture diets provide better
345 oxidative lipid stability in beef rather than grain-based diets containing levels up to 500
346 IU/head/day of synthetic vitamin E supplementation.

347 Dietary treatments did not affect cooking loss, WBSF, and lean objective color of
348 fresh LT. However, as expected, subcutaneous fat was yellower in beef from steers fed
349 roughage-based diets. Similar effects on pigmentation of subcutaneous adipose tissue were
350 reported by others when finishing beef with forage diets (Duckett, Neel, Lewis, Fontenot, &
351 Clapham, 2013; Dunne, O'Mara, Monahan, & Moloney, 2006) and this may be correlated
352 with carotenoid content in adipose tissue (Röhrle et al., 2011). In this study, carotenoid
353 content was 2-fold and 4-fold higher in PAST when compared with SUPP and GRAIN dietary
354 treatments, respectively. However, no differences in yellowness values (b*) were observed
355 when comparing subcutaneous fat objective color of PAST and SUPP-finished beef.
356 Therefore, including WCG as supplementation in grazing-finishing systems does not change
357 yellowness of external fat when compared to finishing diets based on roughage only. Similar
358 results were reported by Röhrle et al. (2011), who demonstrated that changes in yellowness
359 in subcutaneous fat are evident when feeding grain or roughage, but not evident when
360 finishing regimes are based on pasture and pasture plus supplementation with grain.
361 Although yellower fat is perceived by industry personnel and consumers to be undesirable
362 (Dunne, Monahan, O`Mara, & Moloney, 2009), results of this study suggest that higher b*
363 values in subcutaneous fat of steers are associated with better n6/n3 ratio and increased
364 antioxidant content in external fat and lean.

Regarding texture profile, beef from steers finished in PAST and SUPP were more cohesive than beef finished on GRAIN. Cohesiveness is calculated by the ratio of chart areas generated during two compression cycles whereas the area under the peak of the second compression is divided by the area under the peak of the first compression. Cohesiveness is directly associated with tensile and compression strength of meat (Ruiz de Huidobro, Miguel, Blázquez, & Onega, 2005). Values for this parameter were higher for beef finished on pasture, however, no detrimental effects on WBSF and subjective tenderness were observed. Hardness, cohesiveness and chewiness are negatively correlated with overall tenderness parameters (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003). In our study, although increased values of cohesiveness may be associated with pasture-finishing, this minimal variation in texture was not correlated with detrimental effects on objective and subjective tenderness.

Dietary treatment did impact off-flavor intensity. GRAIN-finished samples showed higher scores of off-flavor than PAST-finished. As discussed before, GRAIN-samples showed higher concentrations of volatiles associated with lipid oxidation, which may have contributed to higher off-flavor intensity scores given by panelists who participated in this study. Differently, Resconi, Campo, Font i Furnols, Montossi, and Sañudo (2010) reported no differences in rancid, liver, and fat flavors in beef from steers finished on pasture and with concentrate, whereas Duckett, Neel, Lewis, Fontenot, & Clapham (2013) reported that panelists used in their study reported higher off-flavor intensity in forage-finished beef when compared to concentrate-finished. Possibly, the different results from those studies are related to the acceptability of forage-finished and concentrate-finished beef by panelists from different countries (Realini et al., 2009). Whereas U.S. consumers prefer grain-finished flavor (Duckett, Neel, Lewis, Fontenot, & Clapham, 2013), European consumers tend to

prefer beef finished on pasture or with lower levels of concentrate (Realini et al., 2009). In Brazil, consumers are accustomed to consuming beef from cattle finished on pasture. Therefore, it is possible that higher off-flavor intensity scores of GRAIN-finished samples are associated with Brazilian beef cattle production systems, which are based on pasture feeding (Freitas et al., 2014).

5. Conclusions

Finishing steers on pasture of oats, ryegrass, and clover with or without WCG supplementation increased the deposition of desirable n3 and CLA fatty acids in the lean, which are desirable from a health standpoint. The formation of volatiles from beef finished on legume-grass pastures with or without WCG supplementation were similar, but differed when compared to volatiles formed in beef from steers finished with WCG only. Although color parameters of LT muscle did not differ across treatments, yellowness intensity was greatest in subcutaneous fat from steers finished on legume-grass pasture with or without WCG supplementation. Panelists scored higher off-flavor intensity on beef samples from GRAIN-finished steers. Although removing roughage of finishing diets did not alter cooking loss, WBSF, and color parameters, feeding only grain negatively affected flavor perception when compared to feeding grain plus roughage and roughage only. Overall, beef from steers finished on legume-grass pasture is very similar to beef of steers finished on pasture with 1.4% of BW of WCG supplementation. However, finishing steers with no levels of roughage may compromise flavor and consequently consumer acceptability.

Declaration of interest

The authors declare no conflict of interest associated with this research.

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618 Table 1. Chemical composition of dietary treatments offered to steers finished on three
619 different feeding regimes.

Nutrients	Finishing system		
	GRAIN[1]	SUPP[2]	PAST[3]
Crude protein[4]	14.9	15.9	20.9
Ether extract	2.3	2.5	2.5
Neutral detergent fiber corrected for ash	16.8	30.9	49.5
Ash	3.6	7.2	12.5
Non-fiber carbohydrate	62.2	43.5	14.4
Metabolizable energy[5]	2.89	2.75	2.50
α-tocopherol[6]	2.16	2.76	4.18
Carotenoids	0.62	1.89	3.18
16:0[7]	15.06	16.49	19.31
18:0	3.86	3.83	4.34
18:1n9	29.29	17.96	7.29
18:2n6	43.82	17.96	7.29
18:3n3	2.20	27.22	52.27

620 [1]85% whole corn grain + 15% protein-vitamin-mineral pellet supplement.
621 [2]Pasture and concentrate allowance (50:50 ratio).
622 [3]Pasture only.
623 [4]Crude protein, ether extract, neutral detergent fiber corrected for ash, ash, and non-fiber
624 carbohydrate, all values expressed on a dry matter basis (%).
625 [5]All values expressed in Mcal/kg; ME = TDN (g/kg DM) × 4.4 × 0.82 (NRC, 2000).
626 [6]α-tocopherol and carotenoids expressed in mg/100g.
627 [7]All fatty acids expressed as % of FAME.
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Table 2. Proximate composition and fatty acid profile of beef from steers finished on GRAIN, SUPP, and PAST.

Characteristics	Finishing system				
	GRAIN	SUPP	PAST	*P*-value	SEM
Moisture[1]	75.5[b]	76.12[ab]	77.46[a]	<0.001	0.37
Crude protein[1]	21.25	21.22	21.21	0.990	0.23
Total lipids[1]	2.91[a]	1.89[ab]	1.14[b]	0.002	0.29
Ash[1]	1.03[a]	1.00[ab]	0.99[b]	0.024	0.01
Fatty acids[2]					
14:0	2.75	2.73	2.22	0.348	0.16
16:0	23.42	25.02	23.76	0.434	0.49
17:0	2.28[a]	0.90[c]	1.28[b]	<0.001	0.15
18:0	13.44[b]	16.12[ab]	19.97[a]	0.003	0.92
18:1*trans*[3]	5.16[a]	1.65[b]	2.48[b]	<0.001	0.44
18:1n9	35.12	36.81	33.83	0.376	0.82
18:1n7	1.63[a]	1.31[ab]	1.12[b]	0.002	0.07
18:2*trans*-9 *trans*-12	0.44[a]	0.30[ab]	0.21[b]	0.025	0.03
18:2*cis*-9 *trans*-11	0.21[b]	0.33[a]	0.41[a]	<0.001	0.02
18:2n6	5.67[a]	3.71[b]	4.22[b]	0.021	0.31
18:3n3	0.22[c]	0.90[b]	1.59[a]	<0.001	0.15
20:4n6	1.22	1.71	1.63	0.318	0.13
20:5n3	0.17[b]	0.53[ab]	0.66[a]	0.007	0.07
22:5n3	0.40[b]	0.99[ab]	1.21[a]	0.007	0.12
22:6n3	0.09[b]	0.24[a]	0.10[b]	<0.001	0.02
SFA	42.98	45.45	48.11	0.071	0.96
MUFA	47.72[a]	44.52[ab]	41.07[b]	0.002	0.92
PUFA	9.29	10.02	10.82	0.589	0.59
PUFA/SFA	0.21	0.22	0.23	0.955	0.01
n-6	8.15	6.98	6.79	0.366	0.41
n-3	0.92[b]	2.44[a]	3.60[a]	<0.001	0.35
n-6/n-3	8.86[a]	2.65[b]	1.91[b]	<0.001	0.81

[ab]Means followed by different letters in the same row are significant at the $P < 0.05$.
[1]g/100g.
[2]g/100g FAME.
[3]Sum of C18:1*trans* (C18:1*trans* 9, C18:1*trans*10, C18:1*trans*11).

666 Table 3. Volatile compounds profile of *longissimus thoracis* muscle from steers finished
667 on GRAIN, SUPP, and PAST.

		Finishing system				
Compounds[1]	LRI[2]	GRAIN	SUPP	PAST	*P*-value	SEM
Organic acids						
Acetic acid	1452	25.57	16.70	15.48	0.161	2.35
Butanoic acid	1575	7.82	7.82	3.44	0.290	1.12
Pentanoic acid	1674	4.09	3.91	2.69	0.457	0.48
Hexanoic acid	1787	31.92^{a}	32.48^{a}	10.15^{b}	0.024	4.09
Alcohols						
Ethanol	945	23.93	34.38	18.98	0.468	4.98
1-Penten-3-ol	1157	2.16	1.04	1.29	0.347	0.32
1-Pentanol	1248	14.83^{a}	5.96^{b}	6.44^{b}	<0.001	1.20
1-Hexanol	1354	2.60^{a}	1.26^{b}	1.10^{b}	0.008	0.24
1-Octen-3-ol	1446	7.07^{a}	1.37^{b}	1.66^{b}	<0.001	0.81
Aldehydes						
Acetaldehyde	718	112.40	102.82	126.26	0.308	6.09
2-Methyl propanal	819	59.39^{b}	64.05^{ab}	72.85^{a}	0.013	2.08
Hexanal	1071	492.78^{a}	134.14^{b}	144.92^{b}	0.001	54.55
5-Methyl hexanal	1175	38.67^{a}	24.69^{b}	24.10^{b}	0.005	2.37
Octanal	1284	13.14^{a}	3.98^{b}	4.55^{b}	<0.001	1.33
Nonanal	1400	17.85	13.10	17.30	0.466	1.64
Benzaldehyde	1520	255.52	233.76	248.95	0.766	11.71
Hidrocarbons						
Hexane	600	38.42	57.74	51.53	0.415	5.43
Octane	800	35.92	35.27	34.23	0.954	2.13
Styrene	1255	12.11	7.30	9.69	0.846	3.17
Ester						
Ethylacetate	906	58.80^{b}	76.16^{ab}	117.97^{a}	0.037	10.21
Ketones						
2-Propanone	829	130.64	170.87	108.24	0.701	28.90
2-Butanone	913	280.72^{b}	360.70^{ab}	438.57^{a}	0.007	23.14
2,3-Butanedione	995	174.32	157.95	218.45	0.540	21.86
2,3-Pentanedione	1058	0.24	0.37	0.33	0.582	0.05
2-Butanone, 3-hydroxy	1289	160.13	186.53	125.25	0.350	16.75
Heterocyclic compounds						
2-Pentyl furan	1213	12.17^{a}	1.50^{b}	1.93^{b}	<0.001	1.53
Pyrrole	1497	2.22	2.61	3.14	0.741	0.45
Dihydro-2-methyl,3-furanone	1598	4.67	4.73	4.00	0.862	0.57
Sulphur compounds						
Methanethiol	693	38.75	48.70	58.92	0.195	4.51
Dimethyl sulfide	754	64.89	60.75	64.28	0.514	1.51
Dimethyl disulfide	1060	85.82	72.96	83.50	0.851	9.19
Dimethyl trisulfide	1364	15.73	18.99	20.49	0.219	1.12
Terpenes						
Limonene	1168	1.15	2.18	0.73	0.143	0.31

[ab]Means followed by different letters in the same row are significant at the $P < 0.05$.
[1]Values are expressed in arbitrary area units (peak area) $x10^5$
[2]LRI: linear retention index.

714 Table 4. Cooking loss, WBSF, texture profile, color parameters, and sensory attributes of
715 *longissimus thoracis* muscle from steers finished on GRAIN, SUPP, and PAST.

Attributes	Finishing system				
	GRAIN	SUPP	PAST	*P*-value	SEM
Cooking loss (%)	30.33	31.10	32.08	0.311	0.78
Warner-Bratzler shear force (N)	46.17	47.33	47.97	0.798	1.05
Hardness (N)	313.23	354.86	370.65	0.101	11.47
Cohesiveness	0.44[b]	0.49[a]	0.51[a]	0.004	0.01
Adhesiveness (N.cm)	-0.08	-0.07	-0.08	0.994	0.02
Springiness	1.27	1.15	1.19	0.378	0.03
Chewiness (N)	178.97	203.22	223.07	0.287	11.17
Lean lightness (L*)	40.43	41.32	41.03	0.701	0.41
Lean redness (a*)	22.66	22.93	23.11	0.625	0.17
Lean yellowness (b*)	17.23	17.54	17.55	0.826	0.22
Subcutaneous fat lightness (L*)	72.19	70.41	71.15	0.408	0.52
Subcutaneous fat redness (a*)	9.62	9.47	9.66	0.943	0.22
Subcutaneous fat yellowness index (b*)	15.71[b]	19.91[a]	19.46[a]	<0.001	0.53
Juiciness[1]	4.81	4.24	4.75	0.051	0.12
Initial tenderness[1]	4.80	4.31	4.93	0.068	0.14
Overall tenderness[1]	4.48	4.39	4.87	0.135	0.14
Beef flavor intensity[1]	3.96	4.33	4.15	0.509	0.16
Off-flavor intensity[2]	0.48[a]	0.21[ab]	0.12[b]	0.037	0.06

716 [ab]Means followed by different letters in the same row are significant at the $P < 0.05$.
717 [1]7-point scale: 1 = extremely dry, tough, and bland to 7 = extremely juicy tender and intense.
718 [2]8-point scale: 0 = none, 1 = extremely slight off-flavor to 8 = extremely intense off-flavor.

4.3 MANUSCRITO 3

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Oxidative stability of beef from steers finished exclusively with concentrate, supplemented, or on legume-grass pasture

A.P.B. Fruet[ab*], A. De Mello[b], F. Trombetta[a], F.S. Stefanello[a], C.S. Speroni[a], D.P. De Vargas[c], A.N.M. De Souza[d], A.G. Rosado Júnior[d], C.J. Tonetto[d], J.L. Nörnberg[a]

[a]Department of Science and Food Technology, Federal University of Santa Maria, Santa Maria, 1000 Roraima Av., Santa Maria, RS 97105900, Brazil.
[b]Department of Agriculture, Nutrition, and Veterinary Sciences, University of Nevada, Reno, 1664 N. Virginia St. mail stop 202, Reno, NV 89557, United States.
[c]West University of Santa Catarina, 211 Oiapoc St., São Miguel do Oeste, SC 89900000, Brazil.
[d]Farroupilha Federal Institute, São Vicente do Sul, s/n 20 de Setembro St., São Vicente do Sul, RS 97420000, Brazil.

*Corresponding author: Ana Paula Burin Fruet, Email: ap_burin@hotmail.com. Phone: +55 55 99756760;

Abstract

The objective of this study was to evaluate the effects of three finishing systems based on concentrate and legume-grass pasture on beef. Steers were finished for 91 days with an exclusively whole corn grain-based (GRAIN) diet, grazed on legume-grass pasture plus 1.4% of body weight of whole corn grain supplementation (SUPP), or grazed on legume-grass pasture (PAST) only. Lipid and myoglobin oxidation, pH, objective color, and α-tocopherol concentrations were evaluated on M. *longissimus thoracis* steaks. Dietary treatments did not affect pH and minimally affected protein carbonylation. Steaks from steers fed GRAIN were less red, showed higher lipid oxidation during retail display, and higher metmyoglobin formation from day 7 to 13 when compared to PAST. Levels of α-tocopherol were higher in steaks from steers fed diets containing legume and grass. Inclusion of roughage in finishing diets is essential to maintain retail color and prevent lipid and myoglobin oxidation.

Keywords: Whole corn grain; Legume-grass pasture; Lipid oxidation; α-tocopherol.

1.Introduction

Oxidation in meats leads to detrimental effects on quality attributes including flavor, color, and nutritional values (Petron et al., 2007). Conversion of oxymyoglobin (OMb) to metmyoglobin (MMb) leads to meat discoloration, which negatively affects product acceptability by consumers. Lipid oxidation directly affects product quality and nutritional values due to generation of volatile compounds associated with undesirable flavors and lower availability of desirable fatty acids, respectively (Cheng, 2016). Protein oxidation leads to formation of carbonyls and protein cross-links decreasing solubility, meat tenderness, myofibrillar swelling, and juiciness (Lund, Heinonen, Baron, & Estévez, 2011).

Oxidation can be induced by potential initiators like lipid-derived reactive oxygen species and byproducts of oxidation of OMb (Estévez, 2011; Lund, Heinonen, Baron, & Estévez, 2011), whereas radicals formed from the oxidation of lipids and protein can further accelerate oxidation in a reciprocal manner (Faustmam, Sun, Mancini, & Suman, 2010).

Although oxidative stability of meats depends on several factors, the balance between antioxidant and pro-oxidant compounds in muscle seems to play the most important role in determining oxidation patterns (Descalzo & Sancho, 2008; Ponnampalam et al., 2017). Antioxidants found in muscle usually include endogenous enzymes and non-enzymatic compounds such as vitamin E (Santé-Lhoutellier, Engel, Aubry, & Gatellier, 2008) whereas transition metals, specifically iron, is considered a strong pro-oxidant specie (Papuc, Goran, Predescu, & Nicorescu, 2017).

Dietary treatments may directly affect the balance between antioxidant and pro-oxidant components in muscle (Descalzo & Sancho, 2008; Luciano et al., 2011; Ponnampalam, Butler, McDonagh, Jacobs, & Hopkins, 2012). Extensive feeding systems based on pasture generally promote higher deposition of natural antioxidants in the lean

when compared to concentrate-based diets. This leads to superior oxidative stability of pasture-fed when compared to concentrate-fed beef. (Luciano et al., 2012; Ponnampalam, Butler, McDonagh, Jacobs, & Hopkins, 2012; Wood et al., 2008).

Previous studies showed that pasture is a natural source of antioxidants and preserved roughage, even after being dried or ensiled, still have significant concentration of those compounds (Eriksson & Pickova, 2007; Lindqvist, Nadeau, & Jensen, 2011). Stefanello et al. (2018) reported that silage has higher levels of antioxidants when compared to grains. Possibly, adding silage into grain-based finishing diets may minimize lipid oxidation of beef and lead to similar oxidation stability when compared to forage-finished beef. This is in agreement with previous research conducted by Luciano et al. (2011). Therefore, in order to precisely understand the effects of feeding grain-based in contrast to forage-based diets on oxidative stability of beef and vitamin E deposition in the lean, trials involving feeding exclusive grain-based diets without roughage levels are needed. To our knowledge, there is no previous research that analyzed such effects. Fruet et al. (2016), and Luciano et al. (2012) reported significant differences in lipid oxidation of lamb while evaluating effects of exclusive concentrate-based diets versus forage-based. However effects on vitamin E concentration in the lean and protein oxidation were not evaluated. In this study, we evaluated lipid and protein oxidation, myoglobin stability, and α-tocopherol concentration of beef from steers finished with an exclusively corn-based diet (without any roughage source), on grass-legume pasture plus supplementation of 1.4% of body weight of whole corn grain, and on legume-grass pasture.

2. Materials and methods

2.1 Animals, finishing diets and sample collection

105 For this study, animal handling and welfare procedures approved by the Farroupilha
106 Federal Institute Ethics Committee on the Use of Animals (protocol 003/2015). British and
107 Zebu cross steers (n = 18, initial body weight [BW] 333 ± 27.87 kg), were randomly allocated
108 to three finishing treatments (n=6 per treatment) including grazing on pasture of black oats
109 (*Avena strigosa*), ryegrass (*Lolium multiflorum*), white clover (*Trifolium repens*) and red
110 clover (*Trifolium pretense*) (PAST); grazing on legume-grass pasture plus supplementation
111 with 1.4% of BW of WCG (SUPP); and housed in feedlot pens and fed exclusively with 2.8%
112 BW of whole corn grain (85% of WCG plus 15% of a protein-vitamin-mineral pellet
113 supplement, GRAIN). For dietary treatment GRAIN, the removal of all roughage sources was
114 possible due to the higher particle size of WCG and the addition of 150 mg of ionophore
115 (present in the pellet supplement). Both factors contributed to avoid possible acidosis on
116 steers. Composition of dietary treatments is shown in Table 1. Steers were backgrounded on
117 natural pasture consisting of grasses including *Paspalum notatum*, *P. dilatatum* and
118 *Coelorachis selloana*, *Stipa setigera, S. hyalina*, *Piptochaetium bicolor* and *P. stipoides* as well
119 as legumes including *Trifolium polymorphum* and *Adesmia bicolor.*
120 Steers assigned to grazing treatments were randomly assigned into 12 paddocks
121 measuring 0.6 and 0.4 ha for PAST and SUPP, respectively 6 steers per treatment, one steer
122 per paddock). Steers allocated to treatment GRAIN were confined in a feedlot-covered
123 facility, in individual pens of 13.5 m^2 (n=6). Prior to the experiment, steers were acclimatized
124 to facilities and respective diets during a period of 21 days. Subsequently, steers were fed
125 for 91 days and slaughtered at the Farroupilha Federal Institute abattoir. The carcass weight
126 (kg) of steers fed GRAIN, SUPP, and PAST was 274.1, 279.2, and 257.4, respectively.
127 Carcasses were chilled at 4°C ± 1 and at 24 h *postmortem*, the *longissimus thoracis* (LT)
128 muscle was excised from right carcass sides. Six ribeye steaks of 2.54 cm were fabricated

129 from each LT. Five steaks were packaged in trays overwrapped with O_2 permeable film, kept
130 in refrigerated dark storage at 3°C ± 1, and analyzed at 5 different days for muscle pH, color,
131 lipid and protein oxidation stability. The remaining steak was used for α-tocopherol analysis.
132
133 *2.2 Muscle pH, color stability, lipid oxidation, and protein carbonyl content analysis*
134 Muscle pH, color, lipid, and carbonyl content were measured at day 1 (48 h post
135 mortem) and at days 4, 7, 10, and 13 of storage. The pH values were measured with an
136 electrode probe attached to a pH meter (Testo205, BR), which was calibrated daily prior the
137 analysis by using buffer solutions at pH 7 and 4. For pH analysis 10 g of minced sample was
138 combined with 90 mL of deionized water and homogenized for 30s.
139 Objective color was recorded for L* (lightness; black = 0, white= 100), a* (redness;
140 red = positive values; green= negative values) and b* (yellowness; yellow = positive values;
141 blue = negative values) using a Minolta chromameter (CM-700D; Minolta Inc., Osaka, Japan)
142 with an 8mm-diameter measurement, A illuminant, and a 10° standard observer.
143 Metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) concentrations
144 were calculated by selected wavelengths (AMSA, 2012) as follows:
145
146 Equation 1 (convert the reflectance (R) to reflex attenuance (A)): A = log 1/R;
147 Equation 2: %MMb = {1.395 - [(A572 - A730) / (A525 - A730)]} x 100;
148 Equation 3: %DMb = {2.375 x [1- (A473 - A730) / (A525 - A730)]} x 100; and
149 Equation 4: %OMb = 100 - (%MMb + DMb).
150
151 Lipid oxidation was measured by quantifying thiobarbituric acid reactive substances
152 (TBARS) using the method described by Raharjo, Sofos, and Schmidt (1992). Results were

153 expressed in mg of malonaldehyde (MDA) per 1000 g of meat. Total protein was quantified
154 by following the methodology used by Lowry, Rosebrough, Farr, and Randall (1951).
155 Carbonyl content was measured according to Levine et al. (1990) and results were
156 expressed as nanomoles of 2,4-dinitrophenylhydrazine (DNPH) fixed per milligram of
157 protein.
158
159 *2.3 α-Tocopherol*
160 The α-tocopherol extraction was performed based on the methodology described by
161 Prates, Quaresma, Bessa, Fontes, and Alfaia (2006). Briefly, meat samples were ground,
162 saponified with potassium hydroxide and ethanol, and extracted with a BHT solution in n-
163 hexane. After vortexing, the n-hexane upper layer later was evaporated, suspended in the
164 mobile phase, and transferred into a screw cap vial. Alpha-tocopherol concentration in the
165 muscle was estimated by using a HPLC system (LC-20A Prominence, Shimadzu, Japan)
166 equipped with a quaternary pump, vacuum solvent delivery degasser, and a fluorescence
167 detector (excitation wavelength of 295 nm and emission wavelength of 325 nm). A reverse-
168 phase column (Zorbax C18 – with the corresponding 250 mm x 4.6 mm e 5µm particle size)
169 was used. Acetonitrile, methanol, and methyl tert-butyl ether (65:20:15, v/v) were used as
170 isocratic mobile phase at 1mL/min for 39 min. Total α-tocopherol values were calculated in
171 duplicate for each sample using the standard curve of peak area vs. concentration.
172
173 *2.4 Statistical analysis*
174 Data were analyzed using the SAS® 9.3 package (SAS Institute, Inc., USA) as a
175 completely randomized design. The GLM procedure was used to test the effect of finishing
176 diets on concentration of α-tocopherol in the lean. The MIXED procedure was used to

177 evaluate oxidative stability and pH over the 13 days of refrigerated storage. Fixed effects of
178 finishing diets included GRAIN, SUPP, and PAST, whereas storage days included day 1, 4, 7,
179 10, and 13. Interaction of both effects were also evaluated whereas animal was considered
180 a random effect nested within dietary treatments. In addition, the CORR procedure was
181 used to evaluate correlations among lipid oxidation (LIP), protein carbonyl content (CARB),
182 myoglobin forms (MMb, OMb, and DMb), and α-tocopherol concentration (TOC). Fixed
183 effects and interactions were assessed using the analysis of variance at 5% significance level
184 and predicted means were compared using the Tukey's test.
185
186 **3. Results**
187 *3.1 Color stability and muscle pH*
188 Objective color and pH values are presented in Table 2 and Table 3. No significant
189 effects of finishing diet, storage day, or interaction between fixed effects were observed for
190 L* values. Interaction between fixed effects were observed for a*. As expected, redness
191 decreased along the storage period. However, steaks from steers fed PAST and SUPP were
192 significantly redder than beef from steers fed GRAIN on days 10 and 13 of storage. On day
193 13, PAST steaks had the highest redness values when compared to steaks from other dietary
194 treatments. Individual effects of diet and storage day were observed for b*. Yellowness
195 decreased as storage days increased. Steaks from steers fed PAST were significant yellower
196 when compared to GRAIN steaks. Additionally, there was an effect of storage day on pH,
197 values, which ranged from 5.4 and 5.9.
198 For all myoglobin forms (Table 2), effects of interaction between fixed effects were
199 statistically significant. Beef from steers fed diets with roughage (PAST and SUPP) had
200 greater levels of OMb on days 4 and 10 of storage whereas on day 13, steaks from steers fed

GRAIN had the lowest values. Formation of DMb increased in steaks from steers fed PAST and SUPP along the storage period. On day 13, beef from steers fed PAST and SUPP showed greater levels of DMb when compared to steaks from steers fed GRAIN. Formation of MMb was similar in steaks from all dietary treatments from day 1 to day 4 of storage. Beef from steers fed PAST had the lowest MMb formation on days 7, 10 and 13 when compared to beef from steers fed GRAIN whereas SUPP had lower MMb than GRAIN steaks on day 13.

3.2 Lipid and protein oxidation and muscle α-tocopherol concentration

Significant interactions between finishing diet and storage day were observed for lipid oxidation (TBARS) and formation of carbonyl content (Table 2). Lipid oxidation was significantly higher in steaks from steers fed GRAIN during the whole storage period when compared to steaks from steers finished on PAST and SUPP. Although a significant interaction effect was observed for carbonyl content, values within treatments differed only on day 10 of storage where steaks from GRAIN and SUPP treatments showed greater protein oxidation when compared to steaks from steers fed PAST. Finishing steers with diets containing roughage (PAST and SUPP) significantly increased concentrations of α-tocopherol in the lean when compared to GRAIN diets (Fig. 1). Steaks from steers fed GRAIN had the lowest concentrations of α-tocopherol (2.80, 5.18, and 5.87 μg/g muscle for GRAIN, SUPP, and PAST, respectively, $P < 0.001$).

3.3 Correlation between myoglobin forms, protein carbonyl content, lipid oxidation, and α-tocopherol concentration

Correlations between lipid oxidation, myoglobin forms, protein carbonyl content, and α-tocopherol concentration are shown in Table 4. On day 1, protein carbonyl content

was positively correlated to DMb values (ρ = 0.622) whereas OMb was negatively correlated to MMb values (ρ = -0.878). Lipid oxidation on day 1 was positively correlated to lipid oxidation (ρ = 0.649) and MMb values (ρ = 0.536) from day 13. Lipid oxidation on day 1 was negatively correlated to OMb values (ρ = -0.500) from day 13 and TOC concentration in the lean (ρ = -0.681). On day 13, negative correlations between values of lipid oxidation and OMb (ρ = -0.761), lipid oxidation and DMb (ρ = -0.717), and lipid oxidation and TOC (ρ = -0.778) were observed. Lipid oxidation and MMb values were positively correlated (ρ = 0.852). Oxymyoglobin formation was negatively correlated to MMb (ρ = -0.978) and positively correlated to TOC (ρ = 0.798), whereas MMb values observed on day 13 were negatively correlated to TOC (ρ = -0.824).

4. Discussion

Feeding diets containing roughage significantly improved color and oxidative stability of beef. In this study, cold storage was performed by simulating household practices (Masson, Delarue, & Blumenthal, 2017) as described on item 2.1. After purchasing, consumers usually store uncooked meats in home and freezer refrigerators (Godwin & Coppings, 2005) and heavily rely on meat color as an indicator of product freshness (Suman & Joseph, 2013). Redness is the most important color parameter for fresh beef (Olivera, Bambicha, Laporte, & Cárdenas, 2013) and is inversely correlated to higher concentrations of MMb. In our study, steaks from steers fed PAST and SUPP had improved color stability based on a* values, which were significant higher at the end of the storage period when compared to steaks from steers fed GRAIN (Table 2). Feeding GRAIN negatively affected beef redness on day 13 since a* values were lower (11.36) than values considered to be acceptable by consumers (14.5) (Holman, Van de Ven, Mao, Coombs, & Hopkins, 2017). As

249 levels of forage increased, b* values gradually increased. This is possibly due to greater
250 amount of β-carotene usually found in pasture when compared to concentrates (French et
251 al., 2001).

252 On day 4, formation of DMb was significantly higher in GRAIN steaks when compared
253 to steaks from other treatments. Increase of DMb in beef during storage is associated to
254 muscle reduction capacity of MMb, low oxygen tension, and mitochondrial-mediated
255 conversion of OMb to DMb (Mancini & Hunt, 2005; Ramanathan, Mancini, Joseph, &
256 Suman, 2013). The significant decrease in OMb and increase of DMb in steaks from steers
257 fed GRAIN when compared to PAST may be associated to mitochondrial activity, since no
258 differences in MMb formation was observed in steaks from all treatments during the first 4
259 days of storage. In this study, we did not observe higher MMb formation in GRAIN steaks as
260 levels of MDA increased during the first 4 days (Table 2). Metmyoglobin levels significantly
261 increased only on day 7 when GRAIN and SUPP had higher MMb formation than PAST
262 steaks. This demonstrated that pro-oxidative activity caused by lipid peroxidation
263 byproducts on MMb formation is delayed and occurs later after generation of those
264 byproducts.

265 After day 4, DMb levels in GRAIN steaks gradually decreased whereas the opposite
266 was observed in steaks fed PAST and SUPP. Commonly, OMb is not directly converted to
267 DMb. First, OMb is oxidized to MMb and the formation of DMb depends on the muscle
268 reducing capacity (Mancini & Hunt, 2005). Steaks from steers fed roughage (PAST and SUPP)
269 showed higher levels of DMb on day 13. Possibly, during storage, the ability of reducing
270 MMb to DMb in beef fed PAST and SUPP was superior when compared to beef fed GRAIN.
271 In addition, lower cascade effects from lipid peroxidation byproducts may also have

contributed to lower MMb levels on day 10 and 13 since beef from steers fed PAST and SUPP showed less lipid oxidation.

Lipid oxidation was greater in GRAIN-fed beef when compared to PAST and SUPP-fed throughout the storage period. Beef from steers finished on both treatments based on legume-grass pasture showed higher levels of α-tocopherol in the lean when compared to GRAIN. Roughages contain higher concentrations of natural antioxidants (such as α-tocopherol), when compared to grains. In this study, we observed levels 1.5 times higher of α-tocopherol in beef from steers fed legume-grass when compared to corn. Although α-tocopherol levels in SUPP and PAST diets were different (2.16, 2.76, and 4.18 μg/g DM for GRAIN, SUPP, and PAST), a similar deposition of α-tocopherol in the lean was observed in beef from steers fed both legume-grass based diets (5.87 and 5.18 μg/g muscle for PAST and SUPP respectively). These results are due to better absorption of natural vitamin E sources when compared to synthetic (Descalzo et al., 2005; Ponnampalam et al., 2017) and limitations of maximum absorption and deposition of α-tocopherol in ruminants due to lower intestinal absorption or saturation of plasma lipoproteins, which are carriers of α-tocopherol (Yang, Brewster, Lanari, & Tume, 2002).

Our results have also shown that even levels of α-tocopherol found in beef from steers fed GRAIN (2.8 μg/g muscle) were higher than levels reported by other authors who fed animals with diets containing a mix of grain and roughage sources (Bellés et al., 2018; Descalzo et al., 2005; Luciano et al., 2011; Ponnampalam et al., 2017). Possibly, previous exposure of steers assigned to GRAIN treatment to pasture before entering the experiment may have contributed to higher levels of α-tocopherol in beef from our study when compared to others. Previous research reported that α-tocopherol concentration in Brazilian beef was the highest when compared to beef from Austria, England, France,

296 Germany, United States, and Ireland (Röhrle et al., 2011). This suggests that feedstuffs used
297 in those countries may have lower levels of α-tocopherol, which justifies the significant
298 amount of work done to improve lipid oxidative stability by using supra-nutritional
299 supplementation of vitamin E in grain-based diets (Ponnampalam et al., 2017; Yang,
300 Brewster, Lanari, & Tume, 2002).

301 In order to avoid quality deterioration by minimizing lipid oxidation in meat,
302 desirable levels of muscle α-tocopherol must be above 3.5 μg/g (Ponnampalam et al., 2014).
303 In our study, levels of α-tocopherol in SUPP and PAST-fed beef were significant higher than
304 the value proposed by those authors. However, concentration in GRAIN-fed beef was
305 slightly below the threshold level. This led to higher lipid oxidation in GRAIN-fed beef when
306 compared to SUPP and PAST-fed. On day 13, higher lipid oxidation was observed on steaks
307 from steers fed GRAIN, which could possibly lead to detrimental effects on flavor and
308 decrease acceptability by consumers. Previous research conducted by Campo et al. (2006)
309 suggests that 2 mg MDA/kg muscle may lead to negative effects on sensory attributes. In
310 this study, higher levels of α-tocopherol found in the lean of beef from steers fed PAST and
311 SUPP when compared to GRAIN, plus storage methodology (dark storage), possibly
312 minimized oxidative processes. Therefore, diets without roughage inclusion may lower
313 oxidative stability in beef when compared to diets containing forage.

314 Protein oxidation can be induced by potential initiators like lipid-derived reactive
315 oxygen species and oxidation of OMb (Estévez, 2011; Lund, Heinonen, Baron, & Estévez,
316 2011). Therefore, based on our lipid oxidation results, we expected to observe a similar
317 oxidation pattern of proteins. However, dietary treatments led to minimal effects on
318 carbonyl content of steaks and values presented in this study were lower than values

319 presented by Santé-Lhoutellier, Engel, Aubry, and Gatellier (2008) and Gravador et al.
320 (2015).

321 Correlations analysis showed that CARB was positively correlated to DMb on day 1.
322 Deoxymyoglobin formation may be associated to the initial loss of myoglobin stability due
323 to mitochondrial oxygen metabolism and ability of reducing MMb (Ramanathan, Mancini,
324 Joseph, & Suman 2013). Also, on day 13, negative correlations between DMb and lipid
325 oxidation (ρ = -0.717) and between OMb and lipid oxidation (ρ = -0.761) demonstrated that
326 as expected, higher levels of MMb are formed during storage (ρ = 0.852). In addition, MMb
327 was negatively correlated to TOC (ρ = 0.778) suggesting that antioxidant properties of
328 tocopherol improved myoglobin and lipid stability (Faustman, Sun, Mancini, & Suman,
329 2010). Lipid oxidation was the only variable on the day 1 positively correlated to lipid
330 oxidation and MMb on day 13, and negatively correlated to OMb on day 13. These results
331 suggest that lipid oxidation on day 1 may be used as a prediction factor for further
332 oxidation. Results observed in this research are in agreement with oxidation trends
333 demonstrated by Insausti, Beriain, Lizaso, Carr, and Purroy (2008).
334

335 **5. Conclusion**

336 Beef from steers finished on legume-grass pasture, independently of what level was
337 offered, showed lower lipid and myoglobin oxidation than beef from steers finished with an
338 exclusive whole corn grain-based diet. Beef from steers finished in legume-grass pasture,
339 with or without of supplementation, showed similar α-tocopherol concentrations when
340 compared to beef from steers finished with exclusive concentrate. Results of this
341 experiment demonstrated that including high quality roughage such as combinations of
342 legume and grass in finishing diets is essential to improve oxidative stability of beef.

343 Moreover, final beef quality is associated with oxidative stability and is directly related to
344 different α-tocopherol sources used in finishing diets. Most of available research does not
345 approach the understanding of specific effects of feeding sources containing natural
346 antioxidants. Studies related to the utilization of pasture, inclusion of straw, hay, or silage in
347 finishing diets, as well as, α-tocopherol deposition in the lean must be conducted to
348 understand how the utilization of roughage, fresh or conserved, may improve beef quality.
349
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357
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Table 1. Chemical composition of the feed offered of steers finished in feedlot with exclusively concentrate (GRAIN), legume-grass pasture with (SUPP) or without supplementation (PAST)

Nutrients (% DM)	Finishing system		
	GRAIN[1]	SUPP[2]	PAST
Crude protein	14.9	15.9	20.9
Ether extract	2.3	2.5	2.5
Neutral detergent fibre corrected for ash	16.8	30.9	49.5
Ash	3.6	7.2	12.5
Non-fibre carbohydrate	62.2	43.5	14.4
Metabolizable energy (Mcal/kg)	2.89	2.75	2.50
α-tocopherol (mg/100g of DM)	2.16	2.76	4.18

[1]Feedlot with 85% whole corn grain + 15% protein-vitamin-mineral pellet supplement.
[2]Pasture and concentrate allowance (50:50 ratio).

Table 2. Effect of finishing fed and storage days on color parameters, TBARS value, and carbonyl protein of M. *longissimus thoracis* storage over 13 d at 4 °C of steers finished with exclusively concentrate (GRAIN), legume-grass pasture with (SUPP) or without supplementation (PAST)

	Storage days						
	D1	D4	D7	D10	D13	*P* value	SEM
L*							
GRAIN	40.43	42.17	40.65	41.23	42.24	0.488	0.21
SUPP	41.24	42.11	42.84	41.97	42.92		
PAST	40.94	42.46	42.67	42.62	41.79		
a*							
GRAIN	22.66^{a}	19.21^{b}	19.17^{b}	16.33^{Bc}	11.36^{Cd}	<0.001	0.12
SUPP	22.90^{a}	19.85^{b}	18.83^{bc}	18.17^{Ac}	15.61^{Bd}		
PAST	22.95^{a}	20.18^{b}	19.42^{b}	18.90^{Abc}	17.83^{Ac}		
Oxymyoglobin (%, OMb)							
GRAIN	69.54^{a}	61.66^{Bb}	60.68^{b}	52.59^{Bc}	37.53^{Cd}	<0.001	0.44
SUPP	72.41^{a}	65.31^{ABb}	61.37^{c}	58.15^{Ac}	50.47^{Bd}		
PAST	73.68^{a}	67.07^{Ab}	65.19^{b}	59.35^{Ac}	56.28^{Ac}		
Deoxymyoglobin (%, DMb)							
GRAIN	6.43^{Ad}	9.07^{Aa}	8.81^{Aab}	8.62^{bc}	7.11^{Bc}	0.001	0.17
SUPP	3.99^{Bd}	5.57^{Bc}	6.39^{Bbc}	7.57^{b}	9.16^{Aa}		
PAST	5.74^{Abc}	6.64^{Bbc}	7.89^{ABb}	8.24^{ab}	9.20^{Aa}		
Metmyoglobin (%, MMb)							
GRAIN	24.02^{d}	29.26^{c}	30.50^{Ab}	38.78^{Ab}	55.34^{Aa}	<0.001	0.41
SUPP	23.60^{d}	29.11^{c}	32.23^{Ab}	34.26^{ABb}	40.36^{Ba}		
PAST	20.57^{c}	26.28^{b}	26.90^{Bb}	32.37^{Ba}	34.57^{Ba}		
TBARS (mg of MDA/kg)							
GRAIN	0.29^{Ad}	0.92^{Ac}	0.98^{Abc}	1.28^{Ab}	1.89^{Aa}	<0.001	0.03
SUPP	0.14^{Bb}	0.39^{Ba}	0.44^{Ba}	0.46^{Ba}	0.61^{Ba}		
PAST	0.16^{Bb}	0.26^{Bab}	0.43^{Bab}	0.43^{Bab}	0.59^{Ba}		
Carbonyl protein (nmol/mg protein)							
GRAIN	1.41^{a}	1.40^{a}	1.14^{b}	1.57^{Aa}	1.44^{a}	0.001	0.01
SUPP	1.41^{a}	1.56^{a}	1.04^{b}	1.42^{Aa}	1.54^{a}		
PAST	1.46^{a}	1.45^{a}	1.08^{b}	1.12^{Bb}	1.52^{a}		

$^{A, B, C}$ Predicted means within column with different superscripts are different at $P < 0.05$.
^{a,b,c,d} Predicted means within line with different superscripts are different at $P < 0.05$.

Table 3. Effect of finishing diet or storage days on b* and pH of M. *longissimus thoracis* storage over 13 d at 4 °C of steers finished with exclusively concentrate (GRAIN), legume-grass pasture with (SUPP) or without supplementation (PAST)

	Storage days						Finishing diet				
	D1	D4	D7	D10	D13	*P* value	GRAIN	SUPP	PAST	*P* value	SEM
b*	17.37^{a}	16.06^{b}	15.86^{bc}	15.38^{c}	14.52^{d}	<0.001	15.41^{B}	15.95^{AB}	16.15^{A}	0.051	0.08
pH	5.60^{d}	5.82^{b}	5.89^{a}	5.75^{c}	5.75^{c}	<0.001	5.77	5.75	5.77	0.218	0.01

$^{A, B, C}$ Predicted means within finishing diet with different superscripts are different at *P*<0.05.

^{a,b,c,d} Predicted means within storage days with different superscripts are different at *P*<0.05.

Table 4. Correlation coefficients (ρ) and *P-values* for relationships among values of protein carbonyl content (CARB), lipid oxidation (LIP), oxymyoglobin (OMb), deoxymyoglobin (DMb), and metmyoglobin (MMb) on days 1 (D1) and 13 (D13) of storage, and α-tocopherol concentrations (TOC)

	*Corr/Sign.	CARB D1	OMb D1	DMb D1	MMb D1	LIP D13	CARB D13	OMb D13	DMb D13	MMb D13	TOC
LIP D1	ρ	-0.013	-0.230	0.434	0.012	0.649	-0.243	-0.500	-0.366	0.536	-0.681
	P-value	0.956	0.356	0.071	0.960	0.004	0.329	0.040	0.147	0.026	0.001
CARB D1	ρ	-	-0.099	0.622	-0.206	-0.121	-0.057	0.156	-0.112	-0.118	0.187
	P-value		0.693	0.005	0.411	0.642	0.819	0.549	0.6685	0.650	0.456
OMb D1	ρ	-	-	-0.177	-0.878	-0.443	0.332	0.397	0.331	-0.435	0.404
	P-value			0.480	<0.001	0.074	0.177	0.114	0.193	0.080	0.095
DMb D1	ρ	-	-	-	-0.313	0.322	0.116	-0.323	-0.224	0.344	-0.278
	P-value				0.205	0.207	0.6646	0.205	0.386	0.176	0.263
MMb D1	ρ	-	-	-	-	0.284	-0.044	-0.228	-0.217	0.256	-0.255
	P-value					0.267	0.859	0.377	0.401	0.320	0.305
LIP D13	ρ	-	-	-	-	-	-0.145	-0.761	-0.717	0.852	-0.778
	P-value						0.577	<0.001	0.001	<0.001	<0.001
CARB D13	ρ	-	-	-	-	-	-	-0.033	0.165	-0.005	0.210
	P-value							0.899	0.526	0.984	0.402
OMb D13	ρ	-	-	-	-	-	-	-	0.298	-0.978	0.798
	P-value								0.245	<0.001	<0.001
DMb D13	ρ	-	-	-	-	-	-	-	-	-0.489	0.437
	P-value									0.0464	0.079
MMb D13	*P*	-	-	-	-	-	-	-	-	-	-0.824
	P-value										<0.001

*Corr. (ρ = Pearson's correlation coefficient); Sign. (*P*-value, significant at $P < 0.05$).

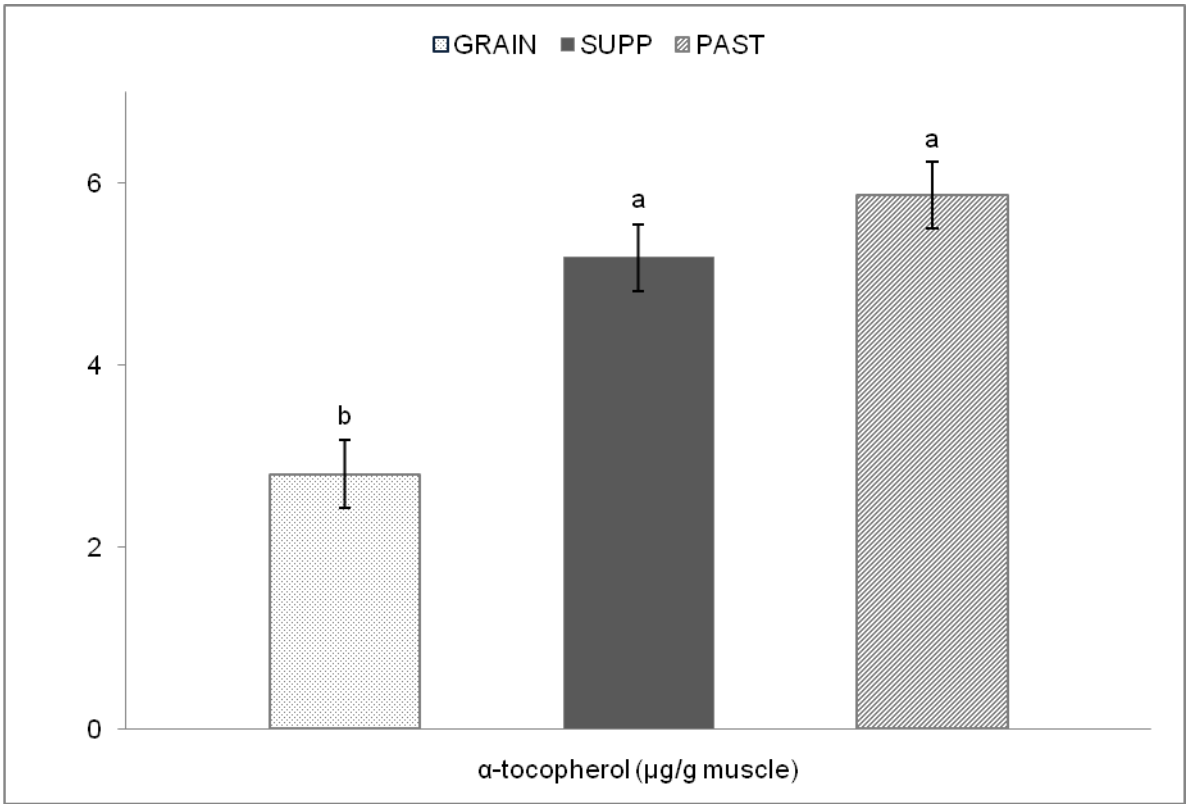

Fig. 1. Muscle α-tocopherol concentration (*P*< 0.001, SEM = 0.37) from steers finished with concentrate only (GRAIN), legume-grass mixture pasture with (SUPP) or without supplementation (PAST).

[a,b] Means within dietary treatments with different superscripts are different.

5. CONSIDERAÇÕES FINAIS

O estudo conduzido demonstrou que dieta consorciada com aveia, azevém, trevo branco e vermelho apresenta adequado requerimento nutricional para terminação de novilhos. A inclusão de concentrado, na forma de grão de milho inteiro, eleva o ganho de peso e deposição de gordura na carcaça e na carne. Não houve diferença no peso de carcaça quente entre novilhos terminados com exclusivo concentrado e suplementação energética em pastagem consorciada. Animais terminados em pastagem consorciada com ou sem suplementação apresentaram perfil lipídico favorável à saúde humana, houve significativa redução da relação n-6:n-3 e aumento da deposição de CLA e n-3. Ainda, a carne de animais terminados em pastagem consorciada apresentou padrão de formação de compostos voláteis diferente da dieta com exclusivo concentrado o que pode ter contribuído para o aumento do escore de off-flavor identificado pelo painel sensorial nas carnes de novilhos terminados com concentrado. A indicação de off-flavor na carne de novilhos terminados em confinamento alto grão sugeri que consumidores do Rio Grande do Sul podem rejeitar tal produto. Independentemente da adição de concentrado, a carne de novilhos terminados em pastagem consorciada apresentou elevado nível de α-tocoferol, menor oxidação lipídica e da mioglobina quando comparado à dieta alto grão.

O sistema de terminação com exclusivo concentrado contribui para incrementar ganho de peso animal e acabamento de carcaças. No entanto, a terminação em pastagem com alto teor nutritivo, composta por gramíneas e leguminosas, confere peso de carcaça quente e deposição de gordura subcutâneo de acordo com o padrão desejável pela indústria brasileira. O sistema de terminação usando 1,4% do PC de grão inteiro de milho em pastagem consorciada contribuiu para aumento de ganho de peso individual e por hectare, assim como, produz carcaça com similar peso à terminação alto grão em confinamento. A carne produzida no sistema de suplementação em pastagem se assemelha às características atribuídas ao produto de animais terminados exclusivamente com pastagem sugerindo que há maior equilíbrio entre desempenho produtivo e qualidade da carne no sistema de terminação quando utilizado a suplementação. Assim, a tomada de decisão por um sistema de terminação dependerá da disponibilidade de área para implementação de pastagem e/ou funcionários, valor da dieta e da necessidade por incorporar características específicas no produto final.

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VITA

Ana Paula Burin Fruet, filha de Anadelci Burin Fruet e Luiz Ernesto dos Santos Fruet, nascida em 11 de dezembro de 1989, em Júlio de Castilhos – RS. Estudou na Escola Estadual Alberto Pasqualini (Júlio de Castilhos – RS) até concluir o ensino fundamental e na Escola Estadual Vicente Dutra (Júlio de Castilhos – RS) onde concluiu o ensino médio em 2006. Em 2008 ingressou no curso de graduação em Medicina Veterinária da Universidade Federal de Santa Maria (Santa Maria - RS). Formou-se médica veterinária em janeiro de 2013. Neste mesmo ano, ingressou no Mestrado do Programa de Pós-graduação em Ciência e Tecnologia de Alimentos da Universidade Federal de Santa Maria, com dedicação exclusiva como bolsista CAPES, sob orientação do Prof. Dr. José Laerte Nörnberg. Em junho de 2013 foi selecionada no Programa Especial de Graduação de Formação de Professores para Educação Profissional da Universidade Federal de Santa Maria, o qual foi concluído em dezembro de 2014. Ainda, no segundo semestre de 2014 ingressou ao doutorado do Programa de Pós-graduação em Ciência e Tecnologia de Alimentos da Universidade Federal de Santa Maria sob orientação do Prof. Dr. José Laerte Nörnberg, como bolsista CAPES. Realizou o experimento de campo do doutorado no Instituto Federal Farroupilha, campus São Vicente do Sul, sob coorientação do Prof. Dr. Alexandre Nunes Motta de Souza. Foi aprovada no Programa de Doutorado Sanduíche no Exterior (PDSE/CAPES), edital n°019 de 2016, e realizou doutorado sanduíche na University of Nevada, Reno, EUA, sob coorientação do Prof. Dr. Amilton de Mello de abril a novembro de 2017. Em novembro de 2017 foi nomeada ao cargo de Fiscal Estadual Agropecuário, da Secretaria da Agricultura, Pecuária e Irrigação do Estado do Rio Grande do Sul, mediante aprovação em concurso público, onde permanece atualmente. Foi submetida à banca de defesa de tese em agosto de 2018.