UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

SISTEMA DE TERMINAÇÃO DE BOVINOS E OVINOS SOBRE A QUALIDADE E AVALIAÇÃO SENSORIAL PELOS CONSUMIDORES DA CARNE

Autor: Carlos Emanuel Eiras
Orientador: Ivanor Nunes do Prado

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"Tese apresentada como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós Graduação em Zootecnia da Universidade Estadual de Maringá – Área de Concentração Produção Animal"

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Dados Internacionais de Catalogação na Publicação (CIP)
(Biblioteca Central - UEM, Maringá, PR, Brasil)

Eiras, Carlos Emanuel
E35s Sistema de terminaçao de bovinos e ovinos sobre a qualidade e avaliação sensorial pelos consumidores da carne / Carlos Emanuel Eiras. -- Maringá, 2016.
xv, 109 f. : il., figs., tabs.

Orientador: Prof. Dr. Ivanor Nunes do Prado.
Tese (doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia, 2016.

1. Casca de algodão - Alimentação animal. 2. Carne - Cor instrumental. 3. Carne - Escatol. 4. Carne bovina - Qualidade. 5. Carne ovina - Qualidade. I. Prado, Ivanor Nunes do, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.085
AMMA-003355

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TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADA em 05 de agosto de 2016.

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“*Veni, vidi, vici*”

Júlio César, 47 a.C

AGRADECIMENTOS

Ao Programa de Pós Graduação em Zootecnia da Universidade Estadual de Maringá, por todas as oportunidades proporcionadas e pelo direcionamento da minha formação acadêmica.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pelo financiamento e concessão das bolsas de estudos no Brasil e no exterior.

Ao *Institut National de la Recherche Agronomique* e seus funcionários por fazerem da minha estada produtiva e prazerosa.

Aos "mestres" que se dedicam a causa e por meio de seus esforços puderam direcionar meus estudos e me permitiram superar meus limites.

Ao professor Ivanor Nunes do Prado, que por meio da amizade construída incentivou o meu crescimento pessoal, além dos inúmeros ensinamentos profissionais. Ao Senhor, meus sinceros agradecimentos pela sua dedicação, ensinamentos em âmbito profissional e pessoal, paciência, amizade e pelos momentos de descontração.

À Sophie Prache, pelos seus ensinamentos, paciência e dedicação à minha formação.

Ao José Carlos da Silva, pilar fundamental para a realização deste estudo. Obrigado pelo seu apoio, amizade e dedicação.

Ao Agnelo Delfino Pinheiro e José Luis Soriani Filho pela colaboração incondicional para a construção deste estudo.

Aos colegas de trabalho por respeitarem minhas limitações e participarem do meu crescimento profissional e pessoal. Obrigado a todos pela oportunidade de ter aprendido diariamente com o nosso convívio.

Aos amigos conquistados durante estes anos que se doaram integralmente, cada qual ao seu modo, a fim de incentivar o meu desenvolvimento pessoal.

A minha grande incentivadora, minha amada mãe, pelo seu esforço e amor incondicional a cada nascer de um novo dia.

A minha companheira de todas às horas Daiane Novais Eiras, pela sua incansável dedicação, paciência e carinho.

BIOGRAFIA

Carlos Emanuel Eiras, filho de João Francisco Almeida Eiras e Lilian Marochi, nasceu no município de Irati (Paraná, Brasil) em 13 de janeiro de 1987.

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Em março de 2013, ingressou no curso de doutorado do Programa de Pós Graduação em Zootecnia da Universidade Estadual de Maringá, concentrando seus estudos na área de produção de ruminantes com ênfase em bovinos de corte. Durante o período entre maio de 2015 e abril de 2016 realizou estágio acadêmico em nível de doutorado no *Institut National de la Recherche Agronomique* (Theix, Auvergne, França).

Em julho de 2016 submeteu-se ao exame geral de qualificação do Programa de Pós Graduação em Zootecnia da Universidade Estadual de Maringá e, em agosto, submeteu-se à defesa da tese.

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RESUMO

O trabalho com ovinos foi realizado para avaliar a concentração de indol, escatol e a cor instrumental do tecido adiposo perirenal e subcutâneo de animais mantidos em três sistemas produtivos, assim como, a avaliação sensorial da carne e a cor do músculo *Longissimus thoracis et lumborum* (Ll) a partir de 24 horas até oito dias após o abate. Foram utilizados 28 cordeiros não castrados da raça Romane distribuídos em três tratamentos: animais mantidos em pastagem de alfafa (A), em confinamento alimentados com ração comercial e feno (S) e animais terminados durante 21 dias em confinamento a partir de um período em pastagem de alfafa (AS). O desempenho dos ovinos mantidos em confinamento foi controlado a fim de manter a mesma velocidade de crescimento entre os tratamentos. Os ovinos foram abatidos com 48,8 kg, 270 cm^3 de volume testicular e 3,56 mm de tecido adiposo subcutâneo. O período de terminação em confinamento reduziu a concentração de indol e escatol no tecido adiposo dos ovinos. Os ovinos terminados em confinamento por 21 dias apresentaram menor intensidade de vermelho e maior luminosidade no tecido adiposo subcutâneo. A luminosidade do músculo Ll foi maior para os ovinos mantidos em confinamento (S); entretanto, os ovinos dos tratamentos A e AS apresentaram maior intensidade de vermelho. Durante os três primeiros dias de exposição da carne, o músculo Ll apresentou maior intensidade de vermelho. A intensidade de amarelo foi alterada somente a partir do sexto dia de exposição. A luminosidade do músculo Ll aumentou a partir do primeiro até o sexto dia de exposição. A maciez e a suculência da carne foram maiores para os ovinos mantidos em pastagem. O período de terminação em confinamento reduziu a presença de odor anormal na carne dos ovinos. A aceitação geral da carne dos ovinos mantidos em confinamento foi menor que a dos animais alimentados em pastagem. Um breve período de terminação em confinamento reduz a concentração de indol e escatol no tecido adiposo de cordeiros e apresenta efeito sobre a avaliação sensorial da carne. No experimento realizado com bovinos o objetivo foi avaliar proporções de casca de algodão em dietas de alto teor de concentrado, tempos de maturação e exposição do

músculo Ll sobre a composição física da carcaça e química da carne, atividade de água, maciez, cor instrumental, oxidação lipídica, atributos visuais e qualidade sensorial da carne, assim como, os hábitos de consumo e perfil dos consumidores. Foram utilizados 30 bovinos cruzados (½ Simental x ½ Nelore) não castrados com peso inicial de 319 ± 12,5 kg. Os tratamentos contendo diferentes proporções de casca de algodão na dieta foram distribuídos às unidades experimentais e estas foram alocadas em baias individuais durante 162 dias, sendo: CH21: 210 g/kg de casca de algodão na MS da dieta, CH27: 270 g/kg de casca de algodão na MS da dieta e CH33: 330 g/kg de casca de algodão na MS da dieta. Após o abate dos bovinos, seções de 2,5 cm do músculo Ll foram embaladas à vácuo, congelados ou mantidos sob refrigeração de 2ºC e ausência de luz por três, sete e 14 dias. Ao mesmo tempo, uma seção do músculo Ll foi mantida sob refrigeração em geladeira frigorífica (4ºC) durante 10 dias para a avaliação visual da carne pelos consumidores. O teor de cinzas, lipídeos totais e perdas por cocção do Ll foi influenciado pela proporção de casca de algodão na dieta. A adição de 330 g/kg de casca de algodão na dieta dos bovinos aumentou o teor de cinzas no Ll, enquanto que reduziu o teor de lipídeos totais e as perdas durante a cocção. A luminosidade e a intensidade de amarelo do Ll foram maiores para os bovinos alimentados com teor intermediário de casca de algodão na dieta. O menor teor de casca de algodão na dieta propiciou menores índices de oxidação lipídica no Ll. A proporção de casca de algodão na dieta não influenciou a força de cisalhamento do Ll. Entretanto, a maturação do Ll durante sete e 14 dias melhorou a maciez da carne. A adição de 330 g/kg de casca de algodão na dieta dos bovinos propiciou melhor aceitação visual da carne pelos consumidores a partir do segundo até o sexto dia de exposição. A aceitação visual e a intenção de compra pelos consumidores reduziram em função do tempo de exposição da carne. A proporção de casca de algodão na dieta não apresentou efeito sobre o flavour, maciez e aceitabilidade geral da carne. A maciez da carne aumentou durante o tempo de maturação (14 dias). Os consumidores de carne avaliados neste estudo habitualmente consomem carne bovina cinco vezes por semana, adquirida fresca em supermercado e consideram a cor como o principal atributo durante a compra do produto. Além disso, apresentam preferência por carne de novilhas terminadas em pastagens. A utilização de até 330 g/kg de casca de algodão na dieta dos bovinos não prejudica a qualidade instrumental e melhora a aceitabilidade visual da carne.

Palavras chave: Casca de algodão, cor instrumental, escatol, maciez

ABSTRACT

The lambs study was aimed to determine the indole and skatole concentrations and the colour of perirenal and subcutaneous fat of lambs raised into three productive systems, as well, the sensory evaluation and the colour of *Longissimus thoracis et lumborum* (Ll) from 24 h to 8 days *post mortem.* Twenty-eighty non-castrated male Romane lambs were assigned into three treatments: A) grazing alfalfa, S) stall-feeding a concentrate-based diet, AS) grazing alfalfa followed by a 21-d concentrate-finishing period in stalls. The concentrate was given at a level that was adjusted to achieve similar growth paths in the three treatments. Lambs were thus slaughtered at mean 48.8 kg, 270 cm^3 of testicular volume and 3.56 mm of subcutaneous fat thickness. The finishing period decreased the indole and skatole concentrations in the fat. The lambs concentrate-fed during 21 days indoors presented lower redness and higher lightness in the subcutaneous fat. Lightness of Ll was higher for stall-fed lambs, however, A and AS lambs presented higher redness. Redness of Ll was higher during the first three days of blooming. Ll yellowness was influenced from sixth day of blooming. Lightness of Ll increased from first to sixth day of blooming. Tenderness and juiciness were higher in the pasture-fed lambs. The finishing period decreased the Ll abnormal odour. Overall acceptability of stall-fed lambs was lower than pasture-fed lambs. The fast-finishing period indoors decrease the indole and skatole concentrations in the lambs' fat and has effect on sensory evaluation. The young bulls study was aimed to evaluate the cottonseed hull level in the high-concentrate diets, aging time and blooming time of Ll on carcass characteristics and chemical composition of meat, water holding capacity, tenderness, instrumental colour, lipid oxidation, visual appraisal and sensory evaluation of the meat, even as, habits and preferences of consumers. Were utilized thirty non-castrated crossbred bulls (½ Simmental vs. ½ Nellore) averaging initial LW 319 $\pm$ 12.5

kg. The cottonseed hull treatments were assigned into the experimental units during 162 days and were allocated in the individual pens, as: CH21: cottonseed hull 210 g kg^{-1} on a DM basis, CH27: cottonseed hull 270 g kg^{-1} on a DM basis, and CH33: cottonseed hull 330 g kg^{-1} on a DM basis. Ll was sectioned into steaks with a 2.5-cm and were individually vacuum packaged, frozen or no-light refrigerated (2ºC) for 3, 7 or 14 days. In the same time, Ll steaks were refrigerated (4ºC) during 10 days to visual appraisal of consumers. Moisture, total lipids and cooking loss was influenced to the cottonseed hull level in the diets. The addition of 330 g kg^{-1} of cottonseed hull in the DM basis increased the Ll moisture and decreased the total lipids and the cooking loss. Lightness and yellowness of Ll were higher to intermediate cottonseed hull level. The lower cottonseed hull level reduced the lipid oxidation of Ll. Shear force was not influenced to the cottonseed hull level. However, the 7 and 14 of aging time increased the tenderness. The addition of 330 g kg^{-1} of cottonseed hull in the DM basis demonstrated better visual appraisal from second to sixth days of blooming by consumers. The visual appraisal and the willingness to buy reduced according blooming time. Flavour, tenderness and overall acceptability were not influenced to the cottonseed hull level in the diets. But, the Ll tenderness increased during the aging time (14 days). The consumers evaluated in this study, usually they consume beef 5 times for week, in fresh cut form, supermarket purchased and the colour was considered as the big one attribute during the meat buy. Besides, they have preferences for meat from heifers finished in pasture. The use of cottonseed hull until 330 g/kg into diet fed by bulls has no prejudicial effect on instrumental quality and improves the visual appraisal of meat.

Keywords: Cottonseed hull, instrumental colour, skatole, tenderness

I - INTRODUÇÃO

A demanda global por produtos de origem animal apresenta implicações sobre a produção mundial de carne e, consequentemente, sobre a eficiência dos sistemas produtivos em países exportadores. A manutenção nos níveis de consumo de carne pela população em países desenvolvidos e a favorável projeção destes índices em países em processo de desenvolvimento esperado para as próximas décadas, indicam uma oportunidade de expansão para a bovinocultura e ovinocultura nos próximos anos (FAO, 2015).

O aumento da renda *per capita* observada em países em processo de desenvolvimento incentivaram uma série mudanças no hábito alimentar da população e permitiu o crescimento exponencial no consumo de carne na última década (FAO, 2015). Enquanto, a estabilidade econômica presente em países desenvolvidos possibilitou a manutenção nos níveis de consumo de carne pela sua população (FAO, 2015). No entanto, as exigências dos consumidores mundiais quanto ao conceito de qualidade de carne caracterizam desafios crescentes a serem atendidos pelos mercados produtores de carne (Blackshaw & Blackshaw, 1994; McAfee et al., 2010).

O conceito de qualidade de carne é subjetivo, multidimensional e dinâmico, sendo influenciado pela classe social, estado econômico e região geográfica de cada mercado consumidor (Ekiz et al., 2012; Zervas & Tsiplakou, 2011). Da mesma forma, a espécie e seus cruzamentos, a idade ao abate e estado de maturação sexual dos animais apresentam efeitos sobre a preferência dos consumidores (Resconi, Escudero, & Campo, 2013; Sink & Caporaso, 1977). Assim, o conceito de qualidade de carne é um fator diretamente relacionado aos hábitos específicos de cada mercado consumidor (Font i Furnols et al., 2011) e o seu consumo é determinado pelo poder aquisitivo de sua população (Ekiz, Yilmaz, Ozcan, & Kocak, 2012).

A busca por fontes alternativas de alimentos e diferentes estratégias de alimentação têm sido adotadas para incrementar os índices produtivos dos sistemas presentes na bovinocultura e ovinocultura mundial e apresentar produtos que atendam às exigências dos mercados consumidores quanto à quantidade e qualidade de produtos ofertados. No entanto, a crescente preocupação com a saúde humana e a segurança alimentar dos produtos são fatores de influência sobre a preferência dos consumidores (Zervas & Tsiplakou, 2011). Assim como, as condições sanitárias e questões relacionadas ao bem estar em que os animais são produzidos e abatidos apresentam-se como critérios de exigência pelos consumidores mundiais.

Neste sentido, os alimentos presentes nas dietas e as estratégias nutricionais utilizadas nos sistemas de produção de ruminantes são fatores decisivos da composição final da carne e estão associados ao conceito de qualidade de carne adotado pelos consumidores (Font i Furnols et al., 2009; Rotta et al., 2009). Entretanto, os atributos sensoriais relacionados à cor, maciez e o flavour da carne são fatores decisivos para a apreciação do produto final pelos consumidores (Resconi et al., 2013; Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). Dessa forma, o equilíbrio entre os índices produtivos destes setores e a qualidade final do produto, com o mínimo efeito dos sistemas de produção sobre o meio ambiente e a redução dos fatores de riscos à saúde humana, têm-se apresentado como um desafio ao mercado mundial da carne.

1.1 Efeitos dos alimentos e estratégias nutricionais sobre a qualidade sensorial da carne de ruminantes

Os ruminantes possuem a capacidade de converter grãos de cereais e forragens em produtos de alto valor biológico utilizados na alimentação humana (Priolo, Micol, & Agabriel, 2001). No entanto, os alimentos presentes nas dietas fornecidas aos animais associam-se a composição nutricional e sensorial do produto final. A composição nutricional das dietas interfere na formação de ácidos graxos voláteis pela microbiota ruminal e apresentam efeito sobre a conformação de carcaça, características físico-químicas e sensoriais da carne (Khan, Jo, & Tariq, 2015).

A utilização de dietas com elevadas concentrações de carboidratos não fibrosos, como as dietas ricas em grãos de cereais, favorece a formação de propionato pelos microrganismos ruminais e uma maior razão acetato: proprionato no rúmen de animais em comparação às pastagens naturais ou fenos (Aurousseau, Bauchart, Calichon, Micol,

& Priolo, 2004; Priolo, Micol, Agabriel, Prache, & Dransfield, 2002). Os ácidos graxos voláteis (AGV) oriundos da fermentação ruminal são aproveitados de diferentes formas pelo metabolismo animal e apresentam influências sobre a composição química e formação de flavour específico na carne de ruminantes (Annison, Lindsay, & Nolan, 2002; Figura 1). Segundo Priolo et al. (2001), o pH da carne é influenciada pela concentração de AGV oriundos da fermentação de alimentos no ambiente ruminal, e apresenta efeito sobre a cor da carne de ruminantes. Da mesma forma, a composição química da carne, a cor e o grau de acabamento em gordura de cobertura das carcaças são afetados pelos alimentos ingeridos e sistemas de produção em que os animais são mantidos (Prache, Bechet, & Theriez, 1990; Ripoll, Albertí, Casasús, & Blanco, 2013).

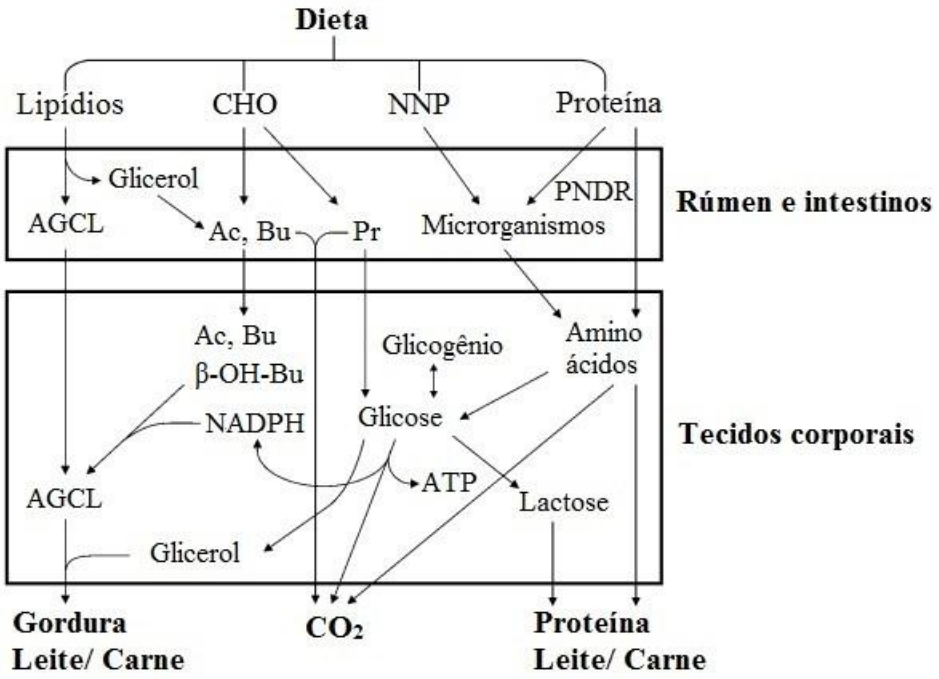

Figura 1. Metabolismo dos componentes presentes nas dietas em produtos finais pelo organismo de ruminantes. Ac: Acetato; AGCL: Ácidos graxos de cadeia longa; ATP: Adenosina trifosfato; Bu: Butirato, β-OH-Bu: Beta-hidoxibutirato; CHO: Carboidratos; CO_2: Dióxido de Carbono; NADPH: Nicotinamida adenina dinucleotideo fosfato; NNP: nitrogênio não proteico; Pr: propionato; PNDR: proteína não degradável no rúmen. Fonte: adaptado de Annison et al. (2002).

A cor da carne é o fator de maior importância no momento da compra pelos consumidores (Calnan, Jacob, Pethick, & Gardner, 2016). No entanto, a ação dos alimentos presente na dieta de ruminantes é considerada de baixa influência sobre este fator, devido à intensa metabolização existente no ambiente ruminal (Priolo et al., 2001). Fatores como o grau de acabamento em gordura de cobertura das carcaças e o pH da carne durante o desenvolvimento do *rigor mortis* são fatores relacionados a cor e a maciez da carne (Calnan et al., 2016). Da mesma forma, a idade do animal, peso da carcaça e a deposição intramuscular de gordura atuam diretamente sobre a cor e maciez final do produto (Brito et al., 2016).

O flavour é um conjunto de impressões sensoriais obtidas pelas papilas gustativas e olfativas durante a degustação da carne, sendo caracterizado pela combinação entre os elementos de "sabor" e "odor" do produto (Khan et al., 2015). Os compostos voláteis são os principais responsáveis pelo flavour existente na carne de ruminantes e apresentam mais de uma origem (Resconi et al., 2013). Fatores transferidos diretamente dos alimentos presentes na dieta, ou ainda, formados a partir de sua metabolização endógena ou pelos microrganismos ruminais são os responsáveis pela formação do flavour presente na carne de bovinos e ovinos (Vasta & Priolo, 2006). Dessa forma, a percepção sensorial obtida pelo consumo da carne de animais alimentados com dietas ricas em grãos de cereais difere do flavour existente na carne de animais produzidos em pastagens (Priolo et al., 2001). Além disso, a existência de compostos voláteis específicos de cada espécie animal apresenta efeitos sobre a preferência dos consumidores (Zervas & Tsiplakou, 2011).

1.2. Qualidade de carne de ovinos criados em pastagens

A carne de animais produzidos em pastagens está associada à melhor concentração de ácidos graxos benéficos à saúde humana quando comparada à carne de animais alimentados com grãos de cereais (Díaz et al., 2002; Padre et al., 2006). Além disso, os sistemas de produção apresentam influência sobre a deposição e coloração do tecido adiposo presente nas carcaças de bovinos e ovinos (Priolo et al., 2002).

A produção de ovinos em pastagens é dependente do valor nutritivo da forragem e sua disponibilidade ao longo do ciclo produtivo (Prado et al., 2002). A consorciação entre gramíneas e leguminosas permite aumentar a ingestão de proteína bruta e

contribui para um maior ganho de peso dos animais, caracterizando o sistema de produção orgânica de carne (Prache, Gatellier, Thomas, Picard, & Bauchart, 2011).

Segundo Inácio, Chalk & Magalhães (2015) a maior fixação de nitrogênio no solo ocasionado por plantas leguminosas reduz a dependência do sistema produtivo ao uso de agentes externos, trazendo benefícios econômicos e incrementando a segurança alimentar dos produtos. No entanto, o alto teor de proteína bruta presentes em plantas leguminosas e a sua extensa metabolização pelo ambiente ruminal são associados à formação de flavour desagradável aos consumidores existente na carne de ovinos (Devincenzi, Prunier, Meteau, Nabinger, & Prache, 2014).

A elevada razão entre proteína/carboidratos não fibrosos presente em pastagens frescas estimula a degradação da proteína pelos microrganismos ruminais, resultando na maior disponibilidade de aminoácidos livres no ambiente ruminal e na menor utilização destes na formação de proteína microbiana devido ao teor insuficiente de energia oriundo do metabolismo dos carboidratos (Schreurs, Lane, Tavendale, Barry, & McNabb, 2008).

A desaminação do aminoácido triptofano eleva a concentração e absorção de 3-metilindol pelo ambiente ruminal (*i. e.* escatol), favorecendo a adesão deste composto no tecido adiposo dos animais (Figura 2). O escatol é um composto volátil associado ao flavour desagradável existente na carne de ovinos, comumente descrito como "sabor de fígado" ou "rançoso" pelos consumidores (Devincenzi et al., 2014). De acordo com Schreurs et al. (2008), o flavour característico da espécie ovina é apreciado por muitos mercados consumidores e não deve ser confundido com "off-flavours" que podem desagradar os consumidores durante a degustação da carne de ovinos. Entretanto, existe uma forte correlação entre a concentração de escatol no tecido adiposo dos animais e o flavour característico presente na espécie ovina.

Young, Berdagué, Viallon, Rousset-Akrim, and Theriez (1997) relataram uma intensificação no flavour da carne de ovinos em função da presença de escatol no tecido adiposo dos animais. No entanto, Devincenzi et al. (2014) observaram a existência de um limiar de detecção na concentração de escatol pelos consumidores, estando entre 0,16 e 0,24 µg de escatol/g de gordura perirenal líquida. A concentração de escatol no tecido adiposo de ovinos está associada à dieta consumida pelos animais, sendo as pastagens formadas por leguminosas as principais responsáveis pelos relatos presentes na literatura (Schreurs et al., 2007a; Schreurs et al., 2007b; Schreurs et al., 2007c).

Em suínos, o escatol é produto da degradação bacteriana do aminoácido triptofano no intestino dos animais, sendo absorvido para a corrente sanguínea e metabolizado no fígado. Portanto, assim como em ovinos, a alimentação apresenta papel importante no controle do escatol na gordura dos animais. No entanto, o maior potencial anabólico de suínos não castrados está associado a um aumento do *turnover* das células intestinais, sendo os resíduos destas células utilizados como fonte de triptofano para a formação de escatol no intestino grosso dos animais. No entanto, esta relação não foi relatada na espécie ovina.

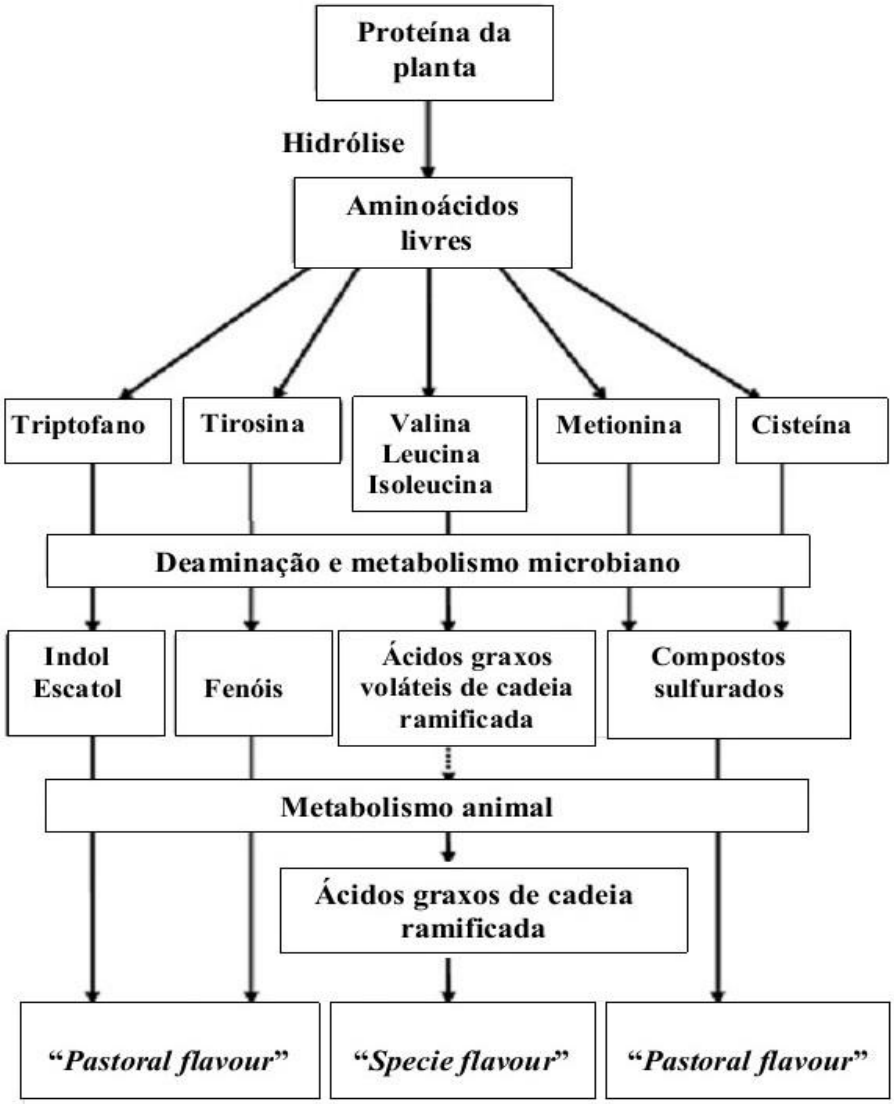

Figura 2. Metabolismo envolvido na formação do flavour presente na carne de ovinos. Fonte: adaptado de Schreurs et al. (2008).

A manipulação dos microrganismos presentes no rúmen por meio do uso de ionóforos ou taninos condensados apresentam-se como estratégias para a redução na

produção ruminal de escatol (Schreurs et al., 2008). Além disso, o aumento na disponibilidade ruminal de carboidratos rapidamente fermentáveis está associado a um melhor aproveitamento dos aminoácidos oriundos da metabolização da proteína pelos microrganismos ruminais.

Devincenzi et al. (2014) observaram a maior concentração de escatol no tecido adiposo perirenal de ovinos alimentados com pastagem de alfafa (25%, 50% e 75% do consumo voluntário de MS) quando comparado com ovinos mantidos em piquetes de gramíneas. Entretanto, a concentração de escatol no tecido adiposo dos animais não diferiu entre os crescentes níveis de ingestão de alfafa.

Os resultados observados pelos autores supracitados não demonstraram efeito da proporção de alfafa ingerida sobre a concentração de escatol no tecido adiposo dos animais. Entretanto, a elevada concentração de proteína bruta presente na alfafa (271 mg/g da MO) pode ter contribuído para formação do aminoácido triptofano no rúmen. Dessa forma, a razão entre proteína e carboidratos não fibrosos na dieta explica a maior concentração de escatol observada no tecido adiposo de ovinos alimentados com níveis de alfafa na dieta.

Além disso, os animais alimentados com nível intermediário de alfafa na dieta (50%) apresentaram maior intensidade de odor desagradável durante a realização da análise sensorial. Os autores atribuíram este efeito a um possível limiar de detecção existente na concentração de escatol durante a avaliação sensorial (0,16 e 0,24 µg de escatol/g de gordura perirenal líquida). Esta relação observada pode ser explicada pela existência de outros compostos voláteis presentes no tecido adiposo dos animais durante o período de engorda dos animais, ou ainda, durante a preparação das amostras (Resconi et al., 2013).

Portanto, o uso de diferentes fontes de forragens na alimentação dos animais trata-se de uma alternativa viável para o controle da formação de escatol no ambiente ruminal de ovinos (Schreurs et al., 2008). No entanto, são limitadas as informações presentes literatura sobre os efeitos do fornecimento de grãos de cereais em forma de suplementação das dietas para animais a pasto, ou ainda, de um curto período de terminação em confinamento sobre a concentração de escatol no tecido adiposo dos animais. Além disso, são escassos os estudos demonstrando a avaliação sensorial da concentração de escatol presente no tecido adiposo de ovinos mantidos em pastagens formadas por leguminosas.

1.3. Dietas de alto teor de concentrado na terminação de bovinos

A crescente demanda mundial tem exigido o uso sustentável dos alimentos, incentivando a busca por ingredientes alternativos a fim de serem utilizados na alimentação animal como forma de evitar a competição com a alimentação humana e reduzir os custos operacionais sem prejudicar o desempenho dos animais (Prado, 2010). No entanto, o mesmo setor exige da cadeia produtiva de carne uma eficiência que permita acompanhar o consumo e a crescente taxa demográfica mundial.

Tradicionalmente, o uso de dietas com elevadas proporções de grãos visa aumentar a taxa de ganho dos animais e a concentração de gordura dos tecidos depositados, devido ao alto nível de energia consumida (Fox, Sniffen, O'Connor, Russell, & Van Soest, 1992). O uso de dietas de alto teor de grãos fornecidas *ad libitum* permite o rápido ganho de peso, a alta eficiência de conversão alimentar e uniformidade no desempenho animais, reduzindo o tempo de terminação para o abate (Woody, Fox, & Black, 1983). Entretanto, além de competir com a alimentação humana, o uso deste sistema de terminação de animais favorece a ingestão de excessivas quantidades de carboidratos prontamente fermentáveis que reduzem o pH ruminal conforme os ácidos graxos voláteis se acumulam, podendo ocasionar problemas de ordem digestiva acompanhados de diminuição no consumo e baixo ganho de peso (Berchielli, Pires, & Oliveira, 2011).

De acordo com Rodrigues et al. (2013), o uso de aditivos em dietas de alto teor de grãos melhora a digestão e a quantidade de nutrientes disponíveis para a absorção pelo trato gastrointestinal de ruminantes, melhorando o desempenho dos animais. O aumento no teor de fibra das dietas também atua no tamponamento do pH ruminal e permite maior equilíbrio na produção de ácidos graxos voláteis pelos microrganismos presentes no rúmen, limitando os efeitos nocivos de uma acidose clínica ou sub-clínica. No entanto, a proporção de volumosos presentes na dieta está relacionada ao teor de energia consumida, sendo associado ao desempenho dos animais e à proporção de tecidos magros ou tecidos gordurosos depositados (Bulle, Ribeiro, Leme, Titto, & Lanna, 2002). Além disso, o uso de volumosos como silagens e fenos elevam o custo de mão de obra e apresentam maior dificuldade de armazenamento nas propriedades.

A busca por alternativas de alimentos de baixo valor comercial e que apresentem características nutricionais que permitam substituir as fontes tradicionais de alimentos na produção de ruminantes têm sido estudadas pela ciência mundial. A grande

diversidade de espécies de plantas, processos de beneficiamento e produtos gerados pela agroindústria brasileira permitiram estabelecer uma variedade de alternativas alimentares a partir dos coprodutos da agroindústria para o setor produtivo da carne. No entanto, a aplicabilidade destas fontes alternativas no sistema produtivo de bovinos não foi totalmente elucidada.

A utilização de dietas secas para animais em confinamento, sem a adição de volumosos frescos ou conservados, apresenta vantagens ao sistema produtivo devido ao fácil armazenamento na propriedade e fornecimento aos animais. Além disso, o baixo índice de deterioração dos alimentos possibilita uma maior segurança e uma vantagem econômica ao decorrer do período de engorda dos animais. No entanto, os estudos avaliando o uso de dietas de alto teor de concentrado a partir de coprodutos da agroindústria sobre a qualidade de carne de bovinos são escassos na literatura.

As fontes de fibra não forragem (FNF) atuam como estratégia de alta viabilidade econômica e nutricional aos sistemas de terminação em confinamento, por se tratarem de coprodutos de menor valor comercial e composição nutricional semelhante aos alimentos tradicionalmente utilizados (Hsu et al., 1987). Além disso, as fontes de FNF se colocam como excelentes alternativas em substituição parcial a alimentos volumosos, principalmente em situações em que existe baixa disponibilidade de forragem devido ao período de secas ou em sistemas de produção em que há limitação de área para produção de alimentos volumosos (Hall & Akinyode, 2000).

A casca de soja e a casca de algodão são consideradas fontes de FNF de excelente utilização pelos microrganismos ruminais, sendo a primeira considerada como um ingrediente volumoso-concentrado e a segunda como um alimento volumoso alternativo para ruminantes (Chizzotti et al., 2005; Hsu et al., 1987).

O termo volumoso-concentrado está associado à função fisiológica da fibra vegetal exercida pelo alimento e sua ação como um grão de cereal em termos de disponibilidade de energia (Swanson, Ko, & Mader, 2007). Portanto, esta pode ser utilizada em substituição parcial à forragem ou mesmo a ingredientes concentrados tradicionais na dieta dos animais. Neste mesmo sentido, a casca de soja auxilia no controle de possíveis impactos negativos ocasionados pela alta ingestão de amido sobre o ambiente ruminal em sistemas de confinamento, como a redução do pH ruminal e inativação de bactérias que degradam a fração fibrosa dos alimentos (Hoover, 1986).

O alto teor de fibra e o baixo valor nutricional presente na casca do algodão permite a sua utilização como alimento volumoso alternativo na dieta de ruminantes

(Chizzotti et al., 2005). O principal componente existente na casca de algodão é a fibra em detergente neutro, assim como, os teores de fibra em detergente ácido e a lignina são elementos marcantes em sua composição.

Normalmente, as frações fibrosas e a lignina são correlacionadas com baixos índices de digestibilidade da matéria seca, reduzindo a ingestão de alimentos pelos animais. De acordo com Berchielli et al. (2011), os baixos índices de digestibilidade presente em alimentos com elevados teores de fibra elevam a distensão física do epitélio ruminal e limitam o espaço disponível dentro do rúmen dos animais, apresentando efeito sobre a ingestão de matéria seca. Entretanto, apesar do seu alto teor em fibra, a casca de algodão apresenta a característica peculiar de não afetar a ingestão de alimentos, devido a sua alta aceitabilidade pelos animais (Hall & Akinyode, 2000).

Os efeitos da terminação em confinamento sobre a qualidade de carne de ruminantes são elucidados pela literatura (Aguayo-Ulloa et al., 2013; Rotta et al., 2009). Da mesma forma, o uso de alimentos alternativos aos tradicionalmente utilizados na terminação dos animais está sendo avaliados a fim de aumentar os recursos disponíveis ao setor produtivo (Eiras et al., 2014a; Marques et al., 2006). Entretanto, a literatura ainda carece de estudos demonstrando o potencial dos coprodutos oriundos da agroindústria em dietas com alto teor de concentrado na terminação de bovinos.

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II - OBJETIVOS GERAIS

- Avaliar a concentração de indol, escatol e a cor instrumental do tecido adiposo de ovinos mantidos em: 1) pastagem de alfafa, 2) em confinamento alimentados com ração comercial e feno e 3) terminados durante 21 dias em confinamento a partir de um período em pastagem de alfafa, e os efeitos destes sistemas produtivos sobre a qualidade sensorial da carne dos animais.

- Avaliar os efeitos de proporções de casca de algodão em dietas de alto teor de concentrado e tempos de maturação e exposição do músculo *Longissimus thoracis et lumborum* de bovinos sobre a composição física da carcaça e química da carne, atividade de água, maciez, oxidação lipídica, atributos visuais e qualidade sensorial da carne, assim como, a intenção de compra, hábitos de consumo e perfil dos consumidores da região norte do Paraná.

- Determinar as alterações presentes na cor instrumental do músculo *Longissimus thoracis et lumborum* de ovinos mantidos em diferentes sistemas produtivos e bovinos alimentados com proporções de casca de algodão em dietas de alto teor de concentrado a partir de 24 horas após o abate dos animais até oito e 14 dias de exposição e maturação da carne, respectivamente.

III - The finishing period indoors reduced the concentration of indole and skatole in the fat of lambs before grazed alfalfa pasture and improved the chop's sensory attributes

Journal: Meat Science

Abstract

Were evaluated the effects of the fast finishing period indoors of lambs before grazed alfalfa pasture on fat indole and skatole concentration and chop sensory attributes. Twenty-eight non-castrated male Romane lambs were assigned into three feeding treatments: (A) lambs pasture-fed alfalfa, (S) lambs stall-fed and (AS) lambs finished indoors with concentrate and straw for 21 days after having grazed alfalfa. The indole and skatole concentration in the fat of lambs was lower after 21 days concentrate-fed indoors. The subcutaneous fat colour was influenced by the finishing period indoors. *Longissimus thoracis et lumborum* (Ll) muscle colour of AS lambs was always closer to A lambs than S lambs. The lambs pasture-fed alfalfa and afterward finished indoors presented lower abnormal odour in the chop and higher flavour liking than S lambs. Overall chop liking of AS lambs were better appreciated that the S lambs during the panels' sessions. The outcome of this study suggests that the panellists' perceptions may be affected to the association between skatole concentrations in the fat and branched-chain fatty acids in the lambs' meat.

Keywords: Blooming, Feeding system, Meat quality, Off-flavour, Stall-fed period

Highlights:

- Fast-finishing indoors does not change the colour parameters of lambs' meat.
- The interaction between skatole and branched-chain fatty acids has influencing on consumers' flavour.

1. Introduction

The sustainable animal production systems have been associated to food healthiness and it has a greater environmental acceptability by consumers in order to use natural resources (Montossi et al., 2013; Ripoll, Albertí & Joy, 2012). The low-input system is based in nitrogen-fixing plants (Devincenzi, Prunier, Meteau, Nabinger & Prache, 2014) and use of organic fertilizers (Inácio, Chalk & Magalhães, 2015) to reduce the dependency on external inputs, such as concentrate feed. This farming livestock system presents a good alternative to indoor lamb production system by has no detrimental effects on live weight at slaughter (Ripoll, Alvarez-Rodriguez, Sanz & Joy, 2014) and decrease the production costs (Zervas & Tsiplakou, 2011). Likewise, the legume-rich pasture has been demonstrated the occurrence of off-flavours in the lamb meat (Devincenzi et al., 2014; Schreurs, Lane, Tavendale, Barry & McNabb, 2008; Schreurs et al., 2007a).

The flavour and colour of lamb meat are influenced by feeding system and their effects are considered to be of greater importance in consumers' judgement of meat quality (Devincenzi et al., 2014; Ripoll et al., 2012). The meat quality has been linked to the oxidative processes of lipids and myoglobin that leads to off-flavour and discolouration of meat (Huang, Andueza, Oliveira, Zawadzki & Prache, 2015; Luciano et al., 2009). Also, the flavour of lamb meat may be affected by microbial deamination and carboxylation of tryptophan from legume-rich pasture to smelling volatile components (Schreurs et al., 2008).

The meat from grazing pasture lambs has higher polyunsaturated fatty acids composition than meat from animals fed concentrate diets (Aurousseau, Bauchart, Calichon, Micol & Priolo, 2004) and this fact can increase the susceptibility of meat oxidative processes (Luciano et al., 2012). However, the dietary antioxidants in the fresh forage may counterbalance the stability of the meat oxidation and reduce their effect on colour and flavour of meat (Carrasco, Panea, Ripoll, Sanz & Joy, 2009). Additionally, the lambs grazing some legume species with high degradable protein content, such as alfalfa (*Medicago sativa*), may reduce the peptides and amino acids incorporation into microbial protein and increase the tryptophan conversion to indole and skatole in the rumen (Deslandes, Gariépy & Houde, 2001; Devincenzi et al., 2014; Schreurs et al., 2008).

The concentration of indole and skatole was described as responsible to the 'animal', 'pastoral' and 'faecal' flavours in the lamb meat (Schreurs et al., 2008). Although the formation of 'pastoral' flavour in the ruminal ambiance of lambs has been described in the literature (Devincenzi et al., 2014; Schreurs et al., 2008; Schreurs et al., 2007a; Schreurs et al., 2007b; Schreurs et al., 2007c), there has not yet been information referent to the finishing period of 21 days indoors on concentration of indole and skatole in the fat and the chop's sensory attributes of lambs before grazed alfalfa pasture. This study was, therefore, undertaken to evaluate the influence of feeding systems on fat and meat colour of lambs' pasture-fed alfalfa, concentrate stall-fed and lambs finished indoors with concentrate and straw for 21 days after having grazed alfalfa.

2. Materials and methods

The study was conducted between May and September 2015 at the Joint Herbivore Research Unit at the INRA Auvergne-Rhône-Alpes Research Centre, France. The animals were handled by specialized staff that ensured their welfare in accordance with European Union Directive No. 609/1986.

2.1. Experimental design, animals and diets

Three treatments were compared from weaning to slaughter: A: grazing alfalfa, S: stall-feeding a concentrate-based diet, AS: grazing alfalfa followed by a 21-d concentrate-finishing period in stalls. During stall-feeding, concentrate was given at a level that was adjusted to achieve similar growth paths in the three treatments, and straw was given *ad libitum*. A and AS lambs grazed together within one group when at pasture.

Were used 30 non-castrated male Romane lambs from 11 rams and 24 dams which were born within 6 days (31 March- 05 April 2015) and suckled by their dams until weaning on 08 June. Each of the three treatments included 10 lambs. Until 11 May, the animals were managed uniformly; they were housed in a sheepfold and received no legumes in their diet. Ewes had access to barley and hay. Lambs had access to a commercial concentrate in an area where the ewes were not allowed. First, 10 and 20 lambs were allocated to the stall and the pasture treatments on the basis of their birth weight and live weight (LW) the day before turning out of the pasture-fed lambs (12 May). Second, 10 pasture-fed lambs were allocated to the AS treatment on the basis of their birth weight and live weight (LW) on 4 August to balance these variables across A and AS treatments.

On 12 May, pasture-fed lambs were allowed to graze with their dams a 1.6 ha cocksfoot (*Dactylis glomerata* L.) pasture which was conterminal to an alfalfa plot. After weaning, the pasture-fed (A and AS) lambs grazed the cocksfoot pasture and were given progressive allowance to the conterminal alfalfa plot for increasing time durations during 6 days. From one week after weaning onwards, they grazed alfalfa exclusively. The alfalfa pasture was grazed rotationally and lambs changed from paddock to paddock to ensure sufficient availability of green leaves.

The 21-d concentrate-finishing period started on 11 August for the five heaviest AS lambs (AS1) and on 25 August for the 4 remaining AS lambs (AS2).

During the stall-feeding period, S and AS lambs received commercial concentrate *ad libitum* straw in collective feeding troughs and racks. The concentrate comprised 302 g/kg barley, 228 g/kg wheat, 142 g/kg beet pulp, 110 g/kg rape cake, 76 g/kg sunflower cake, 37 g/kg wheat red shorts, 30 g/kg wheat bran, 20 g/kg corn grain, 15 g/kg sugarcane molasses, 10 g/kg calcium carbonate, 10 g/kg vegetable fat, 6 g/kg vegetable extracts to prevent renal calculus, 10.5 g/kg trace elements and vitamins, 3 g/kg salt and 0.5 g/kg aroma. The length of the trough was sufficient to avoid between-animal competition. The stall-fed lambs were penned in 3 different groups (S, AS1 and AS2) to bring about a feeding transition for AS lambs and limit between-animal competition for food. The concentrate was distributed every morning at 9 a.m., after the concentrate refusals have been weighed, recorded and discarded. Representative samples of concentrate and straw offered were taken weekly (~ 200 g), dried for 72 h at 60°C and stored until analysis.

A and AS lambs received an antihelmintic drench on 30 June and 4 August. Water and salt block (g/kg as-fed of 60 Ca, 20 P, 10 Mg, 280 Na, 17.5 Zn, 1.5 Fe, 5.5 Mn, 0.03 Co, 0.03 I, 0.01 Se) were made constantly available in all treatments.

2.2 Slaughter procedures

The lambs were slaughtered at the INRA Auvergne-Rhône-Alpes Centre experimental abattoir, according to European Union welfare guidelines on 1st September for the five heaviest lambs in each treatment and on 15 September for the remaining lambs. Lambs were thus slaughtered at mean age 158 (SD6) days, after an experimental period of 78 or 92 days (first and second slaughtering date respectively). The lambs had access to food and water until approximately 30 min before slaughter and were transported by truck to the abattoir located close to the experimental pastures and sheepfold. Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting. The carcasses were placed in a refrigerated room at 4°C until 24 h *post mortem*.

2.3 Measurements

2.3.1 Animal body weight, testicular volume and plasma carotenoid content

Lambs were weighed at birth, at weaning, once weekly in the morning at 8:30 a.m., and the day before slaughter using an electronic scale. Testicular volume was measured four days before slaughter by comparative palpation with an orchidometer. Plasma carotenoid content was measured at slaughter according to the method described by (Dian et al., 2007).

2.3.2 Carcass characteristics and meat and fat sampling

After chilling of the carcass for 24 h at 4°C, carcass weight, meat ultimate pH (pH_u), perirenal fat weight and subcutaneous fat thickness over the last thoracic rib were measured. Samples of subcutaneous fat (from the posterior end of the loin) and of perirenal fat were collected (~30 g) to determine the concentration of indole and skatole.

Fat samples were wrapped, vacuum-packed in sealable polyamide bags and frozen at -20°C for analysis. A two centimetre-thick slice of the left *Longissimus thoracis et lumborum* (Ll) muscle was taken from the last thoracic rib, placed on a polystyrene tray, wrapped in air-permeable film (10.000 cm^3 O_2/m^2 per 24h, polyvinyl chloride film) and stored in darkness at 4°C.

2.3.3 Instrumental colour

The colour coordinates of perirenal and subcutaneous fat were recorded at 24 h *post mortem*. The colour coordinates of Ll muscle were measured 2h post sampling (day 1 after slaughter, D1), then daily until D8, except on D4 and D5. Colour coordinates were expressed as lightness (L^*), redness (a^*) and yellowness (b^*) in the CIELAB uniform colour space, using a MINOLTA CM-700d spectrophotometer (illuminate: D65, observer angle: 10°; Minolta France S.A., Carrières-sur-Seine, France). The overall colour difference between two stimuli (ΔE_{ab}^*) was calculated as $\Delta E_{ab}^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$, where the Δ quantities in the right-hand part of the formula represent the differences between the corresponding coordinates of the two stimuli (Brainard, 2003).

2.3.4 Fat indole and skatole concentrations

The concentrations of indole and skatole in perirenal and subcutaneous fat were measured by HPLC according to the procedure described by Batorek et al. (2012). Concentrations were expressed in µg per gram of the lipid fraction from adipose tissue. The quantification limit was 0.03 µg/g of liquid fat.

2.3.5 Lamb chop sensory evaluation

Lamb chop sensory evaluation was performed by 12 trained panellists according to the rules set out in AFNOR NF ISO V 09 105 at INRA Magneraud Experimental Unit. The panellists were a man aged 69 years and 11 women of an average age of 54 years (range 36-67 years). Lamb chop sensory evaluation was performed on eight chops per lamb in ten panel sessions. At each panel session, each panellist evaluated one lamb per treatment, with lambs from each treatment offered in randomized order, with exception of the last one; where only one A chop was evaluated, because one AS lamb and one S lamb died.

Before evaluation of the experimental chops, three training sessions were held, using additional chops from A lambs with a high skatole concentration in perirenal fat and S lambs with a low skatole concentration in perirenal fat.

Before each session, the chops to be evaluated (two chops from one lamb per treatment) were thawed for 24 h at +4°C. Each chop was individually put into an aluminium foil container covered with aluminium foil. The chops were then contact-grilled ‘bone in’ to an internal temperature of 75°C, and served warm to the 12 panellists. The bone part was removed, then pieces (about 2 cm x 2 cm each) were cut from the lean part (Ll muscle) and pieces (about 1 cm x 2 cm each) were cut from the fat part (the rest of the chop) to provide a piece of both for all the panellists. The panellists were asked to taste each part separately and use 10 cm non-structured line scales to evaluate the intensity of tenderness, juiciness, normal odour, abnormal odour of the lean part and the intensity of normal odour, abnormal odour, six flavour attributes (typical lamb, rancid, liver, milky, fatty and barnyard) and the intensity of their overall liking of the odour and flavour of the fat part, and finally the intensity of their overall liking of the chop. Assessments were subsequently scanned scored as the distance in cm

from the left end of the line using the FIZZ® software (version Fizz Form 2.40G, Biosystemes, Couternon, France). Panellists were asked to drink water and eat toast and a piece of apple between assessments to ensure that each sample was assessed with a cleansed palate.

2.3.6. Forage collection and analyses

Representative samples of alfalfa were collected weekly between week 2 and 13 of the experimental period using the hand plucking technique (Cook, 1964) to simulate the plant parts taken by the lambs. Approximately 200 g of fresh forage was taken and dried for 72 h at 60°C, then milled in a 200 μm outlet mill for ash, crude protein (CP; (AOAC, 1995), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content analyses (Goering & Van Soest, 1970). The organic matter *in vitro* digestibility (OMD) was determined using the pepsin-cellulase method (Aufrere & Michalet-Doreau, 1988).

2.4 Data analysis

The data for animal performances, carcass characteristics, Ll muscle pH_u and fat colour coordinates underwent an ANOVA analysis using a mixed model, with treatment as a fixed factor and slaughter date as a random factor (SAS, 2004). The effect of the treatment on plasma carotenoid and on fat indole and skatole concentrations were analyzed using non-parametric statistics (Kruskal and Wallis test), as the variance for these variables differed between treatments and was not stabilized using the natural logarithmic transformation. The pairwise comparisons were performed using the exact Wilcoxon Two-Sample procedure. The data for Ll muscle colour coordinates underwent an ANOVA analysis using a mixed model, with treatment and measurement time as fixed factors and slaughter date as a random factor, and using repeated measures on one

factor (measurement time). The data for lamb chop sensory evaluation underwent an ANOVA analysis using a mixed model, with treatment and panel session as fixed factors and panellist as a random factor. Were used the Tukey test for pairwise comparisons.

3. Results

3.1. Feed composition

The organic matter *in vitro* digestibility (OMD) of alfalfa averaged 862 mg/g organic matter (OM) (range: from 832 to 920 mg/g OM, Figure 1). The crude protein (CP) content averaged 266 mg/g OM (range: from 206 to 308 mg/g OM; Figure 1). ADF and NDF values of alfalfa averaged 15.1% dry matter (DM) (range: from 10.6 to 16.8% DM) and 34.2% DM (range: from 30.1 to 37.2% DM). Dry matter and CP contents of concentrate and straw were 90.1 and 92.6% of DM and 17.2 and 2.17 mg/g OM.

3.2. Animal performance

During the experiment, one AS lamb and one S lamb died from causes unrelated to the experimental treatments.

Mean LW gain of AS lambs during the stall-finishing period was 5.29 (SD 2.45) kg (range: from 1.80 to 8.80 kg); it was 5.78 (SD 3.12) kg and 4.45 (SD 1.40) kg for AS1 and AS2 lambs, respectively. The average daily gain (ADG) during the last 21 days before slaughter was not different among feeding treatments (P = 0.433), averaging 380, 259 and 330 g/day for A, AS and S lambs, respectively.

Concentrate DM intake per lamb was 83.2 and 21.6 kg for S and AS lambs (24.8 and 17.6 kg for AS1 and AS2 lambs). Lamb LW at slaughter, cold carcass weight and

testicular volume at slaughter did not differ among treatments ($P = 0.441$, $P = 0.067$ and $P = 0.175$, respectively), averaging 48.8 kg, 22.0 kg and 270 cm^3 (Table 1). Subcutaneous fat thickness and perirenal fat weight did not differ among treatments ($P = 0.077$ and $P = 0.052$), averaging 3.56 mm and 373 g. The Ll muscle pH_u was lower ($P < 0.012$) in A lambs than in AS and S lambs for both comparisons; the latter being not significantly different ($P > 0.05$).

3.3. Fat indole and skatole concentrations

Perirenal fat indole concentration was affected by the treatment ($P < 0.005$, Figure 2). Indole was detected in the perirenal fat for 8 A (8/10), 0 AS (0/9) and 2 S (2/9) lambs. It averaged 0.04, 0.00 and 0.02 µg/g liquid fat in A, AS and S lambs, respectively. It was higher ($P < 0.005$) in A lambs than in AS lambs; the other pairwise comparisons being not significant ($P > 0.05$). Indole was not detected in subcutaneous fat, except for the outlier S lamb (0.10 µg/g liquid fat).

Perirenal fat skatole concentration was affected by the treatment ($P < 0.005$); it averaged 0.17, 0.01 and 0.12 µg/g of liquid fat in A, AS and S lambs, respectively (Figure 3). Skatole was detected in the perirenal fat for 9 A (9/10), 1 AS (1/9) and 4 S (4/9) lambs. It was higher in A lambs than in AS and S lambs ($P < 0.001$ and $P < 0.05$), and it was not different between AS and S lambs ($P > 0.05$). We detected 2 outliers, one in the AS treatment (0.08 µg/g of liquid fat) and the other in the S treatment (0.84 µg/g of liquid fat).

Subcutaneous fat skatole concentration was affected ($P < 0.025$) by the treatment; it averaged 0.15, 0.01 and 0.12 µg/g of liquid fat in A, AS and S lambs, respectively (Figure 4). Subcutaneous fat skatole was detected in the 8 A (8/10), 1 AS (1/9) and 4 S (4/9) lambs. It was lower ($P < 0.005$) in AS lambs than in A lambs, the other pairwise

comparisons being not significant ($P > 0.05$). We detected 1 outlier in the S treatment; this lamb presented the highest subcutaneous fat skatole concentrations among all 28 lambs (0.75 µg/g of liquid fat).

3.4. Plasma carotenoid concentration and fat colour

Plasma carotenoid concentration at slaughter was affected by the treatment ($P < 0.001$). It was higher in A lambs (60 µg/L) than in AS (5 µg/L) and S lambs (7 µg/L). The other comparisons being not significant ($P > 0.05$).

Perirenal fat redness and yellowness were affected by the treatment ($P < 0.05$, $P < 0.05$ and $P < 0.001$, Table 2). Redness was lower ($P < 0.01$) and yellowness was higher ($P < 0.05$) in A lambs than in S lambs and AS lambs being intermediate.

Subcutaneous fat lightness and redness were affected by the treatment. Lightness was lower ($P < 0.05$) and redness was higher ($P < 0.005$) in A lambs than in AS lambs. The other pairwise comparisons were not significant.

The overall colour difference (ΔE_{ab}*) between A and AS lambs, A and S lambs and AS and S lambs were 1.57, 3.51 and 2.22, respectively for perirenal fat and 4.89, 2.68 and 2.53 for subcutaneous fat.

3.5. Longissimus thoracis et lumborum muscle colour

There was no interaction among treatment and time of measurement on Ll muscle colour coordinates (Table 3). Lightness, redness and overall colour variation were affected by the feeding treatment ($P < 0.0001$, $P < 0.0001$ and $P = 0.05$, respectively), but yellowness was not ($P > 0.267$). Lightness was higher in S lambs than in A and AS lambs (by 2.59 and 2.52 units, $P < 0.001$ for both, Figure 5), the other pairwise

comparisons being not significant. Redness was lower ($P < 0.0001$) in S lambs than in A and AS lambs (by 0.99 and 1.16 units, for both, Figure 6).

All Ll muscle colour coordinates and overall colour variation were affected by time of measurement ($P < 0.0001$ for all variables). Lightness increased from D1 to D6 (Figure 5), then remained steady from D6 to D8. Redness was higher in D1-D3 than in D6-D8 (Figure 6). Yellowness did not change from D1 to D3, then decreased in D6-D7, and further decreased in D8 (Figure 7).

Overall colour variation during storage (ΔE_{ab}*) was higher in AS lambs than in A and S lambs (by 0.90 and 1.03 units respectively, $P < 0.05$ for both comparisons), the other pairwise comparisons being not significant (Figure 8). $\Delta E_{ab}{}^{*}_{(D1\text{-}Di)}$ increased linearly with time, reaching 5.42, 6.92, 9.22, 9.54 and 10.9 for comparisons made between D1 and Di, i ranging from 2 to 8 (no measurements made for i = 4 and 5).

3.6. Chop sensory evaluation

There was an effect of the treatment on the tenderness and the juiciness of the lean part of the chop ($P < 0.001$ and $P < 0.01$, Table 4). Tenderness was in the order A lambs > AS lambs > S lambs, all pairwise comparisons being significant ($P < 0.005$, $P < 0.001$ and $P < 0.001$ for the comparisons between A lambs and AS lambs, A lambs and S lambs, AS lambs and S lambs respectively). Juiciness was higher in A lambs than in AS and S lambs ($P = 0.038$ and $P < 0.01$). There was no significant effect of the treatment on the intensity of abnormal odour of the lean part ($P = 0.096$).

There was an effect of the treatment on the intensity of the ‘typical lamb’ flavour ($P < 0.05$) of the fat part, which was higher in A lambs than in S lambs ($P < 0.05$), the other comparisons being not significant. There was an effect of the treatment on the intensity of abnormal odour of the fat part ($P < 0.05$): AS lambs presented a lower

intensity than S lambs ($P < 0.05$), the other pairwise comparisons being not significant. There was an effect of the treatment on the intensity of flavour liking of the fat part ($P < 0.05$), which was higher in AS lambs than in S lambs ($P < 0.05$), the other comparisons being not significant.

There was an effect of the treatment on the intensity of chop overall liking ($P < 0.05$), which was higher in AS lambs than in S lambs ($P < 0.05$), the other comparisons being not significant.

4. Discussion

The AS lambs presented a greater variation in the weight gain during the stall-fed period (SD = 2.45). The variability of weight gain in the AS lambs during the concentrate-fed period may be associated to the individual response in front of a new diet and the housing system. According to Silberberg et al. (2013), the shift from high-fibre to high-energy diets in a short transition period may be associated to develop of rumen acidosis. In this study, the readily fermentable carbohydrates in the concentrate might be changed the ruminal pH and the microorganism colonization of rumen during the indoors period; this fact could be affected the feed intake of AS lambs.

There no effect of treatments on animal performances and carcass characteristics. However, the muscle ultimate pH (pH_u) was higher to AS and S lambs than A lambs. We hypostatized that the concentrate-fed competition between the animals may be increased the stress into the stall fed lambs (*i. e.* AS1, AS2 and S lambs) and contributed to the lower muscular glycogen availability.

The concentration of indole in the perirenal fat was lower than that of skatole in this study and was not observed concentrations of indole in the subcutaneous fat. It is likely that indole was effectively metabolized to skatole to the animal. Schreurs et al.

(2007a) have been associated the indole and skatole concentrations in the plasma and fat of lambs to the microbial deamination and decarboxylation of diet to tryptophan acid in the rumen and to the indole and skatole metabolization in the liver. This result is in line with Devincenzi et al. (2014) when observed higher concentration of skatole than that indole in the fat of lambs grazing cocksfoot supplemented with levels of fresh alfalfa (0%, 25%, 50% and 75% of voluntary DM intake). However, we observed lower concentration of indole and skatole in the perirenal fat (0.04 and 0.17 µg/g liquid fat, respectively) than these authors when added higher level of fresh alfalfa (75% of voluntary DM intake) in the diets (0.10 and 0.24 µg/g liquid fat, respectively). The better concentration of indole and skatole observed in our study may be linked to the lower NDF content in the alfalfa pasture-fed than the value observed by these authors (34.2 and 38.4 % DM, respectively).

According to Schreurs et al. (2008), the amount of tryptophan acid might be associated to exceed of peptides and amino acids formation from more rapidly solubilisation and degradation of CP in the ruminal ambiance. The high fibre content and insufficient energy from readily fermentable carbohydrates present in the fresh forage reduce the peptides and amino acids incorporation into microbial protein and increase the tryptophan conversion to skatole in the rumen. In the current study, the lower NDF content and the readily fermentable carbohydrate of alfalfa pasture-fed improved the peptides and amino acids utilization by rumen microbes and reduced the tryptophan acid conversion to skatole.

The AS demonstrated lower concentration of indole and skatole in the fat that A lambs. Surprisingly, the perirenal fat indole and subcutaneous fat skatole were not statistically different between S and A lambs. The 21 days of concentrate-fed period was extremely effective to reduce the concentration of indole and skatole in the fat of

lambs. However, it was higher when the lambs were finished indoors with concentrate and straw for 78 and 92 days.

The readily fermentable carbohydrates present in the concentrate improved the peptides and amino acids incorporation into microbial protein and increase the propionic acid formation by gram-negative rumen bacteria. However, the variability between individual animals in dietary choices may contribute to sub-acidosis installation in the ruminal ambience. The sub-acidosis process might change the pH and micro-organism colonization of rumen. The protozoa and rumen bacteria selection from lower ruminal pH value (5.50) affect the rate of protein degradation and increase the amount of tryptophan available for indole and skatole formation in the rumen (Schreurs et al., 2008; Ushida & Jouany, 1985). The outliers present in the box plots (Figure 2, 3 and 4) demonstrated the variability of individual animals on concentration of indole and skatole in the fat. The possible supposition it is which the individual selectively of animals in dietary choice increased the concentrate intakes and ruminal sub-acidosis was installed. However, no detrimental values were observed on animal performance of AS and S lambs and the referenced outliers did not differ of others experimental repetitions.

The 21 days feeding concentrate and straw was not useful to affect the colour of perirenal fat of lambs. The effects of redness observed between the feeding treatments may be linked to the decreases in the perirenal fat of indoors lambs. According to Zawadzki, Prado, and Prache (2013) the perirenal fat redness might be influenced by increases in the fatness which ‘diluted’ fat-stored haeminic pigments. In this study, the perirenal fat weight was close to significance for the feeding treatments ($P = 0.052$).

The yellowness value in the perirenal fat of grazing lambs may be linked to the carotenoids pigments deposition. Several studies associated the higher yellowness with

carotenoid pigment concentrations in the perirenal fat of grazing lambs (Huang et al., 2015; Prache, Priolo, & Grolier, 2003; Priolo, Micol, Agabriel, Prache, & Dransfield, 2002). Likewise, the subcutaneous fat presents lower carotenoid pigment concentrations than perirenal fat (Kirton, Crane, Paterson, & Clare, 1975). Our result is in line with this affirmation when was observed no effect of feeding treatments on yellowness of subcutaneous fat.

The lightness and the redness of subcutaneous fat were influenced by the period feeding indoors. AS lambs presented higher lightness in the subcutaneous fat than A lambs. However, the redness was higher in the subcutaneous fat of lambs' pasture-fed alfalfa than AS lambs. This may be explained by the fact that haeminic pigments present in the fat of grazing lambs increase the absorption of light. Zawadzki et al. (2013) associated the increases of proportion of light reflected with the dilution of haeminic pigments in the fat of lambs. Although there are differences in the colour of fats, the biological amplitude of perirenal and subcutaneous fat was much lower than the threshold to find differences in the visual appraisals. Schwarz, Cowan, and Beatty (1987), suggested 5.9 as threshold of the overall colour variation (ΔE_{ab}*) to differentiate solid colours on visual appraisals.

The period feeding concentrate and straw indoors did not change the Ll muscle colour of AS lambs. The differences in the colour coordinates of lambs grazing alfalfa pasture (*i.e.* A, AS1, AS2 lambs) and stall-fed lambs may be associated to the higher concentration of haeminic pigments in the muscle of grazing lambs (Carrasco et al., 2009), and the slight difference observed in the pH_u between the feeding treatments. pH_u increasing from 5.4 to 6 have been associated to reducing values in Ll colour of lambs, in order to 0.32 for *L**, 0.42 for *a** and 0.35 points for *b** each 0.1 points of pH_u

increased (Calnan, Jacob, Pethick, & Gardner, 2016). However, 21 days indoors was not useful to reduce the redness of Ll muscle.

According to Ripoll, Joy, and Muñoz (2011) the blooming process presented an important effect on all meat colour. The myoglobin oxygenation it is responsible for increases on all parameters of meat colour between D0 and D3 of blooming time (Carrasco et al., 2009). However, at D3 of blooming the myoglobin oxygenation tends to be stable and, after that time the colour parameters scarcely varies. According to Santé-Lhoutellier, Engel, and Gatellier (2008) the colour parameters of meat lambs present the highest differences at D2 of blooming, after that time an important decrease on redness values of meat lambs demonstrate which the myoglobin oxidation was induced. Whereas, the meat discolouration increases during the blooming time (Carrasco et al., 2009). According to Ripoll, Joy, Muñoz, and Albertí (2008), the lightness is a good indicator to determine the blooming effects on meat colour, especially the meat discolouration.

In the present study, probability, the myoglobin oxygenation was stabilized in D2 and after that time the myoglobin oxidation started in the both feeding treatments. These effects may be observed by decreasing on redness from D2 and the crescent Ll lightness until D8 of blooming time. However, the scarcely variation in the colour parameters after D6 showed that stabilization of myoglobin oxidation occurred between D6 and D7. The concentrations of radical trapping agents (vitamin E, antioxidants and carotenoid pigments) in the alfalfa pasture could influenced the lightness and yellowness values of feeding treatments in D3 of blooming. The transfer of radical trapping agents from forage to animal tissue has been linked to the higher protection of cell membranes and animal tissues to the oxygen radicals (Aurousseau et al., 2004; Huang et al., 2015; Ripoll et al., 2012). The period feeding concentrate and straw indoors restricted the

intake of antioxidant from alfalfa pasture by AS lambs and it made closest the yellowness of Ll muscle of AS and S lambs. However, the lightness of Ll muscle was every closest for AS and A lambs. This can be related to the persistence of haeminic pigments in the muscle of lambs' pasture-fed alfalfa (Ripoll et al., 2012).

The ΔE_{ab}* observed in the Ll of lambs grazing alfalfa pasture (*i.e.* A, AS1 and AS2 lambs) and stall-fed lambs may be associated to lower lightness and higher redness of the feeding treatments. While, the process of oxymyoglobin to metmyoglobin transformation during the blooming time affected the ΔE_{ab}* of Ll muscle. The myoglobin oxygenation occurred between D1-D2 and after this period the oxidation was started. The myoglobin oxidation increased from D3 until D6 and it may explain the difference observed between the ΔE_{ab}*of first three days and the $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)}$. The stabilization of myoglobin oxidation occurred probably between D6 and D7 of blooming and it explain the no difference between, $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)}$ and $\Delta E_{ab}{}^{*}_{(D1\text{-}D7)}$. However, the higher ΔE among D1 and D8 than in $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)}$ it is linked to the metmyoglobin installation.

The feeding treatment presented effect on tenderness and juiciness of chops. The higher fat concentration in the chops of A lambs than in AS and S lambs may have influenced the preference of the panellists.

The panellists were trained and agreed to describe the specific odour and flavour of the chops associated with their skatole concentration. According to Devincenzi et al. (2014), the sensory attributes of skatole in the fat of lambs it is reached to the consumers from 0.16 to 0.24 µg/g of liquid fat. In our case, all feeding treatments presented a below skatole concentration than the plateau perceptions of panellists. However, was observed an effect of the feeding treatments on the flavour liking and abnormal odour in the fat part of chops. The volatiles compounds that no associated to

the skatole concentrations would be affected the panellists' perceptions and could be responsible to the differences in the flavour liking and abnormal odour observed in both lots. Additionally, this result is linked to the difference observed in the 'typical lamb flavour' between the feeding treatments.

The 'typical lamb flavour' it is an important factor for consumer acceptance and may be associated to the propionate originated in the rumen by the fermentation of dietary carbohydrates (Schreurs et al., 2008). The grain-based diets induce greater accumulations in meat of branched-chain fatty acids (BCFA) and these compounds would be considered key in the aroma of sheep meat (Resconi, Escudero, & Campo, 2013). BCFA are formed in adipose tissue from the propionate exceeds the liver metabolization (Vasta & Priolo, 2006). It has also been associated to the oxidative deamination of the branched-chain aminoacids valine, leucine and isoleucine (Duncan & Garton, 1978). In our study, the nutritional value of alfalfa pasture may be linked to the propionate formation in the rumen and the greater amounts of BCFA in the fat of lambs.

The BCFA and the skatole concentrations in the fat of A and S lambs may be influenced the overall chop liking of the panellists. Young, Berdagué, Viallon, Rousset-Akrim, and Theriez (1997) related an interaction between the skatole concentrations and BCFA in the diets that intensifies the perception of sheep meat odour/flavour by consumers. In this study, the reduced skatole concentrations in the fat of AS lambs indicate the lower formation of these compounds in ruminal ambiance and contributed to the appreciation by the panellists.

5. Conclusions

The finishing period indoors reduced the indole and skatole concentrations in the fat of lambs before raised in the alfalfa pasture. The low concentration of skatole observed in the fat of lambs may be contributed to the greater reduction of this compound after 21 days feeding concentrate and straw indoors. However, the effects of a fast-finishing period indoors on a greater concentration of skatole in the fat of lambs are not known. The same, further investigation is required to better understand how a lower finishing period concentrate-fed may affect the concentration of skatole in the fat of lambs.

The Ll colour of grazing lambs was not altered into the fast-finishing period indoors. The visual attributes appreciated by consumers from pasture-fed lambs was always present in the meat of lambs feeding concentrate and straw indoors before raised in the alfalfa pasture.

The chop sensory evaluation suggests that the skatole concentration in the fat of experimental lambs was lower than the plateau of consumers' detection. However, yours association with branched-chain fatty acids had influence on consumers' preference.

Acknowledgements

The authors thank M. Bernard, P. Payard, L. Lavelle, A. Cercy, B. Viallard, I. Constant, S. Faure and all the staff of the INRA-Theix experimental farm, abattoir and laboratory for their collaboration. We also thank A. Lecorgne for analysing the concentrations of indole and skatole in the fat. C.E. Eiras was awarded financial support from the Brazilian Ministry of Science and Technology 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq) - Project PVE 400375/2014-1.

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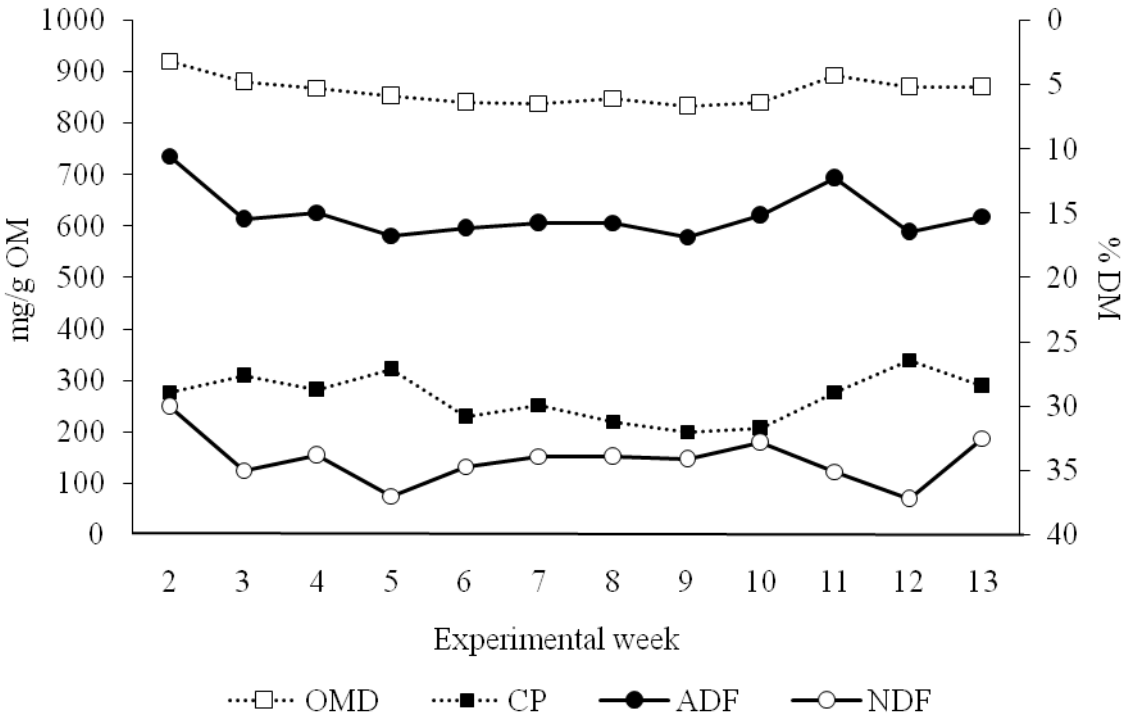

Figure 1. Organic matter *in vitro* digestibility (OMD, mg/g OM), crude protein (CP, mg/g OM), acid detergent fibre (ADF, % DM) and neutral detergent fibre (NDF, % DM) contents in alfalfa offered to grazing lambs.

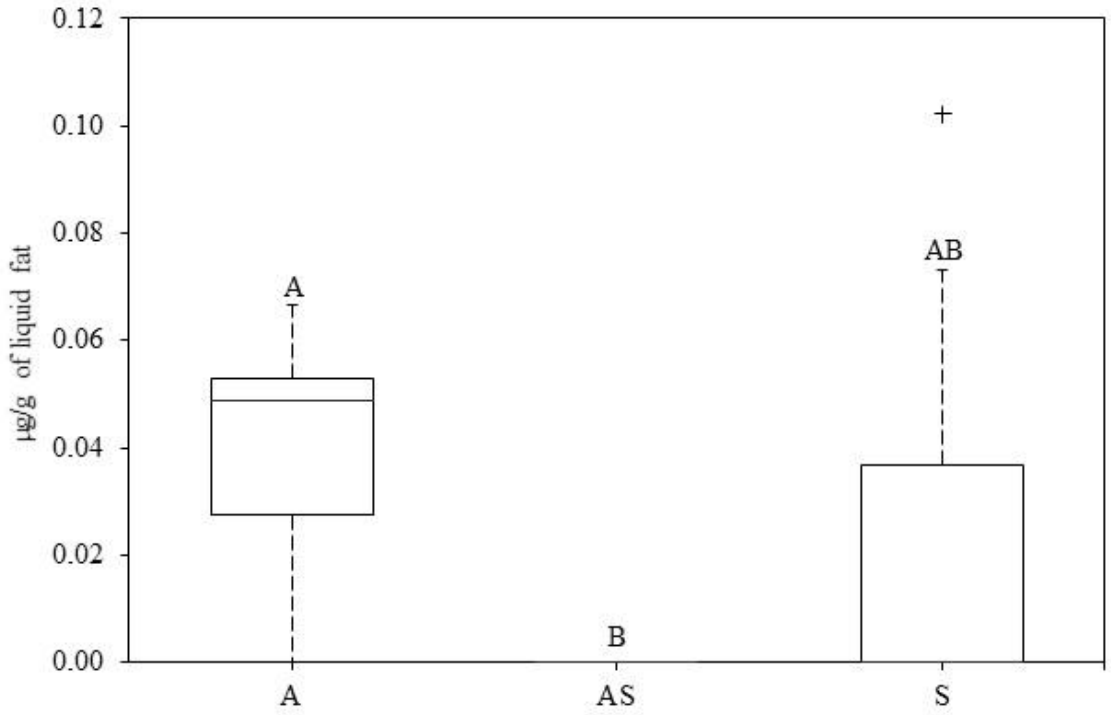

Figure 2. Box plot representation of the perirenal fat indole concentration in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). The box contains the middle 50% of the data, the upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The bold line in the box indicates the median value of the data. Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. The markers (+) indicate outliers. The ends of the vertical lines indicate the minimum and maximum data values. Box plots not bearing a common letter are significantly different (A, B: $P < 0.001$).

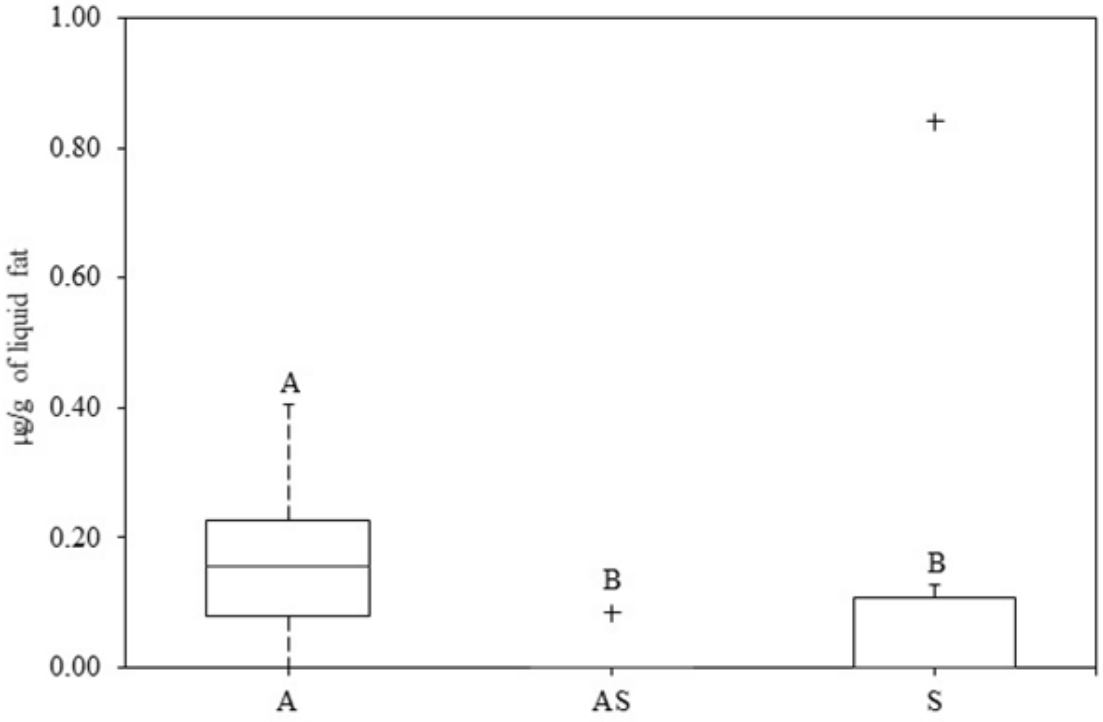

Figure 3. Box plot representation of the perirenal fat skatole concentration in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). The box contains the middle 50% of the data, the upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The bold line in the box indicates the median value of the data. Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. The markers (+) indicate outliers. The ends of the vertical lines indicate the minimum and maximum data values. Box plots not bearing a common letter are significantly different (A, B: $P < 0.05$).

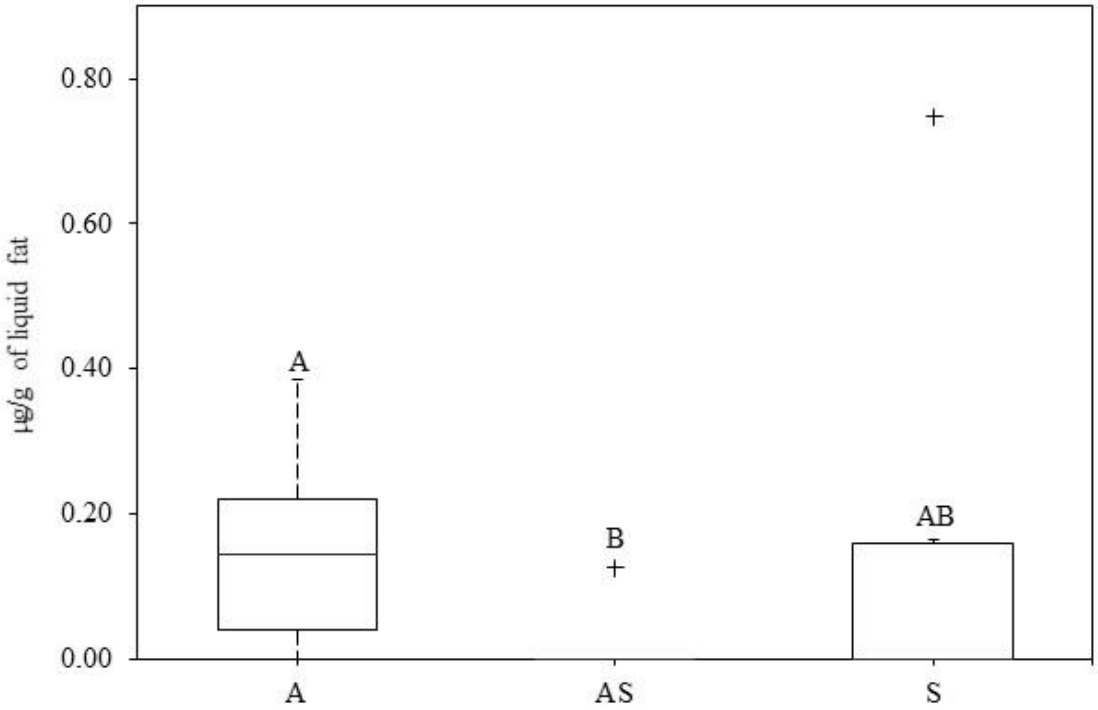

Figure 4. Box plot representation of the subcutaneous fat skatole concentration in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). The box contains the middle 50% of the data, the upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The bold line in the box indicates the median value of the data. Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. The markers (+) indicate outliers. The ends of the vertical lines indicate the minimum and maximum data values. Box plots not bearing a common letter are significantly different (A, B: $P < 0.005$).

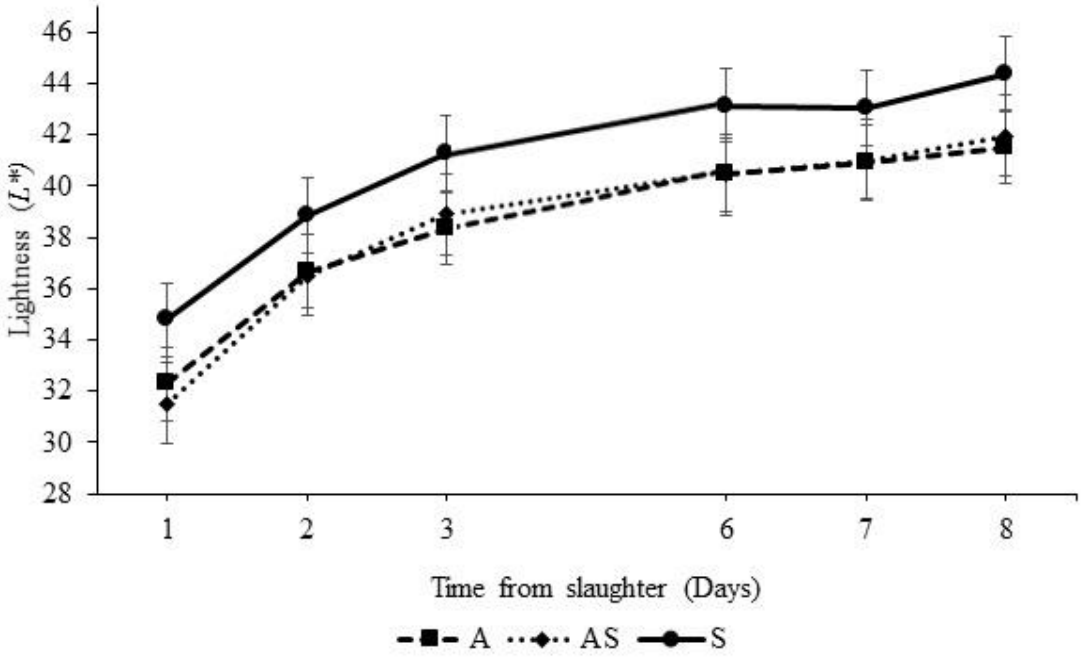

Figure 5. *Longissimus thoracis et lumborum* muscle lightness (*L**) variation, during 8 days (Di, i ranging from 1 to 8) *post mortem*, in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. Pairwise comparisons between treatment groups yielded the following results: A = AS ($P > 0.05$); S > A and AS ($P < 0.0001$ for all comparisons). Pairwise comparisons between days yielded the following results: D1 < D2, D3, D6, D7 and D8 ($P < 0.0001$ for all comparisons); D2 < D3, D6, D7 and D8 ($P < 0.005$ for all comparisons); D3 < D6, D7 and D8 ($P < 0.01$ for all comparisons); D6 = D7 and D8 ($P > 0.05$ for all comparisons); D7 = D8 ($P > 0.05$).

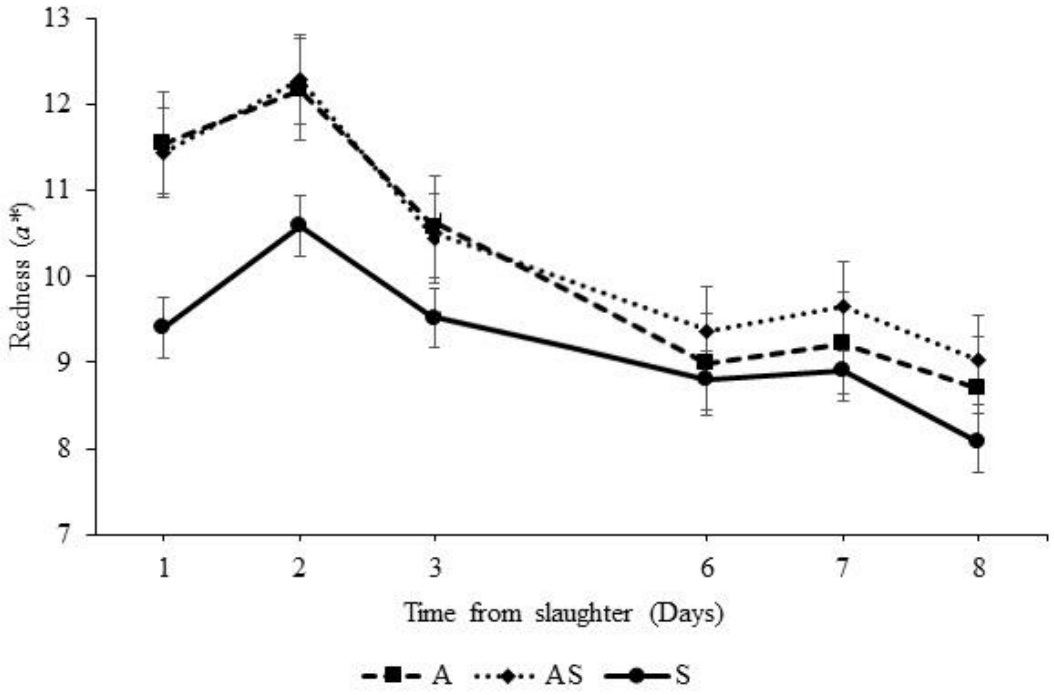

Figure 6. *Longissimus thoracis et lumborum* muscle redness (a*) variation, during 8 days (Di, i ranging from 1 to 8) *post mortem*, in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. Pairwise comparisons between treatment groups yielded the following results: A = AS ($P > 0.05$); S < A and AS ($P < 0.0001$ for all comparisons). Pairwise comparisons between days yielded the following results: D1 < D2 ($P < 0.05$); D1 = D3 ($P > 0.05$); D1 > D6, D7 and D8 ($P < 0.0001$ for all comparisons); D2 > D3, D6, D7 and D8 ($P < 0.001$ for all comparisons); D3 > D6, D7 and D8 ($P > 0.01$ for all comparisons); D6 = D7 and D8 ($P > 0.05$ for both); D7 = D8 ($P > 0.05$).

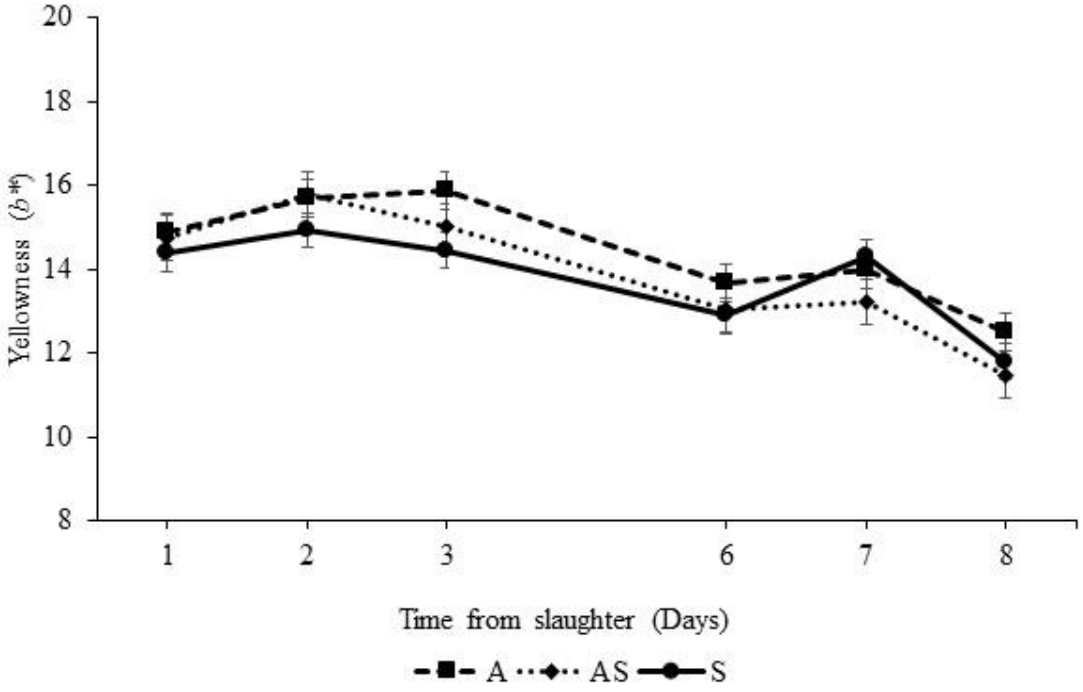

Figure 7. *Longissimus thoracis et lumborum* muscle yellowness (b*) variation, during 8 days (Di, i ranging from 1 to 8) *post mortem*, in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. There was no significant effect of the treatment. Pairwise comparisons between days yielded the following results: D1 = D3 = D7 ($P > 0.05$ for all comparisons); D1 > D6 and D8 ($P < 0.05$ and 0.001, respectively); D2 = D3 ($P > 0.05$); D2 > D6, D7 and D8 ($P < 0.05$ for all comparisons); D3 = D7 ($P > 0.05$); D3 > D6 and D8 ($P < 0.005$ for all comparisons); D6 = D7 and D8 ($P > 0.05$ for all comparisons); D7 > D8 ($P < 0.01$).

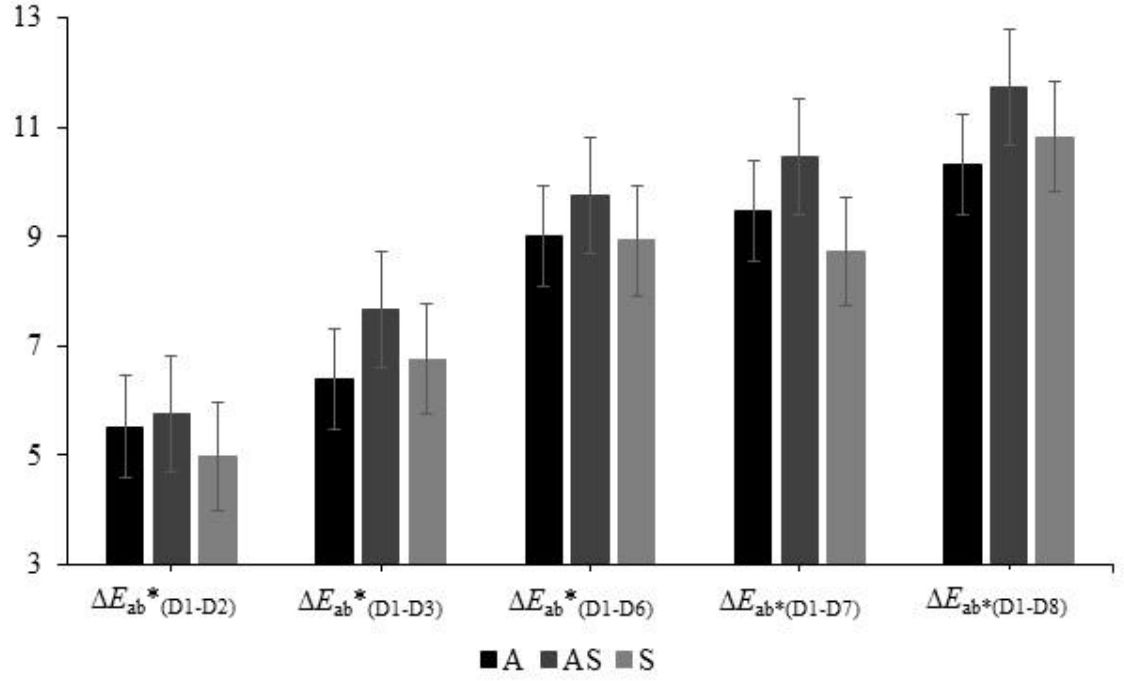

Figure 8. Overall *Longissimus thoracis et lumborum* muscle colour variation (ΔE_{ab}*), during 8 days (Di, i ranging from 2 to 8 days) *post mortem*, in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS). It was calculated as $\Delta E_{ab}{}^{*}_{(D1\text{-}Di)} = ((\Delta L^{*}_{D1\text{-}Di})^2 + (\Delta a^{*}_{D1\text{-}Di})^2 + (\Delta b^{*}_{D1\text{-}Di})^2)^{0.5}$, where $\Delta L^{*}_{D1\text{-}Di}$ $\Delta a^{*}_{D1\text{-}DI}$ and $\Delta b^{*}_{D1\text{-}Di}$ are the differences between *L**, *a** and *b** values measured at Di and their values at D1. Bars represents standard error of the mean (SD/√n), where n is the number of lambs in each group. Pairwise comparisons yielded the following results: $\Delta E_{ab}{}^{*}_{(D1\text{-}D2)} < \Delta E_{ab}{}^{*}_{(D1\text{-}D3)}$ ($P < 0.01$) and $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)}$, $\Delta E_{ab}{}^{*}_{(D1\text{-}D7)}$ and $\Delta E_{ab}{}^{*}_{(D1\text{-}D8)}$ ($P < 0.001$ for all comparisons); $\Delta E_{ab}{}^{*}_{(D1\text{-}D3)} < \Delta E_{ab}{}^{*}_{(D1\text{-}D6)}$, $\Delta E_{ab}{}^{*}_{(D1\text{-}D7)}$ and $\Delta E_{ab}{}^{*}_{(D1\text{-}D8)}$ ($P < 0.001$ for all comparisons); $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)} = \Delta E_{ab}{}^{*}_{(D1\text{-}D7)}$; $\Delta E_{ab}{}^{*}_{(D1\text{-}D6)} < \Delta E_{ab}{}^{*}_{(D1\text{-}D8)}$ ($P < 0.005$); $\Delta E_{ab}{}^{*}_{(D1\text{-}D7)} < \Delta E_{ab}{}^{*}_{(D1\text{-}D8)}$ ($P < 0.025$).

Table 1.

Animal performances, carcass characteristics and muscle ultimate pH (pH_u) of *Longissimus thoracis et lumborum* in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS)

	A	AS	S	SEM	*P*-value
Number of lambs	10	9	9		
Live weight at slaughter (kg)	50.0	48.7	47.7	3.560	0.441
Cold carcass weight (kg)	23.6	21.3	21.1	0.540	0.067
Testicular volume at slaughter (cm^3)	293	279	238	22.3	0.175
Subcutaneous fat thickness (mm)	3.80	3.11	3.78	0.322	0.077
Perirenal fat weight (g)	474	327	317	49	0.052
pH_u	5.60a	5.68b	5.70b	0.025	0.012

SEM: Standard error of the mean

Means in the same row with different letters are significantly different ($P < 0.05$)

Table 2.

Fat colour and firmness in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS)

	A	AS	S	SEM	*P*-value
Perirenal fat colour at 24 h *post mortem*					
Lightness (*L**)	78.82	77.43	76.53	0.834	0.154
Redness (*a**)	1.58b	2.20ab	3.05a	0.677	0.012
Yellowness (*b**)	12.79a	12.41ab	10.57b	0.868	0.018
Subcutaneous fat colour at 24 h *post mortem*					
Lightness (*L**)	65.15b	69.58a	67.25ab	1.195	0.015
Redness (*a**)	4.15a	2.17b	2.94ab	0.386	0.002
Yellowness (*b**)	14.16	13.60	13.01	0.639	0.423
Fat firmness	7.70	6.56	6.78		0.346

SEM: Standard error of the mean

Means in the same row with different letters are significantly different (a, b: $P < 0.05$)

Table 3.

Statistical effects of treatment and measurement time and their interactions on *Longissimus thoracis et lumborum* muscle colour

	P-value		
	Treatment	Measurement time	Interaction
Lightness (L^*)	<0.0001	<0.0001	= 0.9995
Redness (a^*)	<0.0001	<0.0001	= 0.5694
Yellowness (b^*)	=0.2670	<0.0001	= 0.9932
Overall colour variation (ΔE_{ab}^*)	=0.0464	<0.0001	= 0.9841

$\Delta E_{ab}^{*}{}_{(D1\text{-}Di)} = ((\Delta L^{*}{}_{D1\text{-}Di})^2 + (\Delta a^{*}{}_{D1\text{-}Di})^2 + (\Delta b^{*}{}_{D1\text{-}Di})^2)^{0.5}$, where $\Delta L^{*}{}_{D1\text{-}Di}$, $\Delta a^{*}{}_{D1\text{-}Di}$ and $\Delta b^{*}{}_{D1\text{-}Di}$ are the differences between L^*, a^* and b^* values measured at Di and their values at D1.

Table 4.

Sensory evaluation of chops (0-10 scale of increasing intensity) in lambs pasture-fed alfalfa (A), stall-fed concentrate and straw indoors (S), or finished indoors with concentrate and straw for 21 days after having grazed alfalfa (AS)

	Feeding treatment			SEM	*P*-value	
	A	AS	S		Treatment	Session
Lean part						
Tenderness	3.96a	3.57b	2.89c	0.412	<0.0001	0.005
Juiciness	3.01a	2.77b	2.72b	0.314	0.008	0.056
Abnormal odour	1.05	0.79	1.04	0.331	0.096	0.462
Fat part						
Typical lamb flavour	3.67a	3.57ab	3.48b	0.354	0.045	0.022
Rancid flavour	0.75	0.65	0.76	0.215	0.322	0.633
Liver flavour	0.70	0.60	0.59	0.184	0.199	0.479
Milky flavour	1.10	1.04	1.15	0.276	0.580	0.159
Fatty flavour	2.41	2.28	2.33	0.407	0.344	0.184
Barnyard flavour	1.29	1.13	1.16	0.371	0.176	0.019
Flavour liking	2.81ab	3.04a	2.63b	0.434	0.044	0.018
Abnormal odour	1.01ab	0.72b	1.04a	0.310	0.035	0.025
Odour liking	2.79	3.08	2.76	0.082	0.199	0.006
Overall chop liking	3.02ab	3.21a	2.77b	0.447	0.019	< 0.001

SEM: Standard error of the mean

Means in the same row with different letters are significantly different (a, b: $P < 0.05$)

IV - How does the dietary cottonseed hull affect the carcass characteristics and meat quality of young bulls finished in a high-concentrate diet?

Journal: Acta Scientiarium. Animal Science

ABSTRACT. This study evaluated the effects of diets composed by cottonseed hull and meat aging on carcass characteristics and meat quality from young bulls fed on a high-concentrate system. The cottonseed hull treatments were assigned into the experimental units in a complete randomized experimental factorial design during 162 days in the individual pens. (CH21: cottonseed hull 210 g kg^{-1}on DM basis, CH27: cottonseed hull 270 g kg^{-1}on DM basis and CH33: cottonseed hull 330 g kg^{-1}on DM basis) and different aging times (24 hours and 3, 7 and 14 days). Meat from CH27 diets presented smaller Ll moisture content ($P < 0.05$). Total lipids were smaller in CH33 diet ($P < 0.05$). At 24 hours, CH21 diet presented smaller Ll cooking loss than other diets. The increasing aging time reduced the shear force ($P < 0.001$) on the Ll. Meat from CH27 diet presented the highest luminosity ($P < 0.05$) and yellowness values ($P < 0.001$). Three or seven aging days presented smaller values of Ll luminosity. Likewise, the increasing aging time presented greater ($P < 0.05$) yellowness within the Ll. Lipid oxidation was lower from CH21 diet ($P < 0.001$). The fatty acid composition on Ll was similar among diets. The use of cottonseed hull could be useful strategies to improve the meat quality and lean beef production.

Keywords: alternative foods, co-products, feedlot, high-grain, young bulls.

Como a casca de algodão influencia as características da carcaça e a qualidade de carne de bovinos precoces alimentados com dietas de alto teor de concentrado?

RESUMO. Esse estudo avaliou os efeitos de dietas com casca de algodão e maturação da carne sobre as características de carcaça e a qualidade de carne de bovinos precoces alimentados com dietas de alto teor de concentrado. Os tratamentos contendo diferentes proporções de casca de algodão na dieta foram distribuídos às unidades experimentais em design fatorial com três dietas (CH21: 210 g kg^{-1} de casca de algodão na MS da dieta; CH27: 270 g kg^{-1} de casca de algodão na MS da dieta e CH33: 330 g kg^{-1} de casca de algodão na MS

da dieta) e diferentes tempos de maturação (24h, 3, 7 e 14 dias). Os animais foram alocados em baias individuais durante 162 dias. A dieta CH27 apresentou menor teor de umidade na carne ($P < 0,05$). Os lipídios totais foram menores na dieta CH33 ($P < 0,05$). Após 24h, a dieta CH21 mostrou menor perda por cocção no músculo *Longissimus* (ML). O aumento no tempo de maturação reduziu a força de cisalhamento ($P < 0,001$) do ML. A dieta CH27 apresentou maior luminosidade ($P < 0,05$) e intensidade de amarelo ($P < 0,001$). Três ou sete dias de maturação proporcionaram menores valores de luminosidade no ML. O crescente tempo de maturação apresentou maior ($P < 0,05$) intensidade de amarelo no ML. A oxidação de lipídios foi menor na dieta CH21 ($P < 0,001$). A composição de ácidos graxos do ML foi semelhante entre as dietas. O uso da casca de algodão em dietas de alto de alto teor de concentrado pode ser uma estratégia útil para melhorar a produção de carne magra e a qualidade de carne de bovinos.

Palavras-chave: alimento alternativo, co-produtos, confinamento, bovinos precoces.

INTRODUCTION

Beef production and meat quality are influenced by nutritional contents, feeding systems, gender and animal age (Rotta et al., 2009). Brazilian beef production is essentially based on a pasture system, which may increase the slaughter age and affect the meat quality. Nowadays, the Brazilian market has been shifted toward the production of lean beef following market requirements. Currently, the market demands a carcass with a high percentage of lean meat, and the adequate fat distribution determines the market price (Realini et al., 2013). Therefore, highly efficient productive systems should be employed to maximize the growth of beef cattle, increase the meat quality, and reduce the age of cattle at slaughter.

There has been an increase in intensive Brazilian beef cattle production systems, which is supported by the fact that performances and meat quality are affected by this intensification (Maggioni et al., 2010, Prado et al., 2009). Livestock production systems with a high percentage of concentrate and grains that are rich in starch and high ruminal fermentation rates can make up to 80% of such diets (González et al., 2012). However, high costs of cereal grains and the scale of the Brazilian production co-products may be possible alternative feeding strategies. The utilization of co-product foods from agribusinesses may be an option in finishing young bulls in feedlots with a combination of 47% concentrate and 53% corn silage causing no damage in carcass characteristics or meat quality (Eiras et al., 2014).

Likewise, the co-products from Brazilian agribusinesses could potentially be utilized in beef cattle productions in which they are fed in a high-concentrate system.

This kind of co-product is not only abundant in Brazil, but also in other countries of the world as Chine, India, USA, Pakistan, Australia (FAO, 2015) which are the main cotton producers. Consequently results and use can be extrapolated to other regions or beef production systems. Therefore, the objective of the current study was evaluating the effects of cottonseed hull in concentrations from 210 to 330 g kg^{-1} on DM basis of high-concentrate diets on meat with several aging times from crossbred young bulls.

MATERIALS AND METHODS

Local, animals, housing and diets

This experiment was approved by the Ethics Committee of State University of Maringá (# 180/2014), and follows the norms recommended as international guiding principles for Biomedical Research Involving Animals (CIOMS/OMS, 1985).

Thirty crossbred bulls (½ Simmental *vs.* ½ Nellore) were assigned to a randomized complete factorial design experiment composed of three diets with ten animals per group in individual pens (10 m^2 for each animal).

After 15 day diet adaptation period, the bulls were weighed and the study was started at an average initial BW of 319 ± 12.5 kg and an average age of 11 ± 0.8 months. Body weights were recorded monthly and the intake of concentrate was recorded daily until 162 days into the experiment when the bulls reached a final BW of 481 ± 22.8 kg with an average daily gain of 1.0 ± 0.25 kg (Table 1).

The three cottonseed hull diets were assigned into the experimental units: CH21: cottonseed hull 210 g kg^{-1} on DM basis, CH27: cottonseed hull 270 g kg^{-1} on DM basis, and CH33: cottonseed hull 330 g kg^{1} on DM basis (Table 2). The diets were formulated to be isonitrogenous and isoenergetics and to provide a weight gain of 1.0 kg day^{-1} according to the NRC (2000) recommendations (Table 3).

The animals were fed on diets twice a day (08:00 and 16:00 h) to meet the adequate concentrations of nutrients for growing and finishing animals (NRC, 2000). The soybean hull pellets and ground corn were offered *ad libitum* in order to adjust the energetic level of the diets.

Diet chemical analyses

Chemical compositions of ingredients were presented in g kg^{-1} of DM (Table 3). Dry matter was determined after oven-drying at 65°C for 24 h and milling through a 1 mm screen (AOAC, 2005 – ID 934.01). Ash content was measured by combustion at 550°C for 16 h (AOAC, 2005 – ID 942.05) to determine the organic matter (OM). Nitrogen concentration was determined by the Kjeldahl method (AOAC, 2005 – ID 988.05). Following the determination of the nitrogen concentration, the CP was calculated by multiplying the N content by a factor of 6.25. Ether extract content was determined by method ID 920.39 (AOAC, 2005). Neutral detergent fiber (NDF) content was measured using alpha-amylase and was expressed inclusive of residual ash. The acid detergent fiber (ADF) was measured by using the AOAC (2005 - ID 973.18) method and was expressed inclusive of residual ash. Total carbohydrates (TC) were estimated by following the procedure of Sniffen et al. (1992). Non fibrous carbohydrates (NCF) were determined as the difference between TC and NDF. Metabolizable energy of feedstuffs was estimated according to NRC (2000) recommendations.

Slaughter procedure and muscle sampling

The young bulls were slaughtered according to industrial practices in Brazil at a commercial slaughterhouse 80 km from the Iguatemi Experimental Farm. To minimize pre-slaughter stress, the bulls were transported and slaughtered the next day. The animals were fasted from solids for 16 h. On arrival at the slaughterhouse, they were kept in resting pens and were humanely harvested under Brazilian federal inspection according to the Brazilian Regulation of Industrial and Sanitary Inspection of Animal Products. Following slaughter, the carcasses were identified and chilled for 24 h at 4°C.

After chilling, the right half of the carcass was used for the *Longissimus thoracis et lumborum* samples (Ll). The pH in triplicate was measured by using a probe-type portable pH meter (Hanna Instruments, Woonsocket, RI, USA) in the 12^{th} and 13^{th} ribs. The Ll was excised between the 6^{th} and 13^{th} ribs for further laboratory analysis.

Evaluation of carcass characteristics

The whole-carcass composition was assessed from carcass measurements and rib composition. The 6^{th} rib from the right half of the carcass was weighed and dissected into muscle, fat (intramuscular and subcutaneous), bone, and other tissues. The carcass

compositions were calculated as a percentage of the raw weight of the 6th rib and the weight of dissected compounds (Robelin and Geay, 1975).

Subcutaneous fat thickness (mm) was measured with digital callipers and was averaged over three points into Ll from 6th rib.

The Ll samples from the 7th and 13th ribs were sectioned into steaks with a 2.5-cm thickness and were individually vacuum packaged and frozen at –18°C (24 h aging time group). The aging time group was placed in a refrigerator at a temperature of 2ºC for a period of 3, 7, or 14 days and after was frozen at –18°C further analysis.

Chemical composition

The chemical compositions of the Ll (moisture, ash, crude protein, and total lipids) were determined by using a 200 g sample that was thawed at 4°C for 16 h, homogenized, and then analyzed in triplicates by near-infrared spectroscopy (Foss NIR Systems, Inc., USA).

Water holding capacity

Determination of drip loss was calculated from the difference in the raw weight of the Ll samples and the weight after 24 h. The Ll samples were weighed, placed in netting, and suspended in plastic containers for a period of 24 h at a temperature of 4ºC. Drip loss was expressed as a percentage of the Ll initial weight (Honikel, 1998).

For thawing loss, the Ll samples were individually vacuum packaged and frozen at –18°C. Frozen samples were thawed at 4°C for 16 h. Thawing loss was calculated as a percentage of weight loss before and after thawing.

Cooking loss was determined by using an Ll sample that was thawed in a pre-heated grill at 170°C and monitored with a penetration thermocouple until the internal temperature reached 72°C. Ll samples were left to chill at room temperature (25ºC) and were weighed when the steak temperature reached 20°C. Cooking loss was calculated from the difference in the thawed weight of the Ll samples and cooked steaks and was expressed as a percentage of the initial weight.

Warner-Bratzler shear force (WBSF)

The Ll samples were thawed and cooked in a pre-heated grill at 170°C and monitored with a penetration thermocouple until the internal temperature reached 72°C. The Ll samples were divided into eight sub-samples, each one measuring 2.5 cm long and 1.0 cm in diameter. A Warner-Bratzler shear blade was used to measure shear force perpendicularly to the muscular

fiber orientation, according to the principles of Honikel (1998). These cores were sheared by using a Warner-Bratzler probe attached to a TA-TX2i texture analyzer (Stable Micro System, Surrey, United Kingdom) set at a speed of 20 cm min^{-1}.

Instrumental color

Thawed Ll samples were removed from the vacuum packaging and were allowed to bloom for 30 min. CIE L*(lightness), a*(redness), and b* (yellowness) values were measured in triplicate on the surface at three random locations using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan) with illuminant C, an 8 mm aperture, and a 2° observer angle.

Lipid oxidation (TBARS)

The extent of lipid oxidation of the Ll samples was assessed by measuring thiobarbituric acid-reacting substances (TBARS) by using the method described by Botsoglou et al. (1994). The Ll samples were thawed at 4°C for 16 h, homogenized by Ultra-Turrax (90 s, 20000 rpm; Fisher Scientific, Loughborough, UK), and were analyzed in triplicate. TBARS were expressed as mg malonaldeyde kg^{-1} of raw meat.

Fatty acid composition

The Ll fatty acids compositions were obtained by triacylglycerine methylation according to the ISO-R-5509 (1978) method. Fatty acid methyl esters (FAME) were analysed in a gas chromatograph (Varian, Walnut Creek, USA), equipped with a flame ionization detector and a fused silica capillary column CP-7420 (100 m, 0.25 mm, and 0.39 μmo.d., Varian, Walnut Creek, USA) Select Fame. The column temperature was programmed at 165°C for 18 min, 180°C (30°C min^{-1}) for 22 min, and 240°C (15°C min^{-1}) for 30 min with 45-psi of pressure. The injector and detector were kept at 220°C and 245°C, respectively. Gas flows (White Martins, São Paulo, Brazil) were 1.4 mL min^{-1} for carrier gas (H_2); 30 mL min^{-1} for make-up gas (N_2); and 30 mL min^{-1} and 300 mL min^{-1} for H_2 and synthetic flame gas, respectively. The sample was injected by using a split mode 180^{-1}. Fatty acids (FA) were identified by comparing the relative retention time of FAME peaks of the samples with standard FAME 189-19 from Sigma Company, St Louis, USA by spiking the samples with the standard. The peak areas were determined by using Star software (Varian, Walnut Creek, USA).

Statistical Analyses

Data were analyzed by using the GLM procedure of SAS (2004) to perform a randomized complete factorial design experiment with three diets and four meat aging times. The model included the fixed effects of cottonseed hull diets (CH21, CH27, and CH33), aging time (24 h, 3, 7, and 14 aging days), and their interaction by applying the following equation: $Y_{ij}=\mu+A_i+B_j+A_i\times B_j+e_{ij}$; where Y_{ij}=the observed value of the i aging time group effect and j cottonseed hull diets, μ=mean value common to all observations, A_i=fixed effect of aging time group, B_j=fixed effect of cottonseed hull diets, $A_i\times B_j$=interaction between aging time group and cottonseed hull diets, and e_{ij}=the error term. Tukey's test was used to compare treatment means and they were considered to be significantly different when $P < 0.05$.

RESULTS

There was no interaction among diets and meat aging time for any evaluated variables ($P > 0.05$). Thus, effects of diets and meat aging time were presented and discussed as principal effects.

There were no statistical differences for components of the carcass composition among diets ($P > 0.05$; Table 4), presenting all diets similar percentages of muscle, fat, bone and other tissues. The pH_{24h} results in the Ll were similar among the diets ($P > 0.05$; Table 5).

Meat from CH27 diet presented lower ($P = 0.02$; Table 5) moisture contents within the Ll, while the total lipids were reduced in meat from the CH33 diet ($P = 0.03$; Table 5). However, the ash and crude protein of the Ll were similar in meat from the bulls of the three diets ($P > 0.05$; Table 5).

The drip and thawing losses did not change in meat from the bulls fed with three diets ($P > 0.05$; Table 6). Likewise, the aging time did not change the drip and thawing losses ($P > 0.05$; Table 6). On the other hand, the meat from CH21 diets at 24 h presented with lower cooking losses than other diets ($P = 0.004$; Table 6). However, at seven and 14 days, the cooking loss was similar among diets ($P > 0.05$; Table 6).

The diets did not affect WBSF in Ll ($P > 0.05$; Table 6). On the other hand, Ll shear force was affected by aging time ($P = 0.001$; Table 6). The increasing aging time reduced the WBSF by 75% in all diets.

The instrumental colour was affected by diets and aging time ($P = 0.04$; Figure 1). Diet did not change ($P > 0.05$) lightness (L*, Figure 1a), redness (a*, Figure 1b), and yellowness (b*, Figure 1c) values for all diets and at the four aging times studied, except for CH27 diet, which presented greater L* ($P = 0.04$; Figure 1a) and b* ($P = 0.001$; Figure 1c) values at 24 h.

However, the values observed at 14 days were similar to those observed at 24 h. Contrastingly, a* values were greater at 24 h and 14 days and lower at three and seven days.

The lipid oxidation (TBARS) of Ll was lower in meat from the CH21 diet at 24 h (P = 0.001; Figure 1d). However, at 3, 7and 14 days the values were similar among the three diets (P > 0.05; Figure 1d). However, the TBARS values were influenced by aging time (P = 0.001; Figure 1d).

The fatty acid percentages were similar ($P > 0.05$) in the Ll muscle from bulls fed with three diets (Table 7). Similarly, the saturated (SFA), mono-unsaturated (MUFA), and poly-unsaturated fatty acids (PUFA) within Ll were similar (P > 0.05; Table 7) among three diets. Similarly, amounts of *n*-6 and *n*-3 fatty acids were not influenced (P > 0.05; Table 7) by diets. The diets did not affect (P > 0.05; Table 7) the PUFA:SFA and *n*-6:*n*-3 ratios.

DISCUSSION

Percentages of muscle (64.6%), total fat (16.1%), bone (16.1%), and other tissues (3.2%) within the Ll corroborated to compositions reported in other studies with crossbreed bulls (Maggioni et al., 2010). Muscle, fat, and bone compositions of the carcasses are influenced by the animal's breed; however, the fat percentage of the carcasses may be influenced by feed, feeding strategies, animal age, gender and crossbreeding (Campo et al., 2008, Warren et al., 2008). The subcutaneous fat (2.7 mm) presented with lower values than required by the market (3 – 6 mm), but no carcass was penalized by a reduced carcass fat distribution. The lower values that were observed were most likely related to the animal's utilized genetic group (½ Simmental – ½ Nellore). In general, the bulls from crossbreeding between Continental and Zebu breeds presented a low fat thickness (Prado et al., 2008a, Prado et al., 2009, Prado et al., 2008b).

The observed pH_{24h} values (5.7) were considered to be normal pH for bulls that were not stressed at slaughter time (Marıa et al., 2003).

The moisture (71.5%), ash (1.05%), and crude protein (23.4%) contents within the Ll of bulls that were fed with cottonseed hull diets agreed with the characteristics expected of young bulls (Serra et al., 2004), Simmental *vs.* Nellore steers (Padre et al., 2007, Prado et al., 2008a, Prado et al., 2008b), and animals fed with Brazilian co-products (Eiras et al., 2014). The mean values of the total lipids observed in Ll were considered to be below the values that are recommended (3%) by the English Health Department (HMSO, 1994). According to Pensel (1997), intramuscular fat concentrations below the maximum level (5%) are

recommended to prevent coronary heart diseases. Ll total lipid values of bulls that were fed with cottonseed hull diets in a high-concentrate system were similar to those of other studies (2-3%) in which bulls were finished in feedlots (Christensen et al., 2011, Prado et al., 2009, Prado et al., 2008b). The cottonseed hull diets influenced the proportion of soybean hull pellets within the diets and, could be influenced the fiber fermentation within the rumen (Bach et al., 1999). According to the NRC (2000), the non-fiber carbohydrates (*i.e.* pectin) present with high digestibility values in rumen fermentation and demonstrate a high correlation with the formation of acetate fatty acid.

The fiber content in the diets did not affect the drip loss, which was 2.9%. In general, the drip loss varies from 1 to 3% in bulls' carcasses (Frylinck et al., 2013). Thus, the drip loss that was observed in this study may be considered normal in bulls that are finished in feedlots and are slaughtered close to 500 kg of body weight (Waritthitham et al., 2010).

Thawing loss varied from 8 to 11%. The diets and aging time did not affect thawing loss. In general, meat from bulls that were finished in feedlots and fed with a high-concentrate diet presented with thawing losses between 9.4 and 12.4% (Eiras et al., 2014).

The cooking loss that was observed 24 h after slaughter was lower in meat from bulls that were fed with the CH21 (28.3%) diet in comparison to meat from bulls that were fed other diets (31.5%). However, the cooking losses in meat that was aged for 7 and 14 days were similar among the three diets. Thus, in general, the fiber content in the cottonseed hull diets and the aging time did not affect cooking loss. The values that were observed in this study were close to those observed in meat from crossbred bulls that were slaughtered between carcass weights of 269 and 328 kg (Waritthitham et al., 2010).

A Warner-Bratzler shear force (WBSF) value for cooked meat above 6 kgf/ cm^2 has been suggested as the threshold that separates tender and tough meat (Shackelford et al., 1997). In the present study, WBSF of cooked samples varied from 8.6 kgf (24 h of aging time) to 5.5 kgf (7 aging days) and 5.0 kgf (14 aging days). Therefore, the Ll from all diets in the present study may be considered tender after seven days of aging when using this criterion. Therefore, the meat aging time decreased the WBSF and was shown to tenderize the meat, as evaluated by several authors (Frylinck et al., 2013, Hopkins et al., 2013). Only meat from the 24 h aging time, which was included in the study, had WBSF values above 8.0 kg and hence, could be considered extremely tough. Conversely, the diets did not affect the meat WBSF.

The cottonseed hull diets did not affect the lightness (L*), redness (a*), and yellowness (b*) of the Ll by aging time. However, CH27 presented greater L* (38.2 points) and b* (7.41 points) at the 24 h aging time. According to Page et al. (2001), the Ll colour is influenced by

the pH, water activity, and fat composition of the muscle. In this study, the CH27 presented smaller moisture (71.1%) percentages and higher lipid (2.99%) values in the Ll than other diets. In general, until the 7th day of aging, the L* increased while the redness (a*) was reduced in all diets. However, at the 14th aging day, the increasing trend of L* and a* in the Ll were altered by the myoglobin oxidation (Page et al., 2001). The pattern of increasing metmyoglobin formation with aging time was also observed in the evolution of b* values in the Ll.

The lipid oxidation (TBARS) was affected by diets with 24 h aging time. Thus, the extension of lipid oxidation with CH21 was smaller for all aging times. CH21 presented with smaller cottonseed hull, while there was an increased proportion of the soybean hull pellets. According to Brouns et al. (2002), the isoflavonoids that are present in soybean products may reduce the free radical contents and have antioxidant properties in human health. King et al. (1998) observed that lower isoflavonoids values in the milk of cows that were fed silage did not elicit a biological response in humans. However, Lundh et al. (1990) observed significant plasma values of isoflavonoids in cows that were on pasture. Therefore, the increasing soybean hull pellets in the diet due the reducing levels of cottonseed hull could be reduced by the extension of the Ll lipid oxidation of CH21.

The fiber content in the cottonseed hull diets did not affect the individual fatty acid composition within the Ll. However, the composition of saturated (SFA) and polyunsaturated (PUFA) fatty acids was affected. The SFA (41%) percentages were reduced in the Ll of all diets, whereas PUFA (18%) content was increased when comparing several studies with similar conditions. Normally, SFA in Ll varies between 48-50% for ½ European - ½ Nellore bulls that are finished in feedlots (Maggioni et al., 2010) and in bulls with 3 or 4 mm of fat thickness (Albertí, Beriain, Ripoll, Sarriés, Panea, Mendizabal et al., 2014). However, PUFA presents with values between 3.9 – 10.9% for crossbred bulls and 6.5 - 8.9% values for animals in feedlot systems (Rotta et al., 2009). However, Simmental bulls presents with lower SFA means and high PUFA values (Padre et al., 2007; Prado, Marques, Rotta, Prado, Visentainer & Souza, 2009). Thus, the reduction in SFA and increase in PUFA could be related to animal crossbreeding. Conversely, the fiber contents in the diets may influence volatile fatty acids in ruminal fermentation by increasing the acetate acid formation (Bach et al., 1999). According to Pantoja, Firkins, Eastridge & Hull (1994), the fiber sources that were utilized in this experiment could be utilized at adequate levels for fatty acid depositions in milk and most likely in the meat of bulls. Therefore, the elevated insoluble carbohydrate contents in the diets increase the PUFA values (Prado, Marques, et al., 2009; Prado, Oliveira,

et al., 2009) while decreasing the SFA means (Rotta, Valadares Filho, Engle, Costa e Silva, Sathler, Prado et al., 2014) and changing the PUFA:SFA ratio (C. E. Realini, Duckett, Brito, Dalla Rizza & Mattos, 2004).

In general, meat from bulls that are finished in feedlots and fed with high energy density diets present with PUFA:SFA ratios between 0.10 and 0.20 (Rotta et al., 2009; Rotta et al., 2014). Conversely, the observed PUFA:SFA(0.44) in meat in this study is in accord with human health recommendations (0.40) (HMSO, 1994). Thus, the fiber sources in the diets (soybean hull pellets and cottonseed hull) from Brazilian co-products improved the PUFA:SFA ratio of meat, as observed in the meat from bulls that were finished in pasture systems (Padre et al., 2007; C. E. Realini et al., 2004), while the observed meat quality was like that of bulls finished in feedlot systems.

CONCLUSIONS

The use of cottonseed hull from 210 to 330 g kg^{-1} on a DM basis could be useful strategies in high-concentrate system to improve the instrumental meat quality and lean beef production. The lean meat production and the aging time effects on meat quality were obtained according to market demands.

ACKNOWLEDGEMENTS

This work was supported by the Araucaria Foundation and the Brazilian Council for Research and Technological Development (CNPq). The authors gratefully acknowledge the FORTMIX animal technology® (Maringá, Paraná, Brazil) for providing the co-products used in this research. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the Department of Animal Science, Maringá State University, Paraná or Brazil.

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Table 1. The effects of cottonseed hull diets from Brazilian agribusiness on animal performance, feed efficiency and carcass weights of young bulls fed in high-concentrate system

Parameters	Cottonseed hull diets			SEM	*P*-value
	CH21	CH27	CH33		
Initial age, months	10.9	11.3	10.8	0.14	0.63
Final age, months	16.3	16.7	16.2	0.14	0.63
Initial body weight, kg	318	317	318	6.09	0.77
Final body weight, kg	476	483	484	4.14	0.70
Average daily gain, kg day^{-1}	0.98	1.02	1.02	0.04	0.59

a-b: Values with different letters in the same row are different by Tukey test; SEM: Standard error of mean; CH21: cottonseed hull 210 g kg^{-1}on a DM basis; CH27: cottonseed hull 270 g kg^{-1}on a DM basis; CH33: cottonseed hull 330 g kg^{-1}on a DM basis.

Table 2. Compositions of diets provided to high-concentrate system

Ingredients	Cottonseed hull diets (g kg^{-1} of DM)		
	CH21	CH27	CH33
Soybean hull pellets	306	238	181
Ground corn	256	228	194
Sugarcane bagasse pellets	119	119	119
Corn gluten meal	77.9	115	147
Cottonseed hull	210	270	330
Yeast	7.53	7.53	7.53
Urea	5.07	5.07	5.07
Limestone	10.0	10.0	10.0
Mineral salt[1]	7.70	7.70	7.70

[1]Mineral salt composition (kg^{-1}): calcium, 175 g; phosphorus, 100 g; sodium, 114 g; selenium, 15 g; magnesium, 15 g; zinc, 6.004 mg; manganese, 1.250 mg; copper, 1.875 mg; iodine, 180 mg; cobalt, 125 mg; selenium, 30 mg; fluorine (maximum), 1.000 mg; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis.

Table 3. Chemical composition of ingredients and experimental diets

Ingredients	DM	g kg^{-1} on DM								
		Ash	OM	CP	EE	NDF	ADF	TC	NFC	ME
Soybean hull pellets	908	52.9	947.10	123.6	18.9	660	506	804	144	8.62
Ground corn	881	20.1	979.90	102.4	41.4	134	40.8	836	702	14.7
Sugarcane bagasse pellets	923	45.4	954.60	17.4	19.9	854	552	917	25.8	8.58
Corn gluten meal	908	82.5	917.50	234	25.5	410	112	686	275	14.1
Cottonseed hull	908	26.9	973.10	46.7	15.3	897	589	911	14.0	9.20
Yeast	932	46.7	953.27	355	21.3	25.5	9.35	580	554	
Urea	980	987	12.80	2610						
Limestone	984									
Mineral salt	988	997	3.33							
Diets										
CH21	905	51.9	938	110	24.1	559	363	819	256	10.5
CH27	905	52.3	938	110	23.5	579	367	820	237	10.5
CH33	906	52.9	937	110	22.7	604	376	822	214	10.5

DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; TC: Total carbohydrates; NFC: Non-fibre carbohydrates; ME: Metabolizable energy (MJ kg^{-1} DM) was calculated from NRC, 2000 model; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis.

Table 4. Carcass composition of bulls fed with cottonseed hull diets in high-concentrate system

	Cottonseed hull diets			SEM	*P*-value
	CH21	CH27	CH33		
Muscle, %	65.0	65.4	63.3	2.30	0.24
Intermuscular fat, %	10.6	10.5	12.1	0.79	0.98
Subcutaneous fat, %	5.23	5.15	4.65	0.37	0.46
Total fat, %	15.8	15.7	16.7	0.95	0.93
Subcutaneous fat, mm	2.60	2.97	2.46	0.20	0.58
Bone, %	15.9	15.4	17.1	0.62	0.91
Other tissues, %	3.20	3.49	2.90	0.29	0.48

SEM: Standard error of mean; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis.

Table 5. Chemical composition of the *Longissimus* muscle of bulls fed with cottonseed hull diets in high-concentrate system

	Cottonseed hull diets			SEM	*P*-value
	CH21	CH27	CH33		
pH_{24h}	5.79	5.76	5.70	0.01	0.09
Moisture, %	71.7[a]	71.1[b]	71.7[a]	0.10	0.02
Ashes, %	1.07	1.07	1.02	0.02	0.64
Crude protein, %	23.1	23.6	23.5	0.09	0.05
Total lipids, %	2.89[a]	2.99[a]	2.56[b]	0.07	0.03

a-b: Values with different letters in the same row were different by Tukey test ($P < 0.05$); SEM: Standard error of mean; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis.

Table 6. Water holding capacity and Warner-Bratzler shear force within the *Longissimus* muscle of bulls fed with cottonseed hull diets in high concentrate system

	CH21			CH27			CH33			SEM	*P*-value		
	24 h	7 d	14 d	24 h	7 d	14 d	24 h	7 d	14 d		CH	A	CH x A
Drip loss, %	2.31	-	-	3.18	-	-	3.03	-	-	0.15	0.06	-	-
Thawing loss, %	8.63	10.7	9.75	11.2	9.60	8.77	8.17	8.98	9.46	0.47	0.77	0.99	0.52
Cooking loss, %	28.3^{a}	28.5	30.6	32.1^{b}	31.3	33.2	31.1^{b}	30.8	33.1	0.41	0.004	0.06	0.98
WBSF, kgf/ cm²	8.32^{B}	5.00^{A}	4.60^{A}	8.31^{B}	5.64^{A}	4.86^{A}	9.29^{B}	5.79^{A}	5.32^{A}	0.27	0.45	0.001	0.89

a-b: Values with different letters in the same row were different by Tukey test;

A-C: Values with different letters in the same row were different by Tukey test to aging time;

CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis;

24 h: no aging days; 7 d: aging seven days; 14 d: aging 14 day; SEM: Standard error of mean;

CH: cottonseed hull effects; A: aging time effects; CH x A: interaction cottonseed hull x aging time effects; WBSF: Warner-Bratzler shear force**.**

Table 7. Fatty acids composition of the *Longissimus* muscle of bulls fed with cottonseed hull diets in high-concentrate system

Fatty acids, %	Cottonseed hull diets			SEM	*P* - value
	CH21	CH27	CH33		
SFA[1]	40.6	41.2	42.3	0.51	0.45
MUFA[2]	40.6	42.1	39.6	0.68	0.36
PUFA[3]	18.8	16.7	18.1	0.74	0.50
n-6	15.2	12.9	14.8	0.64	0.30
n-3	2.82	3.00	2.57	0.21	0.75
PUFA:SFA	0.47	0.41	0.43	0.02	0.47
n-6:n-3	5.55	5.32	5.93	0.41	0.84
Atherogenic index[4]	0.54	0.53	0.54	0.01	0.95
C 12:0	0.06	0.06	0.06	0.002	0.76
C 14:0	2.17	2.04	1.93	0.07	0.42
C 15:0	0.45	0.51	0.52	0.02	0.44
C 16:0	22.6	22.5	22.9	0.27	0.88
C 17:0	0.99	1.20	1.05	0.27	0.27
C 18:0	13.5	14.0	14.8	0.43	0.49
C 20:0	0.32	0.37	0.41	0.03	0.52
C 22:0	0.08	0.07	0.09	0.007	0.32
C 24:0	0.47	0.50	0.55	0.02	0.43
C 14:1 *n*-7	0.47	0.47	0.46	0.02	0.98
C 14:1 *n*-9	0.18	0.18	0.23	0.01	0.21
C 15:1 *n*-9	1.84	1.39	2.00	0.14	0.20
C 16:1 *n*-9	2.26	2.25	2.15	0.10	0.90
C 16:1 *n*-7	0.40	0.39	0.42	0.009	0.47
C 17:1 *n*-7	1.95	1.59	1.53	0.14	0.44
C 18:1 *n*-7	1.45	1.38	1.39	0.04	0.82
C 18:1 *n*-9	29.9	32.4	29.5	0.66	0.16
C 18:1 *trans*	1.95	1.88	1.76	0.16	0.90
C 20:1 *n*-9	0.19	0.15	0.16	0.01	0.61
C 18:2 *trans*	0.68	0.70	0.67	0.02	0.90
C 18:2 *n*-6	12.4	10.6	11.9	0.49	0.33

C 18:2 *c*-9 *t*-11	0.12	0.09	0.09	0.01	0.60
C 18:3 *n*-6	0.20	0.18	0.19	0.01	0.87
C 18:3 *n*-3	0.81	1.23	0.62	0.17	0.39
C 20:2 *n*-6	2.18	1.70	2.22	0.19	0.50
C 20:3 *n*-3	1.25	1.15	1.18	0.08	0.87
C 20:4 *n*-6	0.41	0.36	0.44	0.06	0.90
C 20:5 *n*-3	0.28	0.20	0.23	0.02	0.48
C 22:6 *n*-3	0.49	0.43	0.55	0.04	0.64

SEM: Standard error of means; CH21: cottonseed hulls 210 g kg^{-1} on a DM basis; CH27: cottonseed hulls 270 g kg^{-1} on a DM basis; CH33: cottonseed hulls 330 g kg^{-1} on a DM basis; [1]Saturated fatty acids ; [2]Mono-unsaturated fatty acids; [3]Poly-unsaturated fatty acids; [4]Atherogenic index: (C14:0 x 4) + C16:0 / (MUFA +∑n-6 + ∑n-3).

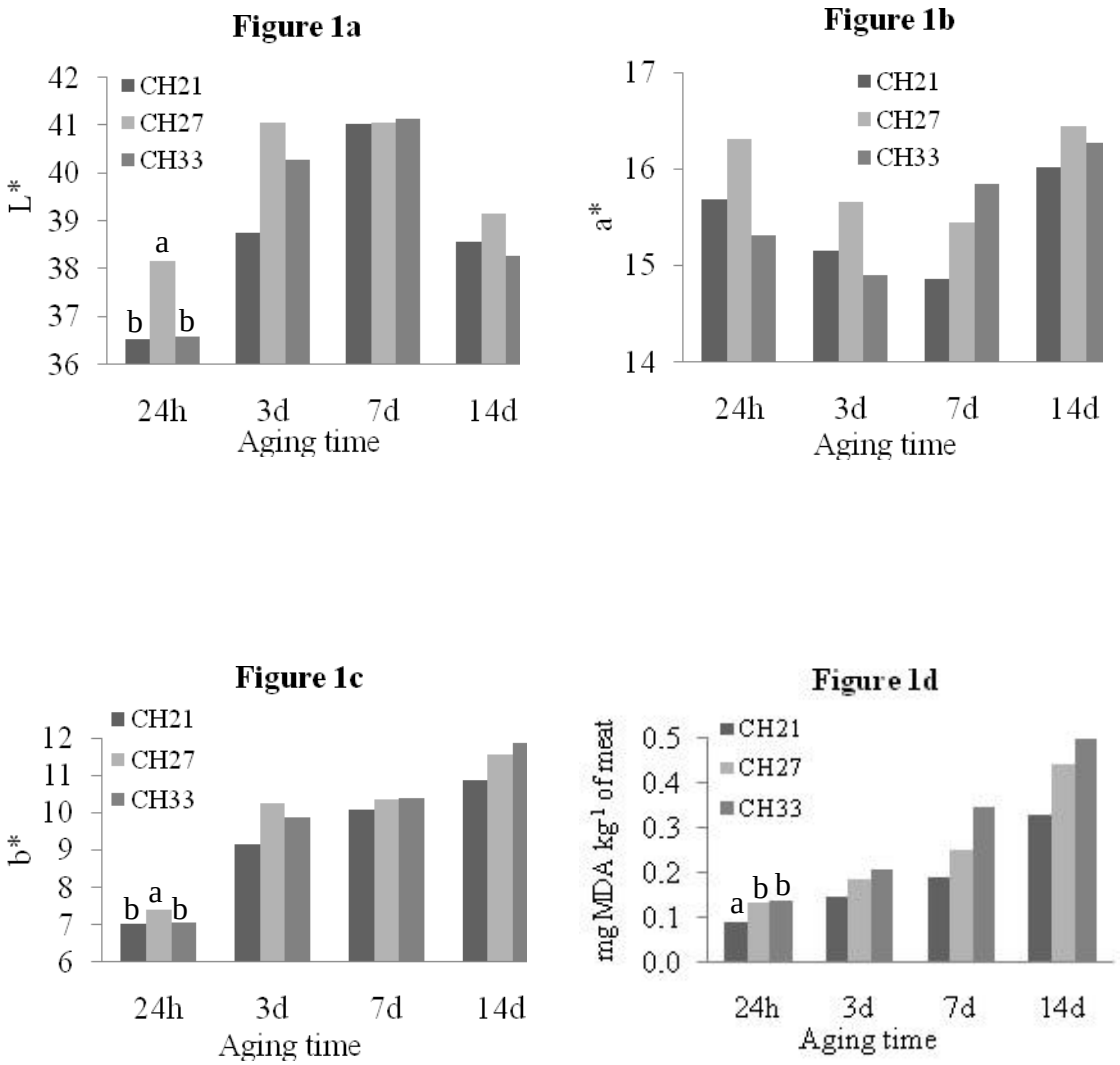

Figure 1. Lightness (L*), redness (a*), yellowness (b*) and TBARS (mg MDA kg^{-1} of raw meat) of the *Longissimus* muscle of bulls fed cottonseed hull diets in high-concentrate system with aging or no aging days.

V - Effects of cottonseed hull levels in the diet and ageing time on visual and sensory meat acceptability from young bulls finished in feedlot

Cottonseed hull levels: consumer acceptability

Journal: Animal. An International Journal of Animal Bioscience

Abstract

Cottonseed hull is a co-product of agribusiness which can be used in beef cattle rations, decreasing the cost of feed. The aim of this study was to evaluate the effects of different cottonseed hull levels, display and ageing times on visual and sensorial meat acceptability. *Longissimus thoracis* et *lumborum* muscle from thirty crossbred young bulls finished on three high concentrate diets (210, 270 or 330 g kg^{-1} of cottonseed hull on DM respectively) were visually evaluated during 10 days of display by 37 appraisers. Tenderness, flavour and overall acceptability from the three diets and three ageing (1, 7 and 14 days) were evaluated by 109 consumers. On the visual study, time of display ($P \leq 0.001$) was more significant factor than diet. Cottonseed hull level had no effect on sensorial analyses, with tenderness acceptability improving with ageing ($P \leq 0.001$). Results indicate the possibility of using the three studied levels of cottonseed hull without damaging consumer meat acceptability.

Keywords: alternative feeds, beef, colour, co-products, consumers test

Implications

To decrease production costs, co-product of agribusiness can be used in beef cattle rations. However quality of the final product should be not reduced. Our results show new data about addition of cottonseed hull on beef rations, complementing the existent information on co-products which help to improve farmers' income. The studied levels did not affect consumer sensory scores but can produce differences on visual appraisal in some intermediate times of display.

Introduction

The co-products from agribusinesses may be an option to obtain higher production efficiency and a greater meat quality (Eiras *et al.,* 2014; Polizel Neto *et al.,* 2014). USA, Brazil, China and India not only are the main beef producers in the world, but also they are, the most important cotton producers with Pakistan (FAOSTAT, 2015). Cottonseed hull is a singular co-product feed. They possess feeding characteristics that are different from most other high fibre feedstuffs. Cottonseed hull is comprised of the seed coat with some attached lint that is separated from the cottonseed kernel during oil production. Their availability, low cost, and excellent mixing characteristics have made them an important source of roughage for ruminants. The main component of cottonseed hull is neutral detergent fibre (NDF) which includes a relatively large proportion of acid detergent lignin. Thus, cottonseed hull inclusion in the diet for ruminants may be interesting to increase the fibre level in the high-concentrate diet.

The effects of those raw materials on production systems are not abundant, specifically consumer acceptability is unknown. Consumers are the last link in

the food chain; and their opinion and acceptability is essential for the development of a new product (Amerine *et al.,* 1965). Diet, production system and animal genotype could imply differences on meat sensory acceptability for consumers (Realini *et al.,* 2009; Realini *et al.,* 2013; Font-i-Furnols and Guerrero, 2014). In the same way, colour is a decisive attribute when consumers make purchase decisions regarding meat (Ripoll *et al.,* 2013). The visual and sensory consumers' acceptability is modified with time of storage, which could be appreciated in different ways depending on the personal background or consumption habits of consumers. Also, ageing time has an influence on global acceptability scores. Usually a study developed in European countries, shows beef liking (overall acceptability) increasing with ageing time (Pérez-Juan *et al.,* 2014).

Brazil is not only one of the principal beef exporters, but is also a large beef consumer; this is due to the population census and socioeconomic changes with an important increase in its internal development (Kirinus *et al.,* 2013). Thus, approximately, 80% of Brazilian beef production is absorbed by the internal market. Owing the scarce number of studies on visual and sensorial acceptability on meat from animal fed with this kind of co-product and the importance of beef consumption in Brazil, a visual and sensorial test were conducted in order to study the acceptability of meat from crossbreed (½ Simmental - ½ Nellore) young bulls fed with the co-product cottonseed hull in a high-concentrate system, after different times of display and ageing.

Materials and methods

Location, animals, diets and handling

This experiment was approved by the Ethics Committee of State University of Maringá (# 180/2014), and follows the norms recommended as international guiding principles for Biomedical Research Involving Animals (CIOMS/OMS, 1985).

Thirty crossbred young bulls (½ Simmental - ½ Nellore) were reared in individual pens. Three cottonseed hull diets (CH21: cottonseed hull 210 g kg^{-1} on a DM basis, CH27: cottonseed hull 270 g kg^{-1} on a DM basis, and CH33: cottonseed hull 330 g kg^{-1} on a DM basis) were assigned into the experimental units, with ten young bulls per group, in a complete randomized experimental factorial design. The cottonseed hull was included in the diets to provide an adequate NDF source for good animal performance. According to Mertens (1987), NDF level in ruminant diets should be below 1.2% of the body weight and above 35% on a dry matter basis.

The diets, offered *ad libitum*, were formulated to be isonitrogenous and isoenergetic and to provide a weight gain of 1.0 kg day^{-1} according to the NRC (2000) recommendations (Table 1). After a 15 day diet adaptation period, young bulls were weighed and the study was started at an average initial body weight of 319 ± 12.5 kg and an average age of 11 ± 0.8 months. Body weights were recorded each 28 days and the intake of concentrate was recorded daily until 162 days of the experiment, when the animals reached a final body weight of 481 ± 22.8 kg. The young bulls were kept in individual pens (10 m^2 for each animal). Data related to animal performance and carcasses are compiled on Table 2, being average daily gain of 1.0 kg ± 0.25 kg.

Diet chemical analyses

Chemical compositions of ingredients were presented in g kg^{-1} of dry matter (DM; Table 1). Dry matter was determined after oven-drying at 65°C for 24 h and milling through a 1 mm screen (AOAC, 2005; ID 934.01). Ash content was measured by combustion at 550°C for 16 h (AOAC, 2005; ID 942.05) to determine the organic matter. Nitrogen concentration was determined by the Kjeldahl method AOAC (2005; ID 988.05). Following the determination of the nitrogen concentration, the crude protein was calculated by multiplying the N content by a factor of 6.25. Ether extract content was determined by method AOAC (2005; ID 920.39). Neutral detergent fibre content was measured according to recommendations by Mertens (2002) while using alpha-amylase. The acid detergent fibre was measured by using the method AOAC (2005; ID 973.18). Total carbohydrates were estimated by the procedure of Sniffen *et al.,* (1992). Non-fibrous carbohydrates were determined as the difference between total carbohydrates and neutral detergent fibre. Metabolizable energy of feedstuffs was estimated according to NRC (2000) recommendations.

Slaughter procedure and muscle sampling

Young bulls were slaughtered at a commercial slaughterhouse 80 km from the feedlot. Upon arrival at the slaughterhouse, they were fasted from solids, kept in resting pens for 16 hours and humanely harvested under Brazilian federal inspection according to the Brazilian RIISPOA – Regulation of Industrial and Sanitary Inspection of Animal Products. Following slaughter, carcasses (265 ± 3.0 kg) were identified and chilled for 24 h at 4°C.

After chilling, *Longissimus thoracis* et *lumborum* (Ll*)* muscle from the right half carcass of each animal was excised between the 6^{th} and 13^{th} ribs for laboratory analysis. The pH (5.75 ± 0.03) was measured by using a probe-type portable pH meter (Hanna Instruments, Woonsocket, RI, USA) in the Ll at the 12^{th} to 13^{th} rib level.

Visual appraisal of the meat and willingness to buy

One Ll steak (2.5 cm of thickness) from the 7^{th} vertebra of each animal was sectioned and packaged in polystyrene trays over wrapped with a retractile films (Goodyear®, Americana, São Paulo, Brazil, with oxygen permeabilities of 8.200 $cm^3/m^2/d$, and rates (RH) of 262 $cm^3/m^2/d$) and stored refrigerated in a illuminated display at 4°C and light (fluorescent lamp, 380 lux, 12 h day^{-1}) simulating typical Brazilian market conditions. Time of display for steaks was 10 days. Selection criteria for participants were availability and motivation to participate on all days of the experiment. The main part of semi-trained appraisers had previously participated on other visual assessments being familiarized with the methodology used for visual colour evaluation. They were asked to evaluate exclusively the colour of the samples and no other factors that can influence on visual appraisal as marbling degree. Each day, the same 37 appraisers (26 women and 11 men, aged from 20 to 40 years) evaluated the visual appraisal, filling a sheet with the score of acceptability of each of the 30 steaks based on a hedonic unstructured numerical scale 9 points scale with only whole numbers from 1 to 9, were used to evaluate colour acceptability of each sample (representing from 1 = dislike extremely to 9 = like extremely) and the willingness to buy each piece (yes/no; Figure 2). In order to avoid any consumer tendency on sample evaluations, the codes placed on the trays that

identified each steak were changed every two days, as well as the location of samples on the meat showcase.

Consumer sensory evaluation

Four steaks (2.5 cm thickness steaks) were cut from Ll between the 8th and 12th ribs, from each animal. Three steaks were vacuum-packed and aged for 1, 7 or 14 days before being frozen at -18°C; they were retained for less than 3 months, and thawed at 4ºC for 24 h prior to analysis, with the fourth steak aged for 1, 7 or 14 days depending on the animal and treatment, in order to equilibrate the statistic sensorial design. Each steak was covered with aluminium foil codified with a random three digit code and cooked in a pre-heated grill (Philco Grill Jumbo Inox, Philco S.A., Brazil) at 200ºC until reaching an internal temperature of 70ºC, monitored with a penetration thermocouple (Incoterm, 145mm, Incoterm LTDA, Brazil). Each steak was cut into ten 2 x 2 cm cubes and kept warm (50ºC) until consumer evaluation (less than 10 minutes after cooking).

The consumer test for beef quality perception was performed during a National Livestock Exhibition in Maringá (Brazil) in a private room adequately adapted to perform a consumer sensory test. One hundred and twenty consumers were selected randomly within quotas of gender and age according to the Brazilian national profile (IBGE, 2010); a summary of the consumer profile is shown in Table 3. Before consumer testing, a questionnaire was applied (Figure 3), including closed questions with multiple choices, based on previous research on beef quality attributes (Banović *et al.,* 2010; Behrens *et al.,* 2010).

Consumer experimental design

Twelve sessions were carried out, each with 10 different consumers. In each session, every consumer evaluated nine samples, one for each type of diet (CH21, CH27, CH33) and ageing time (1, 7 or 14 days). Therefore, one steak from each animal on each experimental diet and with each period of ageing was evaluated.

Samples were served to consumers one at a time, following a randomized design to avoid any order or carry-over effects. Consumers were asked to eat unsalted toasted bread and rinse their mouth with water before evaluating each sample, including the first one. Consumers were only informed that they would be evaluating beef. All consumers were asked to taste the meat samples and evaluate the acceptability of three attributes: tenderness, flavour and overall acceptability; this was achieved using a structured hedonic 9 points scale ranging from (1 = dislike extremely; 9 = like extremely; Figure 4), where a medium level was not included according to methodologies described by Font-i-Furnols *et al.* (2008) and Realini *et al*. (2013).

One hundred and twenty consumers participated on the sensory test; however, consumers with missing data and outliers on the questionnaire were not considered for statistical analyses, leaving one hundred and nine consumers overall.

Statistical analyses

Subsequent visual appraisal and consumer tests were assessed via analysis of variance using General Lineal Model (GLM) procedures with SPSS v15.0 (IBM SPSS Statistics, SPSS Inc., Chicago. USA) for Windows. Visual attributes

were evaluated considering diet and time of display as fixed effects. Likewise, on consumer acceptability, diet and ageing time were considered fixed effects and the consumer was included as a random effect. Differences between means were evaluated using Tukey's test ($P \leq 0.05$).

Willingness to meat buy and the answer related to habit and preferences of consumption were analyzed by frequency of answer for each question.

To identify similarities between consumers on acceptability test, hierarchical cluster analysis was used to determine the different groups of consumers depending on overall acceptability using XLSTAT (v.7.5.3). The number of clusters was selected from the dendrogram, trying to find a compromise between homogeneity within clusters and heterogeneity between clusters. Principal Component Analyze was used to identify the relationship between treatments and meat attributes. Correlations between attributes were evaluated using the Pearson correlation coefficient.

Results and discussion

Visual appraisal of the meat and willingness to buy

Diet effect. There were differences ($P \leq 0.05$) between diets on visual appraisal during the display period from the second to the sixth day (Table 5); beef from CH33 diet presented higher values than those from CH21 diet, showing CH27 diet intermediate values. Thus, cottonseed hull increasing in the diets increased shelf-life, from an appraiser rate acceptability point of view, of meat from bulls fed high-concentrate diets. Typically, increased forage ingestion allows for meat with a redder appearance (Baublits *et al.*, 2004; Mancini and Hunt, 2005). The redder meat can be attributed to increased myoglobin, decreased muscle

glycogen, or both. Animals fed with forages sources presented higher vitamin E content on their meat. So, self-life increase and metmyoglobin is reduced. Consequently oxymyoglobin/ metmyoglobin ratio is higher giving redder colours, which are best accepted. The instrumental colour results from the present study were previously reported in Eiras *et al.* (2016). The L* values observed at 24 h ageing time resulted significant higher ($P \leq 0.050$) in the CH27 (38.1 points) diet, the same that redness (a* = 16.5 points) respect to the others treatments, indicating a lighter and redder meat in CH27 than in the CH21 and CH33 diets.

There were no differences ($P \geq 0.10$) between treatments CH21 and CH27, or CH27 and CH33 related to willingness to purchase. However buy intention was lower for the CH21 treatment compared to CH33 ($P \leq 0.05$), corresponding to its lower scores on visual appraisal. When meat is displayed for 4 days, only the 50% of participants would buy meat from the diet CH21. However, higher levels of cottonseed hull retained an acceptable level of willingness to buy, with over 61-65% buying on the same day of display.

Display time effect. The sensory attributes of meat are important to consumers. Thus, Carpenter *et al*. (2001) found a strong relationship between colour preferences and purchasing decisions of consumers, who discriminated against beef that was not red (e.g., purple or brown). Hence, visual assessments are a gold standard for measuring the success of any new production and consumer preferences and perception (Mancini and Hunt, 2005).

Throughout the 10-d display period, appraiser acceptance of the meat appearance decreased, gradually ($P \leq 0.001$) for the three diets (Table 5). Each day, progressively, appraisers evaluated steaks with a significantly lower score,

except for CH27 and CH33 diets, with values from the second day of display not differing from the first day. Thus, exposure time reduced shelf-life of meat as was previously observed (Vitale *et al*., 2014; Prado *et al.,* 2015). The gradual decline in visual appraisal was expected because oxidative processes are a natural cause of meat deterioration, which is particularly relevant for meat from concentrate-fed animals, because of the lower vitamin E content in not forage diets. The practical exposure maximum time of meat was 4 (for CH21 diet) or 5 days (for CH27 and CH33 diets). After 6 days, all of the scores attributed by appraisers were below 5, which can be considered inappropriate for human consumption.

Willingness to buy presented a similar evolution to visual score, with display being the most important effect ($P \leq 0.001$). During the first three days of display, the percentage of participants that would buy beef from the three diets was high; however, on the fourth (for CH21 diet) and fifth day (for CH27 and CH33 diets), it decreased to less than 50% acceptance, which could be considered the limit of visual acceptability.

Consumer sensory evaluation

There was no interaction ($P \geq 0.05$) between cottonseed hull diet levels and ageing time (Table 6). Thus, the results are presented and discussed separately.

Diet effect. The inclusion of different levels of cottonseed hull in the diet did not affect ($P \geq 0.001$) meat flavour, tenderness or overall acceptabilities (Table 6). Consumers scored acceptability attributes from the three cottonseed hull diets with favourable notes, with values in all cases ranging from 6.7 to 7.2 on a

hedonic 9 points scale. According to the results using the same animals, presented by Eiras *et al.* (2016), different levels of cottonseed hull did not modify fat characteristics (presented the three diets similar subcutaneous fat thickness, percentage of fat on 6th rib dissection), which could affect flavour perception (Kerth and Miller, 2015). However, the slight differences observed between the different levels of cottonseed hull on chemical composition, as percentage of moisture and total lipids, or cooking loss percentage at first day of ageing (Eiras *et al.,* 2016) does not seem to significantly modify the texture of samples, and did not affect any of the studied parameters of consumer acceptability.

Ageing time effect. Ageing time was a significant factor ($P \leq 0.001$) only for tenderness acceptability of meat (Table 6). Scores for flavour or overall acceptabilities did not differ ($P \geq 0.05$) between one, 7 and 14 days of ageing.

Ageing time influences the development of flavour precursors. Usually ageing improves meat acceptability before meat reach long maturations, which could develop off-flavours (Monsón *et al.*, 2005). However in some cattle breeds consumers did not perceive the flavour evolution, and consequently did not report differences on flavour acceptability even between the periods of 1 to 35 days of ageing (Monsón *et al.,* 2005).

According to instrumental values (Eiras *et al.,* 2016), the shear force necessary to cut a piece of beef decreased from the first to the 7th day of ageing, keeping low values until 14 days of ageing (5.3 - 4.6 kg/ cm^2). Similarly to consumers' scores for tenderness acceptability, which were significantly lower on the first day of ageing than those values obtained at 7 or 14 days. The

effect of ageing time on tenderness acceptability results are in agreement with other studies (Monsón *et al.,* 2005; Font-i-Furnols and Guerrero, 2014; Pérez-Juan *et al*., 2014), contributing to eating satisfaction (Font-i-Furnols and Guerrero, 2014) and consumers being willing to pay more if meat tenderness is guaranteed (Realini *et al.,* 2009). However, those consumer studies were developed in European countries with *Bos taurus* breeds. It is known that meat from zebu breeds (*Bos taurus indicus*) due to differences in protein breakdown *post mortem* and calpastatin-calpain activity, could be less tender than those from *Bos Taurus* (O'Connor *et al.,* 1997). Likewise, the crossbreed improved tenderness, as well as meat ageing time (Bianchini *et al.,* 2007). However, data obtained in this work show that consumers preferred texture from beef that had undergone a process of ageing of almost one week. The optimum time of meat ageing is not the same for all cattle breeds (Monsón *et al*., 2005). Bianchini *et al.* (2007) reported that after 7 days of meat ageing, the shear force from purebred *Bos taurus indicus* (Nellore) or *Bos taurus taurus* (Simmental), as well as their respective crossbreed, did not present any statistical differences. Also, Ferraz and Felício (2010) highlighted that in spite of Brazilian meat having a zebu component, it is not as tender, which could affect its acceptability in the international beef market; acceptable levels of tenderness are achieved after 7-14 days of ageing, which would involve the time of transportation to some international markets. Banović *et al.* (2010) reported that in a sensory blind test in Portugal, consumers did not report any differences in sensory dimensions between Brazilian and national beef.

In the current study, according to Pearson coefficients, overall acceptability was more strongly related to tenderness (r = 0.746) than flavour acceptability (r

= 0.487), with a lower correlation existing between texture and flavour (r = 0.205). However, differences in tenderness were not enough to obtain significant different values on overall acceptability between ageing times. As shown in other consumer studies, overall acceptability is not, in general, only modified by tenderness, but is also strongly correlated with flavour (Font-i-Furnols and Guerrero, 2014; Lepper-Blilie *et al.,* 2014). The lack of difference in the overall acceptability could be influenced by the breed involved (crossbreed – *Bos indicus taurus*) and production systems used (feedlot), because these two characteristics result in meat that is more tender than typical beef from pasturage and from older zebu animals; also, meat ageing is not a frequent practice in Brazil, and the population is used to consuming meat in the first few days after slaughter due to their habits (Mazzuchetti, 2004).

Principal component analyses

Information about diets and ageing preferences by consumers is graphically synthesized on the Figure 1. The first two principal component (PC) axes explained 93.55% of the total variance. Attributes of tenderness, flavour and overall acceptability are on the right side of F1, closely located on the graph to the three diets with 7 or 14 days of ageing. Meats with short ageing (1 day) were placed on the left side of F1, inversely related to acceptability attributes. Tenderness and overall acceptability are placed on the same quadrant for beef of any level of inclusion of cottonseed hull (CH21, CH27 and CH33 for both) aged for 14 days; the same happened with flavour acceptability, which is located on the right top quadrant and closely associated to diets with 7 days of ageing, specially low and high levels of cottonseed hull inclusion (for CH21 and

CH33 diets), as well as CH21 aged for 14 days. Intermediary levels of addition of cottonseed hull (for CH27 diet) is inversely correlated with flavour acceptability, presented lower scores in this attribute that the other two diets (for CH21 and CH33 diets), which were inversely correlated to tenderness scores.

Consumer questionnaire

The supplementary questionnaires distributed increase information about consumer habits and preferences of consumption and buying of participants in this study (Table 4). The most frequent consumption of beef was 5 times/week and the percentage obtained (34.9%) agreed with other Brazilian beef studies (Behrens *et al.,* 2010) and the high consumption beef rate of the country (Font-i-Furnols and Guerrero, 2014). According to Kirinus *et al.* (2013) the current socio-economical changes and the increase on the salary of Brazilian population consequently is reflected as an increase on beef consumption decreasing other basic aliment as rice or bean. The majority of consumers in the current study chose to buy their meat from a supermarket (53.2%), following by a butcher (44.0%), with a lower percentage of consumers purchasing the meat from boutiques (2.8%). A similar tendency had been previously reported in Brazil by Kirinus *et al*. (2013) or Mazzuchetti (2004). As Behrens *et al*. (2010) reported the increase of buy meat on supermarkets is consequence of the changes on food habits and modern life style, which demands time saving efforts and reduce time available. Supermarket let buy different kinds of products in the same establishment. Participants in the test preferred buying fresh meat (92.6%) than other packaging forms (3.7% vacuum packed; 1.8% on

tray and 1.8% aged). Nevertheless, buying meat directly on trays is an increasing habit due to it being easier to freeze and store (Velho *et al.,* 2009).

In this study, many of the consumers (40.3%) considered colour to be one of the most important attributes on beef purchase intention, which is in agreement with Ripoll *et al.* (2013). However, the lowest proportion of Brazilian participants (19.3%) considered the price is the most important factor in beef purchasing decisions, as happened with European consumers, who considered others factors such as origin or animal feed to be more important than price when choosing beef (Realini *et al.,* 2013). Regarding the kind of beef preferred, beef from heifers (57.8%) followed by steers (26.6%) was preferred compared to that from cows or bulls.

The traditional Brazilian beef production systems, due to agro-climatic conditions and pasture availability, are under extensive conditions and include zebu breeds (Ferraz and Felício, 2010). The current changes and demands have encouraged the development of highly efficient production systems in order to maximise the potential growth of the animals, reduce the age at slaughter, and increase the meat quality (Prado *et al.,* 2009; Ito *et al.,* 2012). The traditional Brazilian beef production was the best accepted by consumers. Thus, 50.4% of then answered that meat from pastures was the best option for purchase, whereas 27.5% believe that the beef production system does not influence their preferences. The feedlot (22.0%) was the least preferred production system.

Cluster analysis

Regarding beef acceptability, different groups of consumers exist, which constitute significant market segments that demand beef with different characteristics (Oliver *et al.,* 2006); those segments also occur when consumption habits, preferences for meat choice and attitudes to certain meat attributes are considered (Schnettler *et al.,*2009; Realini *et al.,* 2013),which is important to know in order to identify different market niches.

Four different clusters of consumers, related to overall acceptability, were found in the current research (Table 7). The larger cluster (1) of consumers (40.4%) described differences ($P \leq 0.05$) between diets, preferring the lower and intermediate cottonseed hull level (for CH21 and CH27 diets), but the acceptability scores for the three diets were higher than in the other clusters, with an average of 8.1 points on a 9 point scale. Ageing did not have any effect on the cluster (1). This cluster included a similar percentage of men (52.3%) and women (47.7%); however, the age range differed between sexes, with almost 70% of men being aged 18-25 and 41-55 years old, and 70% of women being aged 26-40 years old and 56 or over.

The second largest cluster (2) comprised 37.6% of consumers; this group did not report any differences between diets, with an average acceptability of 6.5 and showing values that were significantly higher ($P \leq 0.05$) for meat aged for 7 days than for 14 days, presenting intermediate values for meat after 1 day of ageing, with 6.5 points of acceptability. A similar number of men (48.8%) and women (51.2%) comprised this group, with more than 50% of men being placed in the age group from 26-40 years old and women being less than 25 years old.

Cluster 3 is formed by a small percentage of consumers (11.9%); however, this segment reported differences between diets ($P \leq 0.01$) and ageing periods ($P \leq 0.001$). Meat from the highest cottonseed hull content (for CH33 diet) and aged 7 or 14 days was preferred, with beef aged for 1 day scoring almost 1.5 point lower than the other ages. This cluster was characterized by people younger than 40 years, with a comparable distribution of sexes (46.2% men and 53.8% women).

The last cluster of consumers (4) was comprised of those who did not report any differences between cottonseed hull diets or meat ageing periods. However, 10.1% of the population rejected the meat produced with any level of co-products, presenting scores that were lower than 4.5 points. The characteristics of this group were that there were no men younger than 26 years old or women between 26 and 40 years old, with the distribution by gender of 54.4% men and 45.5% women.

Conclusions

The addition of cottonseed hull in high-concentrate diets for fed young bulls (½ Simmental - ½ Nellore) produced beef with adequate visual and sensory acceptability for Brazilian consumers. Likewise, 330 g kg^{-1} of cottonseed hull on DM provides the highest meat visual appraisal, packaged with oxygen permeable film, by appraisers during some of the intermediate display times. Also, no differences were reported for acceptance of sensorial attributes. Ageing, with a minimum duration of one week under vacuum conditions only increase tenderness acceptability. The positive results obtained on consumer acceptability are encouraging, as they make the use of this kind of co-product in

beef diets a viable option to reduce production costs. However, taking into account both: profitability of productive parameters and sensory results, it would be recommended until 330 g of cottonseed hull per kg of DM utilised as a non-forage fibre in high-concentrate diets to young bulls in feedlot.

Acknowledgements

This work was supported by the Araucaria Foundation and the Brazilian Council for Research and Technological Development (CNPq). The authors gratefully acknowledge the FORTMIX animal technology® (Maringá, Paraná, Brazil) for providing the co-products used in this research. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the Department of Animal Science, Maringá State University, Paraná or Brazil.

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Table 1. Compositions of ingredients and diets

	Cottonseed hull diets (g kg^{-1} of DM)		
	CH21	CH27	CH33
Ingredients			
Soybean hull pellets	306	238	181
Ground corn	256	228	194
Sugarcane bagasse pellets	119	119	119
Corn gluten meal	77.9	115	147
Cottonseed hull	210	270	330
Yeast	7.53	7.53	7.53
Urea	5.07	5.07	5.07
Limestone	10.0	10.0	10.0
Mineral salt[1]	7.70	7.70	7.70
Chemical Analysis			
Dry matter	905	905	906
Ash	51.9	52.3	52.9
Organic matter	938	938	937
Crude protein	110	110	110
Ether extract	24.1	23.5	22.7
Neutral detergent fibre	559	579	604
Acid detergent fibre	363	367	376
Total carbohydrates	819	820	822
Non-fibre carbohydrates	256	237	214
Metabolizable energy[2]	10.5	10.5	10.5

[1]Mineral salt composition (kg^{-1}): calcium, 175 g; phosphorus, 100 g; sodium, 114 g; selenium, 15 g; magnesium, 15 g; zinc, 6.004 mg; manganese, 1.250 mg; copper, 1.875 mg; iodine, 180 mg; cobalt, 125 mg; selenium, 30 mg; fluorine (maximum), 1.000 mg;[2]Metabolizable energy (MJ kg^{-1} DM) was calculated from NRC, 2000 model; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis.

Table 2. Effects of cottonseed hull levels in high concentrate diets on animal performance and carcass weight of young bulls

	Cottonseed hull diets (g kg^{-1} DM)			SEM	*P*–value
	CH21	CH27	CH33		
Initial age (months)	10.9	11.3	10.8	0.14	0.63
Final age (months)	16.3	16.7	16.2	0.14	0.63
Initial body weight (kg)	318	317	318	6.09	0.77
Final body weight (kg)	476	483	484	4.14	0.70
Average daily gain (kg d^{-1})	0.98	1.02	1.02	0.04	0.59
Cold carcass weight (kg)	258	263	258	2.65	0.55

a-b: Values with different letters in the same row statistically different by Tukey test; SEM: Standard error of mean; CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis. (Source: adapted from Eiras *et al*., 2016).

Table 3. Characteristics (age and gender) of the consumers involved in the trial (n=109 consumers)

	Total population	Men	Women
Age (%)			
< 25 years	27.5	23.6	31.5
26-40 years	29.4	30.9	27.8
41-55 years	22.9	25.5	20.4
> 56 years	20.2	20.0	20.4
Total (%)	100	49.5	50.5

Table 4. Answer of questionnaire about consumer preferences and habits of consumption (n=109 consumers)

Question	Answer	% Consumers
Q1. Frequency of beef consumption?	1 time /week	7.30
	2 times /week	13.8
	3 times /week	22.9
	4 times /week	21.1
	5 or more times /week	34.9
Q2. Place where buy meat?	Butcher	44.0
	Supermarket	53.2
	Meat boutique	2.8
Q3. How do you prefer to buy meat?	Fresh cut	92.6
	Vacuum packed	3.7
	On tray	1.8
	Aged	1.8
Q4. The most important factor when buy meat?	Price	19.3
	Consumption habits	19.3
	Colour	40.3
	Another	21.1
Q5. Which beef cattle category do you prefer?	Steers	26.6
	Bulls	5.5
	Heifers	57.8
	Cows	10.1
Q6. What system of beef cattle production do you prefer?	Pasture	50.4
	Feedlot	22.0
	Little importance	27.5

Table 5. Visual appraisal of meat from young bulls fed with cottonseed hull in high-concentrate system during display time[§] (n=37 appraisers)

Display (d)	CH21	CH27	CH33	SEM	*P*-value
1	7.29 A	7.30 A	7.47 A	0.045	0.236
2	6.85 Bb	7.03 A ab	7.23 Aa	0.043	0.002
3	6.18 Cb	6.50 B a	6.52 Ba	0.041	0.001
4	5.52 Db	5.91 C a	5.99 Ca	0.040	<0.001
5	4.84 Eb	5.10 D a	5.26 Da	0.039	<0.001
6	4.21 Fb	4.31 E ab	4.50 Ea	0.038	0.011
7	3.65 G	3.67 F	3.70 F	0.036	0.900
8	3.13 H	3.11 G	3.05 G	0.036	0.673
9	2.33 I	2.25 H	2.18 H	0.031	0.193
10	1.72 J	1.64 I	1.67 I	0.028	0.564
SEM	0.037	0.038	0.038		
P-value	<0.001	<0.001	<0.001		

CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis; SEM: Standard error of mean; A-J: different letters in the same column indicate significant differences ($P \leq 0.05$) on time of display; a-b: different letters in the same row indicate significant differences ($P \leq 0.05$) on diets; [§]Based on a hedonic 9 points scale (1: dislike extremely; 9: like extremely).

Table 6. Effect of cottonseed hull diets (CH) and ageing time (A) on consumer acceptability of meat from young bulls fed with high concentrate diets (n=109 consumers)[§]

Acceptability	Cottonseed hull diets			Ageing time			SEM	*P*-value		
	CH21	CH27	CH33	1 d	7 d	14 d		CH	A	CH x A
Flavour	6.91	6.78	6.88	6.76	7.03	6.78	0.07	0.511	0.116	0.832
Tenderness	7.03	7.21	7.12	6.83 b	7.20 a	7.33 a	0.06	0.392	0.001	0.784
Overall	6.90	6.99	7.02	6.80	7.06	7.05	0.07	0.617	0.139	0.282

CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis; SEM: Standard error of mean; a-b: different letters in the same row indicate significant differences ($P \leq 0.05$) on ageing time; [§]Based on a hedonic 9 points scale (1: dislike extremely; 9: like extremely).

Table 7. Mean and standard error of the overall acceptability scores of beef from young bulls fed with different levels of cottonseed hull and three ageing times among four consumer groups that were identified by cluster analysis (n=109 consumers)[§]

Overall acceptability			Cottonseed hull diets			Ageing time			SEM	*P*-value		
	n	% Sample	CH21	CH27	CH33	1 d	7 d	14 d		CH	A	CH x A
Cluster 1	44	40.4	8.04 AB	8.27 A	7.98 B	8.02	8.08	8.20	0.054	0.027	0.272	0.097
Cluster 2	41	37.6	6.59	6.58	6.45	6.48 ab	6.88 a	6.27 b	0.100	0.749	0.039	0.490
Cluster 3	13	11.9	6.59 B	6.15 B	7.74 A	5.67 b	7.18 a	7.64 a	0.208	0.004	0.001	<0.001
Cluster 4	11	10.1	3.88	4.39	4.48	4.48	3.60	4.67	0.258	0.550	0.366	0.694

CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis; SEM: Standard error of mean; A-B: different letters in the same row indicate significant differences ($P \leq 0.05$) on diets; a-b: different letters in the same row indicate significant differences ($P \leq 0.05$) on ageing time; [§]Based on a hedonic 9 points scale (1: dislike extremely; 9: like extremely).

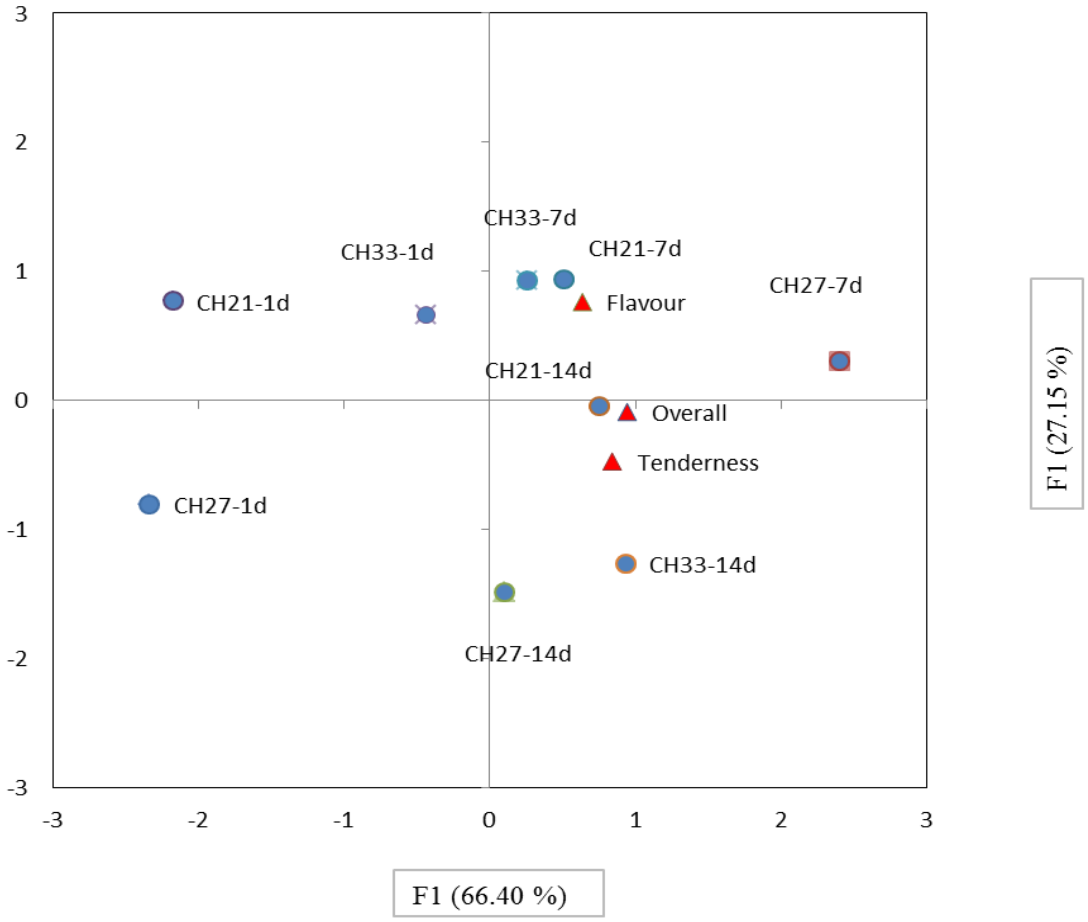

Figure 1. Principal Component Analysis of the scores for tenderness, flavour and overall acceptability of beef from young bulls fed with cottonseed hull (CH21: cottonseed hull 210 g kg^{-1} on a DM basis; CH27: cottonseed hull 270 g kg^{-1} on a DM basis; CH33: cottonseed hull 330 g kg^{-1} on a DM basis) and three ageing times (1, 7 and 14 days).