UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

GLICERINA, ANTIOXIDANTES E CAROTENÓIDES SOBRE A QUALIDADE E TRAÇABILIDADE DA CARNE DE BOVINOS E OVINOS

Autor: Fernando Zawadzki
Orientador: Prof. Dr. Ivanor Nunes do Prado

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Estado do Paraná
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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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Fevereiro - 2013

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

Z39g

Zawadzki, Fernando
Glicerina, antioxidantes e carotenóides sobre a qualidade e traçabilidade da carne de bovinos e ovinos / Fernando Zawadzki. -- Maringá, 2013.
201 f. : il. Algumas color.

Orientador: Prof. Dr. Ivanor Nunes do Prado.
Tese (doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, 2013.

1. Nutrição animal - Glicerina. 2. Nutrição animal - Óleos essenciais. 3. Bovinocultura - Ácidos graxos. 4. Bovinocultura - Músculo longíssimus - Coloração. Ovinocultua - Músculo longíssimus - Coloração. 5. Ovinocultura - Tecido adiposo perirenal e subcutâneo caudal. 6. Ovinos - Espectro de refletância. 7. Ovinos - Carotenóides. 8. Bovinos - Biodisel e Glicerina. 6. Bovinos - Características da carcaça e do músculo longíssimus. 9. Ovinos - Características da carcaça. 10. Carotenóides - Plasma sanguíneo. 11. Cordeiros - Traçabilidade. 12. Nutrição animal - Ácido ricinoleico. 13. Nutrição animal - Ácido anacardico, cardanol e cardol. I. Prado, Ivanor Nunes do, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. III. Título.

CDD 22.ed. 636.2085

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TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADA em 25 de fevereiro de 2013.

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"O êxito na vida não se mede pelo que você conquistou,
mas sim pelas dificuldades que superou no caminho."

(Abraham Lincoln - 1809 - 1865)

A

Deus, por estar sempre por perto, abençoando e iluminando o meu caminho, dando forças para alcançar minhas conquistas.

À

minha mãe, **Madalena Cochinski Zawadzki**, pelo incentivo, amor e carinho que dedicou a mim.

Ao

meu pai, **Olivio Zawadzki**, em sua simplicidade, exemplo de força de trabalho, carinho, amor e pelo grande incentivo e apoio aos meus estudos.

Aos

meus irmãos, **Carlos Eduardo Zawadzki** e **Márcio Édson Zawadzki**, pela amizade, carinho e apoio nos momentos difíceis.

Aos

meus avós, **Daniel Kochinski†** e **Julia Augustin Kochinski†**, e **José Zawadzki†** e **Ana Zawadzki†**, sempre me guiando de longe, mas presentes.

A

vocês, que em muitos momentos renunciaram aos seus sonhos para que os meus pudessem ser realizados.

Deixo o meu **OBRIGADO** e **CARINHO** a todos.

A vocês dedico esta **CONQUISTA!**

AGRADECIMENTOS

Ao Professor, Dr. Ivanor Nunes do Prado,

... na qualificação de pesquisador, quero ostentar meus parabéns pela iniciativa e persistência na elaboração de projetos, sem dúvida pelas muitas conquistas com aprovação de projetos, bolsas de Doutorado, Mestrado e Iniciação Científica, nos diversos órgãos de fomento de pesquisa, parabenizo pelo sucesso.

... na qualificação de Professor, agradeço pela orientação, ensinamentos, exemplo de determinação, empenho e participação nas atividades realizadas, por acreditar e me incentivar, como também pela paciência nos momentos difíceis, e em especial pela amizade construída ao longo destes anos, deixo meu agradecimento e orgulho ser seu aluno.

Ao amigo Jair de Araújo Marques† e família Rosa Saito Marques†, Thauany Saito Marques†. Saudade sempre existirá, mas os ensinamentos permaneceram. Dedico esta conquista a vocês, pois vocês fizeram parte deste resultado.

À Universidade Estadual de Maringá (UEM), especialmente ao Programa de Pós-graduação em Zootecnia (PPZ) pela oportunidade de realização deste curso, pela atuação idônea e pelo nível de excelência no ensino, pesquisa e extensão.

Ao Instituto Agronômico do Paraná (IAPAR), especialmente à direção e funcionários da Fazenda Modelo de Ponta Grossa – Paraná, pela oportunidade de realização do experimento no setor de bovinocultura de corte e pela disponibilização de recursos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão das bolsas de Doutorado no Programa de Pós-graduação em Zootecnia e bolsa Doutorado Sanduíche no Exterior.

À empresa Oligo Basics – Indústria e Comércio de Ração Ltda., Cascavel – Paraná, especialmente ao Joan Torrent, Lucio Aurélio Lopes de Oliveria e Evilasio Melo, pela cooperação e disponibilização de recursos para realização do projeto.

Ao Dr. José Luiz Moletta e Dr. Daniel Perotto, pelo apoio para execução deste projeto na Fazenda Modelo de Ponta Grossa – Paraná, pela confiança depositada à minha pessoa, pelos conselhos, orientações na execução do experimento e pela forte amizade construída ao longo desses anos.

Ao Departamento de Química, especialmente ao Professor Dr. Jesuí Vergílio Visentainer , Elton Guntendorfer Bonafe pela cooperação nas análises da composição de ácidos graxos e pela amizade construída durante esses anos de trabalho.

Ao Departamento de Ciências de Alimentos, em especial à Professora Dr. Grasiele Sacamaral Madrona, Silvana Aparecida da Silva Corradini, Camila Barbosa Carvalho, Lucinéia Aparecida Cestari, Rafaela Vacondio e Raíssa Sant'Ana Bueno pela colaboração e auxílio nas analises laboratoriais.

Aos amigos(as), Adriana Martins, Beatriz Silva Lima, Bruna Bonini Sestari, Carlos Alberto Fugita, Carlos Emanuel Eiras, Daniele Maggione Cheffer, Dayane Cristina Rivaroli, José Carlos da Silva, Juliana Akamine Torrecilhas, Lorrayny Galoro da Silva, Maria Carla de Oliveira Pires, Maribel Velandia Velero, Marival Gustavo de Oliveira, Maryon Guilherme Strack, Milene Puntel Osmari, Mônica Chavez Françoso, Olga Barreto da Cruz, Renato Manarelli Martins, Roberto Haryoshi Ito e Rodolpho Martin do Prado, pelo companheirismo, pelos momentos de descontração, pelo apoio nos momentos difíceis, pelo auxílio no desenvolvimento deste trabalho e pela amizade que construímos.

Aos amigos(as) Silvia Cristina de Aguiar, Néna Echassoux, Marcelo Nascimento de Oliveira, Joel Carneiro dos Santos Filho, Antonia Fernandes dos Santos, Heitor Liberiano, Emilie Raymond, Guillaume Vaz, Natália Vaz, Thaís Devincenzi, Lisandre Oliveira pela amizade construida durante o periodo de estadia na França, pelo apoio e realização deste projeto.

Aos técnicos do Laboratório de Análises de Nutrição Animal, Cleuza Volpato, Creuza de Souza Azevedo pelo auxílio e cooperação nas análises laboratoriais e ao secretário do PPZ Denilson dos Santos Vicentin pelo auxílo nos documentos durante o período de estudo.

Aos meus amigos Gustavo Arruda Bezerra, Guimadimar Cortez, Ivana Cintra Reiniz, Luiz Delfin de Mattos Junior, Rosana Afonso, Silvio Vieira Dauricio e à Bibiana Saito Marques, pelo apoio e amizade ao longo destes anos.

A todos os que acreditaram e confiaram na concretização deste trabalho, direta ou indiretamente, deixo aqui, o meu **OBRIGADO** e **CARINHO**.

REMERCIEMENTS

A Madame le Docteur Sophie PRACHE.
Chercheur à l'INRA, Theix – France,
qui m'a encadré et conseilé pour la realisation de ce travail,
qui me fait l'honneur de participer de mon jury de these,
et un très grand plaisir de pouvoir travailler avec vous.
Sincères remerciements.

A monsieur le Docteur Bruno MARTIN.
Checheur à l'INRA, Theix – France,
puor avoir m'accepté dans l'equipe Systèmes Biotechniques d'Elevage - SYBEL
Sincères remerciements.

À l'Institut National de la Recherche Agronomique – INRA,
pour accuellier et permettez-moi de developer ce projet de rechercher.
Sincères remerciements.

A l'equipe de l'installation experimentale en particuliuer Frederic ANGLARD, Pascal PAYARD, Cédric ROUGHEOL, Lionel CAVELLE, Bruno FREDOT et Etienne AISTER.

A l'equipe de l'abattoir experimentale Cédric COUSTET, Simon COLLANGE, Julien MONGIAT et Denis ROUX.

A l'equipe SYBEL en particulier Isabelle CONSTANT, Sandrine FAURE et Anne-Marie CHANEL.

A l'equipe Relations Animale-Plante et Aliments – RAPA en particulier Donato ANDUEZA et Fabienne PICARD.

A l'equipe Animal Muscle Viande – AMUVI en particulier David CHADEYRON et Jean-Luc MONTEL.

Je remercie a toute et a tous pour leur aide dans la réalisation de ce projet.

Sincères remerciements.

Au Pôle informatique en particulier Christiane ESPINASSE, pour accuellier dans votre bureau à l'INRA.

Sincères remerciements.

A l'equipe de Systèmes d'Elevage, Coordinations, Territoires – SELCET (METAFORT) en particulier Marcelo NASCIMENTO DE OLIVEIRA, Jean-Yves PAILLEUX, Cyrille RIGOLOT, Xavier COQUIL, Stéphane INGRAND, Yanik CURIER, Sylvie COURNUT, Nathalie HOSTIOU, Ildiko BALAZSA-BAJUSZ, Matthieu BOUCHON, et Benoit DEDIEU.

A Inès SNEESENS, Claudio POZO, Merien TOUATI, Claire COLLAS, Jose M. RODRIGUEZ, Lahlou BAHLOUL et Gonzalo CANTALAPIEDRA.

Je remercie a tous pour l'amitié que nous avons construi au cours de mon séjour en France.

Merci a tous.

BIOGRAFIA

FERNANDO ZAWADZKI, filho de Olivio Zawadzki e Madalena Cochinski Zawadzki, nasceu em Araruna – Paraná – Brasil, em 20 de Janeiro de 1980.

Em fevereiro de 1995, iniciou o curso Técnico em Contabilidade no Colégio Estadual Princesa Isabel, na cidade de Araruna – Paraná.

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Em Junho de 2011, foi aprovado no Exame Geral de Qualificação.

Em Julho de 2011 a Junho de 2012, realizou estágio de Doutorado Sanduíche no Exterior no Institut National de la Recherche Agronomique – INRA, Theix – France, Unité Mixte de Recherhes sur les Herbivores – Systèmes Biotechiques d'Elevage (SYBEL).

Em fevereiro de 2013, concluiu o curso de Doutorado em Zootecnia pela Universidade Estadual de Maringá – àrea de concentração: Produção Animal.

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RESUMO

No presente estudo, foram realizados dois experimentos: Exp. 1 foi avaliada a substituição parcial do milho pela glicerina (812 g de glicerol por kg/MS) e a adição óleos funcionais (óleo de caju e mamona) extraídos de plantas produzidas do norte do Brasil; e no Exp. 2 foi avaliado o efeito de uma dieta rica em carotenóides com baixo nível (L, 100 g/animal/dia) e alto nível (H, 400 g/animal/dia) de cevada, durante 75 dias de confinamento. No Exp. 1, avaliou-se o desempenho animal, ingestão de nutrientes, digestibilidade aparente, características da carcaça, qualidade do músculo *Longissimus*, composição de ácidos graxos da gordura perirenal, gordura subcutânea e do músculo *Longissimus*. O milho grão foi substituído por 203 g/kg/MS dia de glicerina e/ou adição de óleos funcionais (3 g/animal/dia). Utilizou-se 32 touros da raça Purunã (¼ Aberdeen Angus + ¼ Caracu + ¼ Canchim + ¼ Charolês) com 8 meses de idade e peso vivo inicial de 206,1 kg ± 20,0, terminados em confinamento por 252 dias. As dietas avaliadas foram: sem glicerina (CON), com óleos funcionais (FOL), com glicerina (GLY) e glicerina + óleos funcionais (GOF). O peso e rendimento de carcaça quente foram superiores para os animais alimentados com as dietas FOL, GLY e GFO (+ 5,0% e + 3,7%, respectivamente), em comparação à dieta CON. As dietas com glicerina melhoraram a conversão alimentar da matéria seca (+ 8,9%), em relação às dietas sem glicerina. As dietas GLY e GFO reduziram o consumo de fibra em detergente neutro (10,0 e 18,9%), em relação às dietas CON e FOL, respectivamente. A dieta FOL aumenta o teor do ácido esteárico (+ 15,3% *vs.* GLY e GFO), α-linolênico (+ 27,7% *vs.* GFO), somatório dos ácidos graxos saturados (+ 6,78% *vs.* GLY) e dos ácidos graxos poli-insaturados (+ 34,4% *vs.* GFO), entretanto, reduziu o somatório dos ácidos graxos monoinsaturados (- 10,0% *vs.* GLI e GFO) no músculo *Longissimus*. Dietas com glicerina reduziu o ácido linoléico (- 21,6% *vs.* CON e FOL) no músculo *Longissimus*. Dietas contendo glicerina e óleos funcionais não alteraram o desempenho animal, além disso, melhora o peso de carcaça, o consumo de ração e a digestibilidade aparente.

Portanto, até 20% da matéria seca da dieta pode ser substituída pela glicerina com a adição de óleos funcionais na dieta de touros terminados em confinamento. De modo geral, os óleos funcionais adicionados ou não em dietas sem glicerina melhoram a composição de ácidos graxos. No Exp. 2, foram investigadas as alterações da concentração plasmática de carotenóides, as características do espectro de refletância e cor do tecido adiposo de 24 cordeiros da raça Romane machos inteiros, confinados em baias individuais. A concentração plasmática de carotenóides no abate foi de 16% menor em cordeiros alimentados na dieta H. Cordeiros terminados na dieta H apresentaram carcaças mais pesadas e maior proporção de gordura do que cordeiros L. Os parâmetros de cor amarela e vermelha da gordura perirenal foram ligeiramente menores em cordeiros terminados na dieta H. O valor absoluto médio da integral (AVMI) foi calculado a partir do espectro de refletância do tecido adiposo na banda de 450-510 nm, a qual não foi influenciada pelos tratamentos. Os parâmetros de cor amarela, croma e AVMI da gordura subcutânea não foram afetados pelos tratamentos, entretanto, diminuiram com peso vivo inicial dos animais.

Palavras-chave: ácidos graxos, biocombustíveis, carcaça, carotenóides, cor, cordeiros, glicerina, gordura, refletância, touros.

ABSTRACT

In this study two experiments were conducted. Exp. 1 evaluated the effects of partial replacement of corn grain by glycerine (812 g of glycerol per kg/DM) and the addition of functional oils (cashew and castor oil) extracted of plants grown in northern Brazil; and Exp. 2 evaluated the effects of a diet rich in carotenoid with low (L, 100 g/lamb/day) and high-level (H, 400 g/lamb/day) of barley supplementation during 75 days of confinement before slaughter. In Exp. 1, animal performance, feed intake, apparent digestibility, carcass characteristics, quality of the Longissimus muscle, fatty acid composition of perirenal fat, subcutaneous and *Longissimus* muscle fat were evaluated of Purunã bulls finished in feedlot. The corn grain was replaced by glycerine at 203 g/kg of DM/day and/or by functional oils at a dose of 3 g/animal/day. 32 8-month-old Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolaise + ¼ Canchim) were used, weighing 206.1 kg (SD 20.0) and finished for 252 days in feedlots. The following diets were tested: without glycerine (CON), with functional oils (FOL), with glycerine (GLY) and with glycerine + functional oils (GFO). The hot carcass weight and the hot carcass dressing were higher for the animals fed the FOL, GLY and GFO (+ 5.0% and + 3.7%, respectively) diets compared to those fed the CON diet. Diets with glycerine improved dry matter conversion (+ 8.9%) in relation to diets without glycerine. The GLY and GFO diets reduced the neutral detergent fibre intake (10.0 and 18.9%) in comparison to the CON and FOL diets, respectively. Diets did not change lauric, myristic and palmitic acids on *Longissimus* muscle and subcutaneous fat. FOL diet increases the content of steraric acid (+ 15.3% *vs.* GLY and GFO), α-linolenic (+ 27.7% *vs.* GFO), saturated fatty acids (+ 6.78% *vs.* GLY), polyunsaturated fatty acid (+ 34.4% *vs.* GFO), and decreases monounsaturated fatty acids (- 10.0% *vs.* GLY and GFO) on *Longissimus* muscle. Diets with glycerine reduced linoleic acid (- 21.6% *vs.* CON and FOL) on *Longissimus* muscle. Diets containing glycerine and functional oils

did not change animal performance; furthermore, they improved carcass weight, feed intake and apparent digestibility. Thus, up to 20% of the DM of the diet can be replaced by glycerine with the addition of functional oils from cashew and castor plants to the diets of bulls finished in feedlot for 252 days and will provide a high-density energetic diet. In general, FO addition or not in diets without glycerine improve fatty acids on all tissues. In Exp. 2, changes in plasma carotenoid concentration, fat reflectance spectrum characteristics and color were investigated in 24 Romane male lambs kept in individual pens. Plasma carotenoid concentration at slaughter was 16% lower in H lambs than L lambs. H lambs had heavier and fatter carcasses than L lambs. Yellowness and redness of perirenal fat were slightly lower in H lambs than in L lambs. The mean absolute value of the integral (AVMI) was calculated from the reflectance spectrum of the fat in the 450–510 nm band which was not affected by the treatment. Yellowness, chroma and AVMI of subcutaneous fat were not affected by the treatment but decreased with initial live weight of animals.

Keywords: biofuels, bulls, carcass, carotenoid, fat color, fatty acids, glycerine, reflectance, lambs, plant oils

OBJETIVOS

Experimento 1:

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre o desempenho de bovinos Purunã terminados em confinamento.

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre a ingestão de nutrientes e conversão alimentar de bovinos Purunã terminados em confinamento.

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre as características de carcaça, composição química do músculo *Longissimus* de bovinos Purunã terminados em confinamento.

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre a proporção de gordura, músculo e osso da carcaça de bovinos Purunã terminados em confinamento.

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre os parâmetros de cor L*, a* e b* no músculo *Longissimus* de bovinos Purunã terminados em confinamento.

Avaliar a substituição parcial do milho pela glicerina e/ou adição de óleos funcionais sobre a composição de ácidos graxos na gordura perirenal, subcutânea e no músculo *Longissimus* de bovinos Purunã terminados em confinamento.

Experimento 2:

Avaliar os efeitos da suplementação de cevada sobre a concentração plasmatica de corotenoides de cordeiros terminados com dieta rica de carotenoides.

Avaliar os efeitos da suplementação de cevada sobre a deposição de gordura perirenal de cordeiros terminados em dieta rica em carotenoides.

Avaliar os efeitos da suplementação de cevada sobre os parâmetros de cor L*, a* e b* na gordara perirenal e caudal de cordeiros terminados em dieta rica em carotenoides.

Avaliar os efeitos da suplementação de cevada sobre as características do espectro de refletância de cordeiros terminados em dieta rica em carotenoides.

I – REVISÃO BIBLIOGRÁFICA

1. Introdução

Coprodutos da agroindústria (Farias et al., 2012a, Françozo et al., 2013 in press, Gomes et al., 2011, Gunn et al., 2010a, Mach et al., 2009, Parsons et al., 2009) e aditivos (Benchaar et al., 2006, Kazama et al., 2011, Martineau et al., 2008, Meyer et al., 2009, Prado et al., 2010, Valero et al., 2011b) são amplamente utilizados na alimentação de ruminantes com objetivo de melhorar a eficiência alimentar e reduzir os custos operacionais (Prado e Souza, 2007, Prado, 2010). A terminação de bovinos em confinamento é um sistema eficiente para garantir um bom desempenho animal e melhorar a qualidade da carne (Maggioni et al., 2010, Abrahão et al., 2005, Zawadzki et al., 2011a, Zawadzki et al., 2011b, Valero et al., 2011a), por outro lado gera custos elevados para o produtor, os quais trabalham com margens estreitas de lucro. A baixa rentabilidade do sistema exige a adoção de novas tecnologias para ser competitivo no mercado (Zawadzki et al., 2011b, Zawadzki et al., 2011a, Françozo et al., 2013 in press, Farias et al., 2012a). Entretanto, da mesma forma, o mercado consumidor nos países desenvolvidos estão mais exigentes com relação ao impacto ocasionado pela alimentação sobre a qualidade da carne (McAfee et al., 2010, Williamson et al., 2005, Melton, 1990), bem estar animal (Baumont et al., 2000, Stricklin e Kautz-Scanavy, 1984, Tarrant, 1989, Blackshaw e Blackshaw, 1994) e com a utilização de aditivos na alimentação animal (Benchaar et al., 2008, Guzmán-Blanco et al., 2000). Desta forma, novos conceitos de produção animal estão sendo adotados com o objetivo de repassar informações suficientes da origem do produto, e com certificação comprovada para o consumidor final (Prache, 2009, Dunne et al., 2009). Assim sendo, Prache e Theriez (1999) propõem como ferramenta analítica uma equação sobre espectro translatado na faixa de 410 para 510 nm com auxílio de métodos baseados em propriedades ópticas, ou

seja, a espectroscopia no infravermelho próximo – NIRS (400 a 2500 nm) e/ou espectroscopia no visível – VIRS (400 a 700 nm) para autenticar dietas de ruminantes.

2. Glicerina na dieta de ruminantes

2.1 Aspectos mercadológicos da cadeia produtiva do milho

O milho grão possui características nutricionais de excelente qualidade para alimentação animal. Utilizado em larga escala na formulação de dietas para animais de produção, sua proporção na formulação de dietas varia de acordo com a exigência nutricional da categoria animal (NRC, 2000). A utilização de cereais na dieta de bovinos proporciona excelente fonte de amido para o crescimento e manutenção dos microrganismos ruminais, os quais utilizam na produção de ácidos graxos voláteis (Van Soest, 1994).

De acordo com AGRIANUAL (2012), a produção mundial de milho em 2011 foi de 864,3 milhões de toneladas. Dentre os países produtores de milho, os EUA produziram 340,4 milhões de toneladas, que corresponde 39,3% do milho produzido no mundo. Em 2011 o Brasil produziu aproximadamente 56,5 milhões de toneladas, das quais 68,5 % é direcionada à nutrição animal. Já os EUA direcionaram 43,4% para nutrição animal e 35,1% para produção de etanol (FAPRI, 2012).

O direcionamento da produção milho para o setor bioenergético reduz a disponibilidade do produto para os demais segmentos da indústria (Ajanovic, 2011, Gorter e Just, 2010, Timilsina e Shrestha, 2011), oscilando o preço da commodity no mercado internacional (Figura 01), principalmente em períodos com déficit de produção ocasionados por problemas climáticos. A produção de milho no Brasil em 2012 foi de aproximadamente 51,0 milhões/ton., enquanto que a demanda interna foi de 48,3 milhões/ton. (AGRIANUAL, 2012), Figura 2. Aproximadamente, 68,5% da demanda interna do milho são direcionados à alimentação animal que corresponde a 38,7 milhões/ton. (FAPRI, 2012).

A demanda de milho na cadeia produtiva da carne bovina brasileira é extremamente baixa se comparado ao sistema de produção dos EUA. Entretanto, o uso de grãos de cereais como o milho tem sido a principal fonte de energia em dietas na terminação de bovinos em sistemas intensivos.

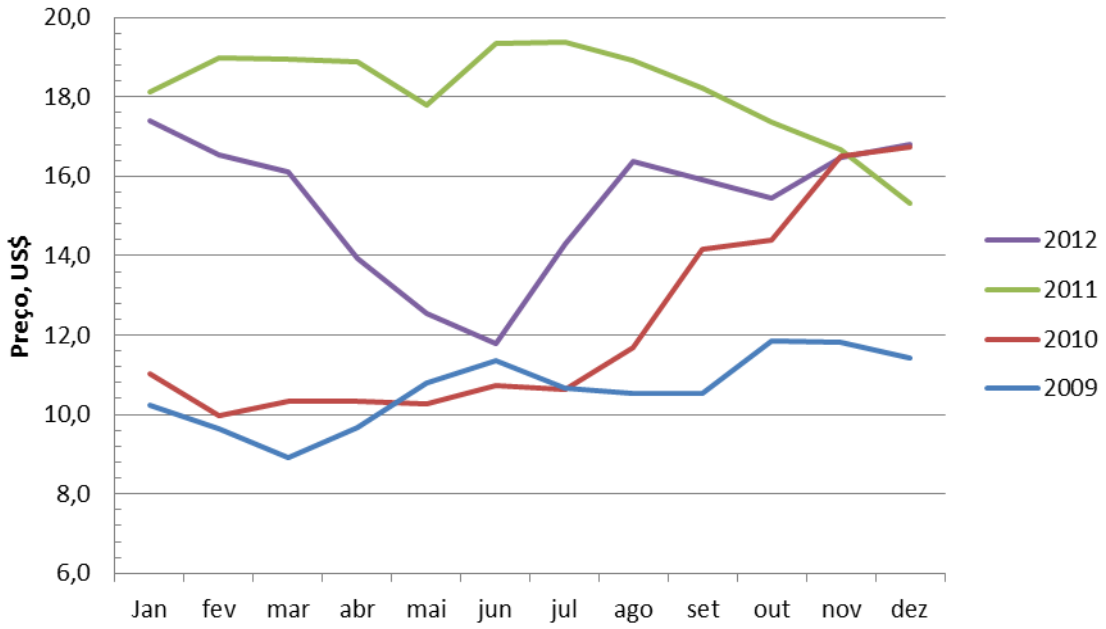


Figura 01. Variação do preço do milho grão saca 60 kg nos últimos 4 anos. Fonte: CEPEA (2013).

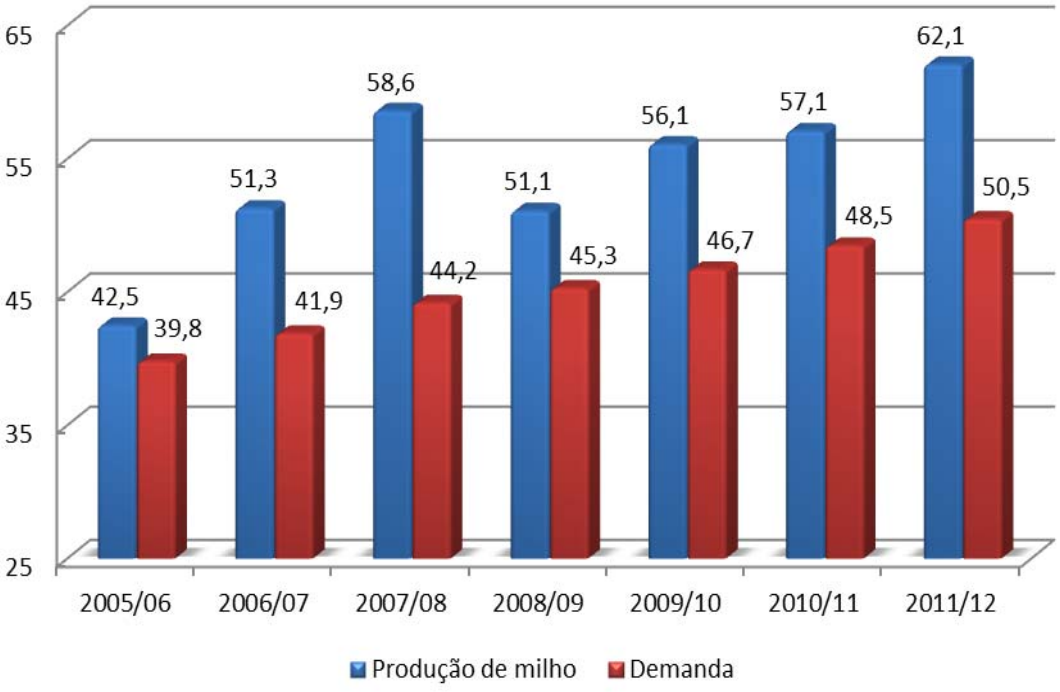


Figura 2. Produção e demanda de milho no Brasil (milhões de toneladas). Fonte: AGRIANUAL (2012).

De acordo com ANUALPEC (2013), apenas 1,7% do rebanho bovino é terminado em sistemas intensivos, e 1,46%, sistemas semi-intensivos, que corresponde aproximadamente 2,5 a 3 milhões de cabeças. No entanto, o custo de produção animal

dia/confinado nos sistemas de produção intensivos é alto, com baixas margens de lucro. Com a oscilação dos preços das commodities ou permanência de preços elevados justifica-se a necessidade de utilizar fontes alternativas em substituição parcial dos produtos convencionais. Neste contexto, a utilização de dietas contendo grãos de cereais convencionais eleva o custo de produção, desfavorecendo a competitividade da carne bovina no mercado.

2.2 Aspectos mercadológicos da cadeia produtiva do biodiesel

A indústria do biodiesel cresceu exponencialmente nos últimos anos para atender à demanda do mercado por uma fonte renovável de energia (Ajanovic, 2011, Govind Rao et al., 1969, ANP, 2013, Visser et al., 2011). De acordo com a Lei-11.097 (2005), o Art. 2º estabelece a adição de 5% em volume ao óleo diesel. Valor considerado como percentual mínimo obrigatório para a adição de biodíesel no óleo díesel comercializado para consumidor final.

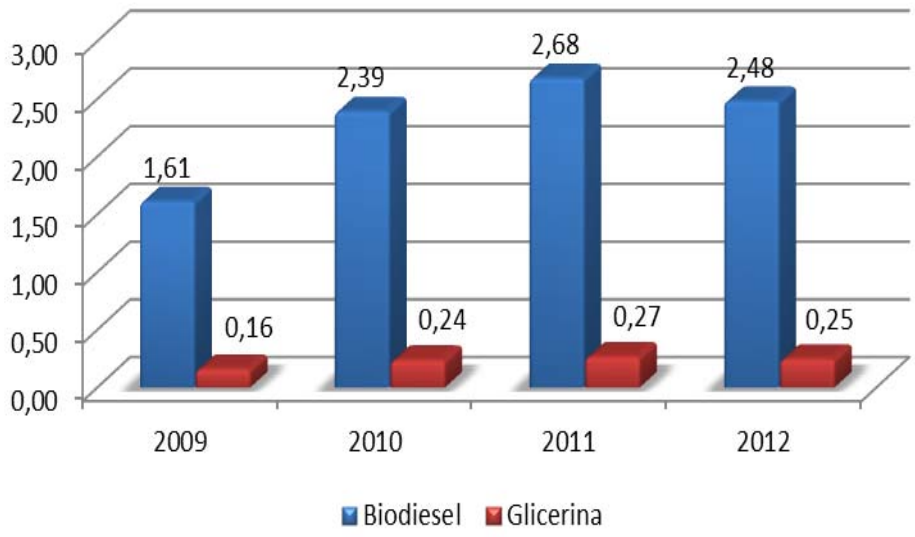


Figura 3. Produção de biodíesel e glicerina no Brasil. Fonte: ANP (2013). 2012 - produção referente aos meses de janeiro a novembro de 2012. ²Estimativa da produção de acordo com (Dasari et al., 2005).

Desta forma, o Brasil deverá produzir aproximadamente 2,25 bilhões de litros de biodiesel para cumprir com a legislação (Lei-11.097, 2005), que colocará no mercado um excedente de 225 milhões de litros de glicerina. Em 2012, o Brasil produziu o

equivalente a 2,48 bilhões de litros de biodíesel e 250 milhões de litros de glicerina (Figura 3).

De acordo com Dasari et al. (2005), para cada 10 litros de biodíesel produzido, gera-se um excedente de 1 L do coproduto glicerina. A glicerina é obtida após processo de transesterificação do óleo convencional com auxílio de ésteres metílicos (Ooi et al., 2004), que normalmente utiliza o metanol por apresentar baixo custo. Nas plantas industriais atuais, a principal matéria prima utilizada para produção de biodíesel são os óleos de origem vegetal (Ajanovic, 2011). Por outro lado outros produtos como gorduras de origem animal, óleos e gorduras residuais podem ser utilizados (ANP, 2013).

O teor de glicerol presente na glicerina varia de acordo com sua classificação, a qual pode ser classificada em baixa, média e alta pureza (Hippen et al., 2008). A glicerina de alta pureza disponível no mercado apresenta em média 99,8% de glicerina e 2,5% de água (Hippen et al., 2008). De acordo com Behr et al. (2007) e Knothe e Ramos (2006), a utilização de glicerina de alta pureza atende à demanda da indústria de alimentos por apresentar características atóxicas. E pode ser utilizado como aditivo no setor farmacêutico na constituição de xaropes, cápsulas, anestésicos, cremes e pomada; na indústria de cosméticos para fabricação de loções pós-barba, creme dental e para pele; e na indústria química para síntese de propileno glicol, formaldeído entre outros seguimentos (Behr et al., 2007, Knothe e Ramos, 2006).

De acordo com a resolução 21-CFR-Part-182 (2013), a glicerina é reconhecida como substância atóxica “Generally Regarded as Safe - GRAS”. No Brasil, a utilização de glicerina como aditivo na alimentação humana e animal é assegurada pela Resolução-nº386 (1999). Segundo Paule (2010), o glicerol disponibilizado para alimentação animal deve conter um padrão mínimo de qualidade. Para cada 1 kg de glicerina, deve conter no mínimo 800 g de glicerol e valores máximos 130 g de umidade e 150 mg de metanol (Paule, 2010).

Entretanto, no Brasil, a maioria das plantas industriais de produção do biodíesel não refina a glicerina bruta para obter glicerina de alta pureza, assim sendo, disponibiliza para o mercado maiores quantidades de glicerina de baixa e média pureza em função do alto custo para purificar a glicerina (Ooi et al., 2004). De acordo com Hippen et al. (2008), a glicerina de baixa a média pureza possui aproximadamente 63,3% a 85,3% de glicerol, sendo os demais constituintes água, lipídeos, fósforo, sódio

e metanol. Desta forma, o excedente de glicerina de média pureza no mercado torna-se atrativo para utilização em outros setores da cadeia produtiva.

2.3 Aspectos econômicos da substituição parcial do milho pela glicerina

A glicerina apresenta características energéticas semelhantes ao milho grão, desta forma várias pesquisas foram desenvolvidas utilizando a glicerina na alimentação animal (Mach et al., 2009, Wang et al., 2009a, Wang et al., 2009b, Parsons et al., 2009, Farias et al., 2012a, Farias et al., 2012b, Françozo et al., 2013 in press). Anteriormente citado, a composição química do glicerol varia em função de sua classificação (Hippen et al., 2008). De acordo com Paule (2010), a glicerina deve conter um padrão mínimo de glicerol e outros compostos, sendo adequado para alimentação animal.

A glicerina de média pureza apresenta aproximadamente 81,2% de glicerol, 4,76% de cinzas, 0,33% de metano, 0,01% de proteína bruta, 0,33% de extrato etéreo, 232 mg/kg de água, 11634,4 mg/kg de sódio, 79,1 mg/kg de potássio, 35,8 mg/kg de cálcio, 16,3 mg/kg de magnésio, 239,8 mg/kg de fósforo e 3656 cal/g de energia bruta (TECPAR, 2010, Fundação-ABC, 2010, LANA, 2010). De acordo com a literatura (Ooi et al., 2004, Hippen et al., 2008), a presença proteína bruta é ausente ou apresenta concentrações insignificativas, da mesma forma para gordura e fibra. No entanto a glicerina de média pureza apresenta valores energéticos (3656 cal/g) semelhantes ao do milho grão, caracterizando o produto como fonte energética.

A inclusão de glicerina substituindo parcialmente o milho, trabalhos anteriores demonstram a aplicabilidade da glicerina na alimentação de bovinos (Ferraro et al., 2009, Abo El-Nor et al., 2010, AbuGhazaleh et al., 2011). Entretanto, pela baixa ou ausência de proteína, a proporção da fonte protéica deve ser acrescida a partir de outra matéria prima. A inclusão de glicerina em substituição parcial do milho reduz o uso do milho grão na dieta, no entanto é necessário acrescentar à fonte de proteína para atender exigências nutricionais da categoria animal (NRC, 2000). De acordo com Lage et al. (2010), a inclusão de até 6% de glicerina bruta otimiza a conversão alimentar dos animais e reduz o custo do ganho de carcaça quando o preço do coproduto representa até 70% do preço do milho.

Neste contexto a viabilidade econômica e aplicabilidade da glicerina na alimentação animal devem ser cuidadosamente estudadas com base na análise de preço de diferentes fontes energéticas e protéicas presentes no mercado. A glicerina de média

pureza é um coproduto a ser utilizado para reduzir custo da alimentação animal em substituição de ingredientes energéticos nos períodos de oscilação dos preços.

2.4 Glicerina na alimentação de ruminantes, fermentação ruminal e metabolismo

A glicerina tem sido utilizada na alimentação animal para minimizar problemas metabólicos na dieta de vacas leiteiras no período de transição (Goff e Horst, 2001, DeFrain et al., 2004, Hippen et al., 2008). A glicerina na dieta de ruminantes é utilizada como fonte energética na substituição parcial de cereais. No rumem, o glicerol é utilizado pelos microrganismos ruminais para formação de ácidos graxos voláteis (Abo El-Nor et al., 2010, AbuGhazaleh et al., 2011, Wang et al., 2009a, Ferraro et al., 2009).

De acordo com Abo El-Nor et al. (2010), a inclusão de glicerina, não altera o pH do liquido ruminal, a concentração molar de propionato e NH_3-N, como também a digestibilidade da matéria seca. Entretanto, há redução do ácido acetato e aumento dos ácidos butírico, valerato e isovalerato. Por outro lado, a inclusão de 72 e 108g/kg de MS, a digestibilidade da fibra em detergente neutro, a relação acetato:propionato, concentração de DNA para *Fibrisolvens B.* e *Selenomonas ruminantium* foram influenciados negativamente.

Em outro trabalho, Parsons e Drouillard (2009) relatam que a inclusão de 2, ou 4% de glicerina bruta em dietas de alto concentrado reduz as concentrações de butirato, valerato e acetato, entretanto a concentração de propionato não foi influenciada. De acordo com AbuGhazaleh et al. (2011), a inclusão de 15, 30 e 45% de glicerina purificada (0,995g/g de glicerol) em substituição parcial do milho (30 a 45%), reduz a concentração de acetado, enquanto que a concentração de propionato aumenta ao nível de 45%. Além disso, a inclusão de 30 e 45% de glicerina reduziu as concentrações de DNA para as bactérias *Buturivibrio fibrisovens* e *Selenomonas ruminantium*, enquanto que *Albus Ruminococcus* e *dextrinosolvens Succinivibrio* não apresentaram diferenças.

Segundo Ferraro et al. (2009), a produção de gases *in vitro* utilizando glicerol como fonte energética resultou em maior volume de gás, por outro lado a metabolização foi mais lenta em comparação às outras fontes (propilenoglicol e melaço). Além de reduzir a produção de acetato com ligeiro aumento dos ácidos propiônico e butirico. Em outro trabalho, Wang et al. (2009a) relatam redução do pH ruminal e aumento da concentração de AGV's totais ao avaliar diferentes níveis de glicerina (100, 200 e 300 g

de glicerol). Segundo os autores, a relação acetato:propionato diminuiu linearmente como a suplementação de glicerol em função do aumento do propionato.

Gunn et al. (2010b) avaliando a inclusão de 15, 30 ou 45% de glicerina bruta com base na matéria seca, relatam que as concentrações séricas de glicose e insulina diminuíram com a inclusão de glicerina, entretanto as concentrações de *β*-hidróxibutirato foram superiores para os tratamentos com glicerina. Segundo os mesmos autores, o peso de carcaça quente e área de olho de lombo não apresentaram diferenças, porém a espessura de gordura decresceu com o aumento do nível de glicerina.

Wang et al. (2009b) relatam que a degradação ruminal da FDN do milho melhorou, no entanto a degrabilidade da proteína bruta reduziu em função do aumento de inclusão de glicerol. Além disso, a excreção urinária de purinas, a digestibilidade da matéria orgânica, proteína bruta e fibra em detergente neutro foram influenciadas com a inclusão de glicerol. Segundo os autores, os resultados indicam que a suplementação de glicerol melhora a fermentação ruminal com aumento da produção do ácido propiônico e a digestibilidade do alimento no trato digestivo de bovinos de corte.

Lage et al. (2010), avaliando a inclusão de 3, 6, 9 e 12% de glicerina bruta na dieta de cordeiros machos, relatam que a inclusão de até 6% de glicerina bruta otmiza a conversão alimentar dos animais e reduz o custo do ganho de carcaça quando o preço do coproduto representa até 70% do preço do milho. Por outro lado, Lage et al. (2010) reportam efeito negativo sobre o desempenho, consumo, digestibilidade e para as características quantitativas das carcaças.

De acordo com Mach et al. (2009), a inclusão 8% de glicerina não influenciou as avaliações de carcaça e qualidade da carne, entretanto, houve redução do pH ruminal com maior concentração de AGV's totais, insulina sérica e glicose. Da mesma forma, na dieta de 12%, não apresentou diferença no desempenho, nas variáveis de carcaça e qualidade da carne (Mach et al., 2009).

DeFrain et al. (2004) relatam maior concentração de AGVs totais e ácido propiônico, com redução da razão acetato:propionato. Segundo Schröder e Südekum (1999), o ácido butírico é convertido no epitélio ruminal e omasal em *β*-hidróxibutirato em função de sua toxidade. Osborne et al. (2009), avaliando metabólitos sanguíneos, observaram aumento na concentração sérica de *β*-hidroxibutirato com a inclusão de glicerina em comparação ao controle e óleo de soja.

De acordo com Zawadzki et al. (2010), o glicerol não metabolizado pelos microrganismos no rumém é absorvido à corrente sanguínea e metametabolizado no

fígado em glicose ou na síntese de triacilglicerol. Com a ação da enzima glicerol-cinase, o glicerol livre é fosforilado no fígado a glicerol-3-fosfato e destinado à formação de triacilgliceróis, fosfolipídeos ou glicose, em conjunto com ácidos graxos livres (Motta, 2009, Nelson e Cox, 2004). Os adipócitos são desprovidos de glicerol-cinase e obtém o glicerol-3-fosfato exclusivamente pela reação do glicerol-3-fosfato-desidrogenase. No fígado, rim e intestino delgado, ocorrem a fosforilação do glicerol livre em presença de glicerol-cinase. Os triacilgliceróis são sintetizados pela adição de acil-CoA graxo ao glicerol-3-fosfato ou à diidroxiacetona-fosfato (Motta, 2009, Nelson e Cox, 2004).

Os acil-CoA empregados na síntese dos triacilgliceróis são provenientes de ácidos graxos livres ativados pela ação das acil−CoA−sintetases. O glicerol-3-fosfato além de sua formação com o glicerol livre é sintetizado a partir da diidroxiacetona-fosfato gerada na glicólise ou formado a partir do glicerol pela ação da glicerol-cinase. A diidroxiacetona-fosfato é transformada em glicerol-3-fosfato em reação catalisada pela enzima glicerol-3-fosfato-desidrogenase (Motta, 2009, Nelson e Cox, 2004).

Entretanto, o direcionamento do glicerol para formação de triacilgliceróis somente ocorrerá em função dos níveis adequados de glicose circulante. Em níveis adequados de glicose aumentará a deposição de gordura que se torna interessante para atender às exigências do mercado com carcaças de melhor qualidade.

3. Extratos vegetais de plantas na alimentação animal

O uso rotineiro de antibióticos e promotores de crescimento na alimentação animal tem preocupado a saúde pública (Benchaar et al., 2008). As restrições impostas à utilização de antibióticos na alimentação animal têm como base preocupações no desenvolvimento de microrganismos resistentes pelo uso inadequado de ionóforos comprometendo a ação terapêutica dos antibióticos em humanos (Guzmán-Blanco et al., 2000, Russell e Houlihan, 2003, Dewulf et al., 2007).

Em ruminantes, a inclusão de ionóforos na dieta tem como objetivo manipular a fermentação ruminal para melhorar os processos benéficos (seleção das bactérias gram negativas) e minimizar ou excluir processos ineficientes (produção de gás metano – CH^4 e gás carbônico – CO^2). De modo geral, a ação dos ionóforos sobre as bactérias gram-positivas atuam modificando o fluxo de íons na membrana celular (Bergen e Bates, 1984, Russell e Strobel, 1989).

A ação dos ionóforos sobre a população de bactérias gram-positivas (*Peptostreptococcus anaerobius*, *Clostridium sticklandii* e *Clostridium aminophilum*) desempenha papel importante na fermentação de aminoácidos e na redução da produção de amônia ruminal (Russell e Strobel, 1989, Russell e Wallace, 1997). Por outro lado, a seleção das bactérias gram-negativas está relacionada à dupla camada de membrana celular, constituídas por lipoproteínas e lipopolissacarídeos que impedem a passagem das moléculas da monensina (Russell e Wallace, 1997).

Extratos naturais de plantas contêm uma ampla variedade de compostos com diferentes funções e mecanismos de ação (Benchaar et al., 2008). Os compostos naturais atuam de forma específica de acordo com sua estrutura química, ligando-se a sítios específicos na célula bacteriana, acarretando na desintegração da membrana citoplasmática, alteração do fluxo de elétrons e/ou coagulação do conteúdo celular (Benchaar et al., 2008, Burt, 2004).

Dentre os compostos que apresentam características de ação antimicrobiana presentes nas plantas, encontramos a classe dos compostos fenólicos (fenóis simples – *cetocol*, ácidos fenólicos – *ácido anacárdico, cinâmico, caféico e rícininoleico,* quinonas – *hipericina*, flavonóis – *totarol*, taninos – *elagitanina*, cumarinas – *warfarin*); óleos essenciais e terpenóides (*capsaicina, thimol, mentol, carvacrol, cânfora* e *eugenol*); alcaloides (*berberina, piperinae* e *teofilina*); polipetídeos e lectinas (*manose-aglutinina, fabatina e thionina*); e poliacetilenos (*heptadeca-dieno-diol*), cada um com seu respectivo mecanismo de ação (Burt, 2004, Benchaar et al., 2008, Peres et al., 1997, King e Tempesta, 1994, Perrett et al., 1995, Kubo et al., 1992, Stern et al., 1996, Cichewicz e Thorpe, 1996, Freiburghaus et al., 1996, Meyer et al., 1997).

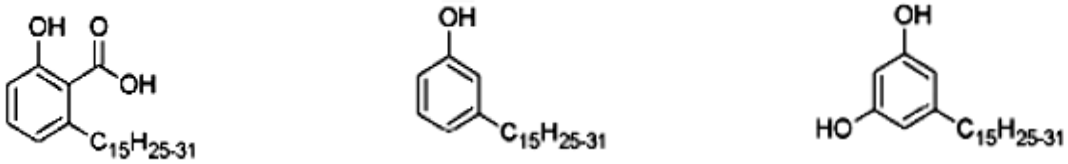
De acordo com Oldoni (2007), os compostos fenólicos determinam sua capacidade de atuar em função do grau de metoxilação e o número de hidroxilas para atuarem como agentes redutores contra o estresse oxidativo. O termo ácido fenólico é utilizado a fenóis associados a um ácido carboxílico funcional (Oldoni, 2007).

3.1 Caju (*Anacardium occidentale*) e óleo manona (*Ricinus communis* L) como aditivos na terminação de bovinos

O cajueiro (*Anacardium occidentale* L.) é uma planta nativa da Amazônia e Nordeste do Brasil (Mazzetto et al., 2009, Ohler, 1979). Durante o processo de beneficiamento da amêndoa, origina-se liquido da castanha de caju (LCC), o qual

possui várias aplicações na indústria (Gedam e Sampathkumaran, 1986, Trevisan et al., 2006, Calo et al., 2007). Por outro lado o LCC possui altas concentrações de lipídeos fenólicos, dentre eles o *ácido anacárdico, cardol e cardonol* (Mazzetto et al., 2009), Figura 4.

As concentrações dos ácidos variam em função do processo de obtenção da amêndoa (Mazzetto et al., 2009, Lubi e Thachil, 2000, Das et al., 2004). De acordo com Mazzetto et al. (2009), a concentração dos ácidos no LLC natural varia de 71,7 a 82,0% para o *ácido anacárdico*, de 13,8 a 20,1% para o *ácido cardol* e 1,6 a 9,2% para o *ácido cardonol* no processo de extração a frio.



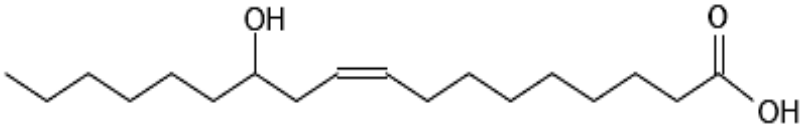
Ácido anacárdico *Cardol* *Cardonal*

Figura 4. Estrutura química dos principais compostos presentes no óleo de *Anacardium occidentale*. Fonte: (Mazzetto et al., 2009).

Entretanto o LLC técnico utilizando o processo de extração térmico, os teores variam de 1,1 a 1,7% para *ácido anacárdico*, enquanto que para *o ácido cardol* de 3,8 a 18,8% e para o *ácido cardanol* 67,8 a 94,6%. De modo geral, o LLC técnico é obtido com temperaturas elevadas que pode acarretar na alteração da estrutura química dos ácidos graxos pela reação de descarboxilação, originando maiores teores do *ácido cardonol* (Mazzetto et al., 2009).

A planta mamona denominada de *Ricinus communis* L (Weiss, 1983) está disseminada principalmente na região Nordeste do Brasil pelas características de adaptação ao clima seco e com elevadas temperaturas (Nóbrega, 2008, Devide et al., 2010). De acordo com Costa et al. (2004), o teor de óleo extraído da semente da mamona varia de 35 a 55%, apresentando altas concentrações do *ácido ricinoléico* (cis-12-hydroxyoctadeca-ácido-9-enóico) Figura 5. A concentração do *ácido ricinoléico* no óleo da semente de *Ricinus communis* L. corresponde de 85 a 90%, (Vaisman et al., 2008), seguido de outros ácidos graxos em menor proporção como o *ácido linoléico*

(4,2%), *ácido oléico* (3,0%), *esteárico* (1,0%), *palmítico* (1,0%), *ácido hidroxi esteárico* (0,7%), *ácido linolênico* (0,3%) e *ácido eicosanóico* (0,3%) (Ogunniyi, 2006).



Ácido ricinoléico

Figura 5. Estrutura química do principal composto do óleo de *Ricinus communis* L.
Fonte: (Ogunniyi, 2006).

De acordo com Ogunniyi (2006), o processo de extração do óleo de mamona pode ser obtido por prensagem mecânica ou com a utilização de solventes. No entanto, o processo de obtenção do óleo pode alterar a composição química dos compostos presentes no óleo de mamona (Ogunniyi, 2006). Entre os compostos presentes no óleo de mamona, o principal constituinte é o *ácido ricinoléico*, o qual possui hidroxila em sua estrutura química e confere maior densidade e viscosidade quando comparado aos outros óleos (Beltrão e Oliveira, 2009, Costa, 2006, Costa et al., 2009). A versatilidade do *ácido ricinoléico* permite a utilização do óleo na indústria farmacêutica e cosmética para fabricação de impermeabilizantes, lubrificantes, tintas, sabões, aditivos para polímeros e na produção do biodíesel (Costa et al., 2004, Chechetto et al., 2010, Beltrão e Oliveira, 2009, Silva et al., 2010, Zuchi et al., 2010).

Os compostos presentes no caju e no óleo de mamona apresentam características desejáveis para o setor industrial em diversos seguimentos. Entretanto, seus compostos podem ser utilizados como produtos bioativos, atuando como agentes antimicrobianos. De modo geral, compostos com hidroxila em sua estrutura permite interação com proteínas da membrana celular bacteriana ocorrendo à ruptura e morte do microrganismo (Novak et al., 1961, Mason e Wasserman, 1987, Toda et al., 1992, Kubo et al., 2003, Benchaar et al., 2008, Burt, 2004).

Os compostos presentes no *Anacardium occidentale* L são compostos dos *ácidos anacárdico, cardol e cardonol* os quais possuem hidroxila em sua estrutura química (Trevisan et al., 2006) os quais desempenham atividade antimicrobiana (Himejima e Kubo, 1991, Kubo et al., 1992, Kubo et al., 1993, Muroi et al., 1993, Muroi e Kubo, 1993, Nagabhushana et al., 1995, Kubo et al., 2003) e ação antioxidante (Kubo et al.,

2006). Segundo Muroi e Kubo (1993), os *ácidos anacárdicos* possuem atividade antimicrobiana, principalmente em bactérias gram-positivas.

De acordo com Lima et al. (2000), os *ácidos anacárdico, cardol e cardonol* presentes no óleo da casca da castanha de caju apresentaram atividade antimicrobiana sobre os microrganismos *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans* e *Candida utilis* e com maior atividade inibitória sobre a bactéria gram-positiva *Streptococcus mutans*. Da mesma forma, Muroi e Kubo (1993) relatam atividade antibicrobiana dos ácidos anacárdicos sobre as bactérias gram-positivas.

O óleo de *Ricinus communis* L. é composto por 85-90% do *ácido ricinoléico* (Vaisman et al., 2008), sendo o mesmo caracterizado pela presença de uma hidroxila (cis-12-hydroxyoctadeca-9-enoic acid), o qual desempenha ação antimicrobiana semelhante a um ionóforo divalente e ação anti-inflamatória (Maenz e Forsyth, 1982, Novak et al., 1961).

O sinergismo dos compostos presentes no óleo de *Anacardium occidentale* e *Ricinus communis L*. apresentam um grande potencial para ser utilizado como aditivo na manipulação da fermentação ruminal, em substituição dos ionóforos convencionais utilizados na terminação de bovinos. A adição de óleos funcionais (*Anacardium occidentale* e *Ricinus communis L*) na dieta de bovinos auxiliam o processo de fermentação e manutenção do pH ruminal para melhorar a eficiência microbiana.

De acordo com Coneglian (2009), a adição de óleos funcionais em dieta de alto grão não influencia a concentração dos ácidos propiônico e ácido butítrico, entretanto, a adição de 1g/dia de óleos funcionais aumenta a produção do ácido acético e proporciona maior concentração de ácidos graxos voláteis totais. Da mesma forma, Coneglian (2009) relata que a inclusão de 1g/dia de óleos funcionais proporciona a relação de 57:27:16 para acetato:propionato:butirato, enquanto os demais tratamentos apresentaram 51:33:16, respectivamente.

Coneglian (2009) relata que a adição de 2 e 4g/dia dos óleos funcionais aumenta em 3,4% no pH ruminal, quando comparado à dieta com monensina sódica. Nos resultados obtidos por Coneglian (2009), verifica-se melhor digestibilidade da MS e PB com inclusão de 2 e 4g/dia de óleos funcionais, em comparação aos níveis de 1 e 8g/dia.

Assim sendo, a funcionalidade dos compostos presentes nos óleos *Anacardium occidentale* e *Ricinus communis* L. desempenha importantes funções para auxiliar os processos de fermentação ruminal pela ação antimicrobiana, além de maximizar o desempenho de bovinos terminados em sistemas intensivos de produção.

4. Autenticação de dietas em ruminantes

A qualidade da carne é um fator determinante para o tempo de prateleira e para decisão de compra do consumidor. Por outro lado, além da qualidade do produto, nos últimos anos os consumidores procuram obter informações mais detalhadas sobre a origem dos produtos. Desta forma, nas últimas décadas, estudos foram aplicados para o desenvolvimento de ferramentas analíticas com o objetivo de traçar o histórico alimentar dos animais ruminantes (Prache et al., 1990, Prache e Theriez, 1999). Deste modo, a palavra traçabilidade tem sido utilizada para descrever o histórico do animal. De acordo com ISO 8402, a palavra traçabilidade pode ser definida como a habilidade de traçar a história, aplicabilidade ou localização real por registro e identificação.

Para autenticar o histórico alimentar de ovinos, compostos presentes nos alimentos, podem ser utilizados como marcadores naturais (Prache e Theriez, 1999). De acordo com (Prache et al., 2005), vários marcadores foram investigados para serem utilizaos como biomarcadores presentes nas plantas (pigmentos carotenóides, terpenos e compostos fenólicos), metabólicos do metabolismo do animal (ácidos graxos) e marcadores físicos (isótopo ^{15}N). Dentre os marcadores presentes nas plantas, os pigmentos carotenóides apresentam características desejáveis para discriminar dietas à base de forragens *vs.* concentrado (Dian et al., 2007b).

4.1 Concentração de carotenoides nos alimentos

A concentração de carotenóides nas forragens verdes depende da síntese e degradação (Nozière et al., 2006). Os pigmentos carotenóides são sintetizados naturalmente nas plantas, os quais conferem coloração amarela (xantofila), laranja (caroteno) e vermelha (licopeno) (Farré et al., 2010). Por outro lado, além de conferir coloração, os pigmentos são responsáveis pela absorção da energia solar para ser utilizada no processo de fotossíntese nas plantas (Farré et al., 2010).

Por outro lado, a concentração dos pigmentos carotenóides é influenciada diretamente pelo processo de conservação dos alimentos (Kalač e McDonald, 1981, Nozière et al., 2006, Dunne et al., 2009). De modo geral, as forragens verdes possuem altas concentrações de carotenóides, os quais variam entre 430 a 700 μg/g, de acordo com do período do ano (Prache et al., 2003), em menor proporção para silagens (140 a

240 μg/g), fenos de 55 a 140 μg/g (Nozière et al., 2006), concentrados 33,2 (Oliveira et al., 2012b) e palha que varia de 2 μg/g (Dian et al., 2007a) a 12,8 μg/g (Oliveira et al., 2012b). Por outro lado, a alfafa peletizada possui elevada concentração de carotenóides que varia de 280 μg/g (Dian et al., 2007a) a 920 μg/g (Oliveira et al., 2012b).

De modo geral, a degradação dos carotenóides ocorre pela oxidação, quando exposto à luz solar (Nozière et al., 2006). Dentre os carotenóides, os principais compostos analisados são a luteína, β-caroteno, zeaxanthina, 13-cis *β*-caroteno, 9-*cis β*-caroteno, violaxantina e neoxantina. A luteína apresenta maior concentração, seguida de *β*-caroteno no concentrato, alfafa, palha ou cevada (Oliveira et al., 2012b).

4.2 Digestão, absorção e metabolismo de carotenóides

Nozière et al. (2006) e Dunne et al. (2009) relatam degradação dos carotenóides no rumem. Nozière et al. (2006) cita dados de N. Cardinalut et al. (não publicados), reportam desaparecimento de 50% da luteína adicionada na dieta. Por outro lado, além da degradação, (Cardinault et al., 2006) relatam que os microrganismos ruminais têm capacidade de sintetizar *β*-caroteno e formas conjugadas de xantofilis. Os pigmentos carotenóides oriundos da dieta ou da produção pelos microrganismos ruminais, são incorporados em micellas no lúmen intestinal, absorvidos nos enterócitos e associados a lipoproteínas para serem transportados aos tecidos (Furr e Clark, 1997).

Em bovinos, os carotenóides absorvidos são associados com a lipoproteína de alta densidade – HDL (Bierer et al., 1995). Por outro lado, em ovinos, os carotenóides são associados com a lipoproteína de baixa densidade – LDL e de lipoproteína de densidade muita baixa – VLDL (Yang et al., 1992). Além destas associações, os carotenóis também estão associados aos quilomicrons, os quais representam o primeiro modo de transporte no plasma e distribuído nos tecidos (Nozière et al., 2006). Nos enterócitos e hepatócitos, os carotenóides são convertidos em vitamina A (Borel et al., 2005). De acordo com (Yang e Tume, 1993), em ovinos, há maior atividade da enzima β-carotene 15,15'-monooxigenase nos enterócitos que converte β-caroteno em vitamina A e menor atividade desta enzima em bovinos. Desta forma, em ovinos, haverá maior concentração plasmática de luteína circulante (Prache et al., 2003), enquanto em bovinos, maior concentração de *β*-caroteno (Nozière et al., 2006).

Segundo (Oliveira et al., 2012b), a concentração plasmática de carotenóides se estabiliza em 6 dias para ovinos, após a ingestão de dietas ricas em carotenóides. Da

mesma forma, (Prache et al., 2003) relata que a persistência de carotenóides no sangue, em ovinos em dietas ricas em carotenóides, reduz em 15 dias após receberem dietas com baixo teor de carotenoides. Estes são responsáveis pela cor do tecido adiposo em bovinos (Dunne et al., 2009) e ovinos (Dian et al., 2007b, Dian et al., 2007a, Prache et al., 2003).

4.3 Ferramentas analíticas para autenticar dietas de ruminantes

Nos últimos anos, métodos baseados em propriedades ópticas têm sido utilizados para autenticar dietas de ovinos (Prache, 2007, Prache et al., 1990, Prache et al., 2005, Prache e Theriez, 1999). Dentre os métodos baseados em propriedades ópticas, a espectroscopia no infravermelho próximo – NIRS (400 a 2500 nm) e/ou espectroscopia no visível – VIRS (400 a 700 nm) tem auxiliado para determinar os pigmentos carotenóides presentes no tecido adiposo de ovinos (Prache, 2009, Dian et al., 2008, Dian et al., 2007b, Dian et al., 2007a) e bovinos (Dunne et al., 2009, Röhrle et al., 2011), os quais se baseiam na capacidade de absorção da luz pelos pigmentos carotenóides (CIE, 1986).

A avaliação da cor baseada no sistema CIE (1986) que avalia a cor pela refletância da luz em três dimensões: L * representa luminosidade (100 = corresponde ao branco e 0 = corresponde ao preto) e a* (- a* representa o verde e + a* o vermelho) e b* (- b* representa o azul e + b* o amarelo) que representam a tonalidade de cor. Os pigmentos carotenóides depositados no tecido adiposo conferem a cor amarela. Desta forma, Prache e Theriez (1999) propõem uma equação matemática para quantificar os pigmentos carotenóides no tecido adiposo perirenal e caudal de ovinos a partir da absorção de luz pelos pigmentos carotenóides. De acordo com Prache e Theriez (1999), a equação matemática propõe determinar a intensidade de luz absorvida pelos carotenóides no espectro de refletância translatado entre 450 a 510 nm. O mensuramento do espectro (400 a 700 nm) no tecido adiposo perirenal e caudal utiliza a espectroscopia no visível – VIRS, a qual possui característica não invasiva e de fácil utilização (Prache, 2007).

A concentração de pigmentos carotenóides no tecido adiposo pode ser influenciada pela dieta (Dian et al., 2007b, Dian et al., 2007a), raça (Prache et al., 2010) e localidade do tecido (Priolo et al., 2002, Dian et al., 2007b). De acordo com Dian et al. (2007a) ao avaliar níveis de alfafa desidratada com diferentes concentrações de

pigmentos carotenóides, os resultados obtidos com a equação proposta por Prache e Theriez (1999) mostram que as dietas sem alfafa desidratada (com baixa concentração de pigmentos carotenoides) o valor absoluto do index varia de 114 para 236 unidades, enquanto o nível mais alto de alfafa desidratada varia de 218 para 391 unidades. Enquanto que ovinos terminados em pastagem, o valor absoluto do index varia entre 130 para 471 unidades no tecido adiposo subcutâneo e 195 para 629 unidades no tecido adiposo perirenal (Dian et al., 2007a).

Em outro experimento, Oliveira et al. (2012a) compara seis períodos de terminação (0, 15, 30, 45, 60 e 75 dias) em dieta de alta concentração de pigmentos carotenóides, respectivamente, o valor absoluto do index no tecido adiposo perirenal foi 176 para 288; 146 para 344; 197 para 336; 214 para 421; 225 para386 e 210 para 485 unidades, enquanto que, no tecido adiposo subcutâneo, variou de 151 para 225; 98 para 308; 179 para 345; 169 para 317; 191 para 316 e 206 para 389 unidades, respectivamente. Da mesma forma, avaliando o índice b*, os valores foram 10,25; 12,22; 12,53; 12,47; 13,23 e 15,40 para o tecido adiposo subcutâneo e 11,71; 13,15; 12,04; 14,37; 12,48 e 13,27 para o tecido adiposo perirenal, respectivamente para os seis períodos. De acordo com Oliveira et al. (2012a), a concentração de carotenóides e a média de b* (cor amarela) no tecido adiposo subcutâneo aumentam linearmente com a duração de consumo de alfafa, com alta concentração de pigmentos carotenóides; por outro lado, no tecido adiposo, obteve um comportamento curvilíneo.

Dian et al. (2008), utilizando a espectroscopia no infravermelho próximo – NIRS (400 a 2500 nm) e/ou espectroscopia no visível – VIRS (400 a 700 nm) em 120 ovinos em pastagem *vs.* 139 ovinos confinados, a média do valor absoluto do index no tecido adiposo perirenal para ovinos terminados em pastagem foi de 269 unidades, enquanto em confinamento, foi de 129 unidades. De acordo com Dian et al. (2008), os métodos NIRS e VIRS permitem classificar as carcaças de ovinos terminados em pastagem *vs.* confinados em dieta de baixa concentração de pigmentos carotenoóides *vs.* pastagem.

Entretanto, como mencionamos anteriormente, alfafa peletizada contém alto teor de carotenóides (Oliveira et al., 2012b, Oliveira et al., 2012a). A alfafa peletizada é um ótimo alimento protéico, podendo ser utilizado na terminação de ovinos em confinamento. Assim sendo, em animais terminados em dietas com diferentes teores de pigmentos carotenóides ainda não é possível autenticar a dieta pelo método proposto por Prache e Theriez (1999) e caracterizar as carcaças no momento do abate. São necessários mais estudos avaliando diferentes condições alimentares.

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II – Replacing of corn by glycerine and functional oils (cashew nut shell liquid and castor oil) as alternative additives in the diets of crossbred bulls finished in feedlot: animal performance, feed intake and apparent digestibility

ABSTRACT

This work was conducted to study the effects of replacing corn grain by glycerine (812 g of glycerol per kg/DM) and functional oils (extracted in northern Brazil from cashew nut shell liquid and castor oils) on the animal performance, feed intake and apparent digestibility of Purunã bulls finished in a feedlot. The corn grain was replaced by glycerine at a dose of 203 g/kg of DM/day and/or by functional oils at a dose of 3 g/animal/day. The study used 328-month-old Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolaise + ¼ Canchim) weighing 206 kg (SD 20.0) that were finished for 252 days in feedlot. The following diets were tested: without glycerine (CON), with functional oils (FOL), with glycerine (GLY) and with glycerine + functional oils (GFO). FBW (kg) and ADG were similar among the animals fed the various diets. However, the HCW and the HCD were higher for the animals fed the FOL, GLY and GFO (+ 5.14% and + 2.76%, respectively) diets in comparison with those fed the CON diet. The FOL diet increased the intake of DM (kg or % BW) and nutrients and improved the FE. DM conversion in the diets with glycerine improved (+ 9.42%) compared to the diets without glycerine. The GLY and GFO diets reduced the NDF intake (- 10.0 and - 18.8%) in comparison to the CON and FOL diets, respectively. However, bulls had a high intake of digestible (+ 22.6%) and metabolisable energy (+

Abbreviations: FBW, final body weight; ADG, average daily gain; HCW, hot carcass weight; HCD, hot carcass dressing; ADFi, indigestible neutral detergent fibre; ADF, acid detergent fibre; ADG, average daily gain; CP, crude protein; DM, dry matter; DMI, dry matter intake; EE, extract ether; FBW, final body weight; FE, feed efficiency; HCD, hot carcass dressing; HCW, hot carcass weight; NDF, neutral detergent fibre; NFC, non-fibre carbohydrates; OM, organic matter; TC, total carbohydrates; TDN, total digestible nutrients.

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22.6%) compared to those fed diets without glycerine. Diets containing glycerine and functional oils did not change the animal performance; furthermore, they improved the carcass weight, the feed intake and the apparent digestibility.

Keywords: bio-fuels, bulls, energy, feed efficiency, plants extract

1. Introduction

Intensive systems of animal production improve animal performance and meat quality (Ducatti et al., 2009; Fugita et al., 2012; Prado et al., 2008a; Prado et al., 2009a); however, they demand higher investments and technological alternatives (Capper and Hayes, 2012). In recent years, beef cattle producers have operated with a narrow profit margin. The cost of beef production has increased due to the supply and demand of the products used in diet formulations. Last year, the high cost of the ingredients was due to the influence of the climate on the grain yield (Fisher et al., 2012; Henry et al., 2012), the high demand for food production (Berry et al., 2012; Zilberman et al., 2012) and the production of ethanol as a renewable energy source (Mata et al., 2012; Zhang et al., 2012). Previous studies have evaluated co-products (Dian et al., 2010; Eiras et al., 2013a; b; Farias et al., 2012) and essentials oils (Benchaar et al., 2007; Benchaar and Greathead, 2011; Burt, 2004; Coneglian, 2009; Yang et al., 2010) as alternative additives to manipulate microbial fermentation in the rumen.

The biodiesel industry has rapidly expanded worldwide in the last few years (FAPRI, 2012). In 2011, the biodiesel industry produced approximately 2 billion L of glycerine worldwide, with the Brazilian market producing approximately 2.6 million L (FAPRI, 2012). Glycerine is formed during the transesterification process in biodiesel production (Meher et al., 2006). Research has demonstrated that glycerine is an

excellent energy source and can partially replace corn grain in ruminant diets (Eiras et al., 2013b; Lee et al., 2011). Others studies have reported that it has no effect on (Eiras et al., 2013b; Françozo et al., 2013; Mach et al., 2009) or decreases (Farias et al., 2012; Parsons and Drouillard, 2009) the DMI when fed to beef cattle.

Glycerol is involved in the process of gluconeogenesis (Krehbiel, 2008), improves the synthesis of glucose in the liver (Chung et al., 2007), provides energy for cellular metabolism (Goff and Horst, 2001) and improves fat deposition (Mach et al., 2009). In the ruminal metabolism, glycerol is a carbon source that microorganisms use to synthesise volatile fatty acids (Abo El-Nor et al., 2010). According to Rémond et al. (1993), the inclusion of glycerol caused a 27% decrease in acetic acid and a 37% increase of propionic acid. Moreover, Lee et al. (2011) reported that glycerol is rapidly fermented to propionate in the rumen. The increase in propionate has an interesting effect on animal performance because the majority of the propionate is utilised for gluconeogenesis in the liver (Forbes, 1988).

In contrast, functional oils contain secondary metabolites (terpenoids, phenolic compounds and others) that show antimicrobial activity toward gram-positive and gram-negative bacteria (Benchaar et al., 2008; Burt, 2004). Cashew nut shell liquid and castor oil contain a high percentage of compounds with characteristics that confer antimicrobial activity. According to Dorman and Deans (2000), this antimicrobial activity can be attributed to structural hydroxyl groups that have a high affinity for the lipids of bacterial cell membranes. Castor oil contains a high percentage of ricinoleic acid (Morris, 1967; Naughton, 2000), which has antimicrobial properties (Novak et al., 1961; Shin et al., 2004), as do their analogous derivatives (Narasimhan et al., 2007). Cashew nut shell liquid contain a high percentage of anacardic acid and a lower proportion of cardol and cardanol (Trevisan et al., 2006). Certain compounds in cashew

nut shell liquid confer antibacterial (Himejima and Kubo, 1991; Kubo et al., 2003; Muroi et al., 1993), antioxidant (Andrade et al., 2011) and anti-parasitic (Kubo et al., 1999) activities.

Previous studies (Gill et al., 2002) have demonstrated that synergism between the compounds increases the antibacterial activity. Thus, the synergism between the compounds in cashew nut shell liquid (anacardic acid, cardol, and cardanol) and castor oil (ricinoleic acid) may improve the antimicrobial effect. According to Coneglian (2009), effects similar to those of the combination of the compounds extracted from cashew nut shell liquid and castor oil were observed through the use of 4 g of sodium monensin/day in the high-grain diet of bulls. However, the effects on animal performance and feed efficiency in beef cattle being finished in feedlot are unclear. Thus, this work was conducted to study the effects of the partial replacement of corn grain by glycerine (812 g of glycerol per kg/DM) and/or functional oils based on the extracts of anacardic acid and ricinoleic acid that were produced in northern Brazil on animal performance, feed intake, feed efficiency and apparent digestibility of Purunã bulls finished in a feedlot.

2. Material and Methods

2.1. Locale, animals and diets

The experiment was conducted at the Experimental Farm of the Agronomic Institute of Paraná, in the city of Ponta Grossa, Paraná, south Brazil, and followed the guidelines for biomedical research with animals (CIOMS/OMS, 1985).

Thirty two Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were selected at birth (39.3 kg; SD 5.19) at 04 july 2008, and kept in a

pasture of *Hemarthria altissima* from birth until they were allocated into individual pens (8 m^2 for each animal) in a feedlot system (191.5 kg; SD 11.3) at 23 may 2009. During an adaption period of 21 days before the start of the experiment, the bulls were fed corn silage and concentrate in separate troughs (40:60 ratio, respectively). The concentrate contained soybean meal, corn grain and mineral salt *ad libitum*. At the start of the experiment (13 June 2009), Purunã bulls were assigned to birth BW and LW 206 kg (SD 20.0). The experimental design included four treatments with eight replications: CON – a diet without glycerine; FOL – a diet containing functional oils (3 g/animal/day); GLY – a diet containing glycerine (20.1% glycerine of DM basis), and GFO – a diet containing glycerol (20.1% glycerine of DM basis) and functional oils (3 g/animal/day), for 252 days before slaughter. The bulls were fed the concentrate and corn silage in separate troughs, with corn silage available *ad libitum*. Half of the feed was offered in the morning at 0800 h and the other half in the afternoon at 1600 h. The bulls were given access to a diet that was formulated to produce a weight gain of 1.2 kg/day to meet the requirements for fattening beef cattle (NRC, 2000). The concentrate intake was fixed at 1.4% of BW and adjusted at 28 days intervals. Samples of the corn silage and concentrate offered to the bulls were collected twice weekly to estimate the percentage of DM. All of the diets were formulated to be isonitrogenous (Table 3). Water and mineral salts were given *ad libitum*. The mineral salts contained (g/kg; as-fed) 150 Ca, 88.0 P, 0.08 Co, 1.45 Cu, 10.0 S, 1.0 Fe, 0.88 F, 0.06 I, 10.0 Mg, 1.10 Mn, 0.02 Se, 120 Na and 3.40 Zn.

The glycerine was produced in a soy-diesel facility, and the chemical composition was determined at the Institute of Technology of Paraná (TECPAR, Table 1). Glycerine was used as an energetic feed ingredient in the current study; therefore, to obtain four isoenergetic diets, the glycerine level was counterbalanced, predominantly

by decreasing the content of corn grain (Table 2). The functional oils (FO) contained ricinoleic acid, anacardic acid, cardanol and cardol. The ricinoleic acid was obtained from castor oil (extracted from castor seed) and the anacardic acid, cardanol and cardol derived from the cashew nut shell liquid; both were produced in northern Brazil. Vermiculite was used for solidification of the FO. In the FO contained 9% of ricinoleic acid, 4% of cardol and 20% of cardanol of level guarantee. The mixture of the FO was produced in the Analysis Laboratory of Oligo Basics Agroindustrial Ltd.

2.2. Animal performance and feed intake

The bulls were weighed at the beginning of the experiment and thereafter at 28-d intervals, after fasting from solid food for a period of 16 hours, to evaluate animal performance. The daily feed intake was estimated by the difference between the supplied feed and the refused feed left in the trough. The refused feed represented 5% of the total. During the collection period, samples of the supplied feed and the refused feed were collected and a representative composite sample was drafted per animal fed each diet.

2.3. Apparent digestibility in the entire digestive tract

Faecal collection was performed for a period of five days from the 196th to the 240th day of the feedlot experiment to obtain the apparent digestibility coefficient of the dry matter and other nutrients. Faecal samples (approximately 200 g wet weight) were collected for each bull at 2 to 3-h after feeding and pooled by bulls for each period. After being dried at 55°C for 72 h, the samples were ground in a feed mill and passed through a 1-mm sieve for chemical analyses. To estimate the flux of the faecal dry matter, indigestible neutral detergent fibre (ADFi) was used as an internal marker. The

samples were milled through a 2-mm sieve, packed (20 mg of DM/cm²) in 4 cm x 5 cm TNT bags (cloth non cloth) that had been previously weighed, and incubated for 288 hours in the rumen of a fistulated Purunã bull (Casali et al., 2008) that was fed a mixed diet of equal parts forage (corn silage) and concentrates (the same one used in this study). After incubation, the bags were removed, washed with water until total cleaned, dried in a ventilated oven (55°C for 72 hours) and then dried in an oven at 105ºC.

The ADFi was estimated from the difference in weight before and after the ruminal incubation of the samples. Faecal excretion was calculated using the following equations: FE = ADFi/ADFiCF, where FE = faecal excretion (kg/day), ADFi = ADFi intake (kg/day) and ADFiCF = ADFi concentration in faeces (kg/day). The apparent digestibility coefficients (ADC) for the DM and other nutrients were estimated according to the formula: DC = [(Intake – Excreted) / Intake] x 100.

2.4. Chemical analyses

The DM content of the samples was determined by drying them at 135 °C for 3 h according to the AOAC (1990) method. The OM content was calculated as the difference between the DM content and the ash content, with ash determined using combustion at 550 °C for 5 h. The NDF and ADF contents were determined using the methods described by Van Soest et al. (1991), using heat stable alpha-amylase for solubilisation of the amylaceous compounds (Mertens, 2002) and sodium sulphite in the NDF procedure, and the contents are expressed inclusive of residual ash. The N content in the samples was determined using the Kjeldahl method (AOAC, 1990). The total carbohydrates (TC) were obtained by applying the following equation: TC = 100 – (% CP +% EE +% Ash) (Sniffen et al., 1992). The non-fibre carbohydrates (NFC) were determined from the difference between the TC and NDF.

The total digestible nutrients (TDN) content of the diets was obtained using the methodology described by Kearl (1982): silage = -17.2649 + 1.2120 (% CP) + 0.8352 (% ENN) + 2.4637 (% EE) + 0.4475 (% CF); energetic foods = 40.2625 + 0.1969 (% CP) + 0.4228 (% ENN) + 1.1903 (% EE) + 0.1379 (% CF) and protein foods = 40.3227+0.5398 (% CP) + 0.4448 (% ENN) + 1.4218 (% EE) – 0.7007 (% CF). The samples were analysed in the Laboratory of Feed Analyses and Animal Nutrition at the State University of Maringá.

The gross energy contained in the feed and faeces was determined by analysing the dried samples (in duplicate) using a bomb calorimeter Parr® 6200 (Parr Instrument Company – USA). The calculation for the gross heat of combustion is $H_c = (WT – e_1 – e_2 – e_3) / m$, where H_c = gross heat of combustion; T = observed temperature rise; W = energy equivalent of the calorimeter being used; e_1 = heat produced by burning the nitrogen portion of the air trapped in the bomb to form nitric acid; e_2 = heat produced by the formation of sulphuric acid from the reaction of sulphur dioxide, water and oxygen; e_3 = heat produced by the heating wire and cotton thread; and m = mass of the sample. The digestible energy is then calculated by subtracting the gross energy excreted from the gross energy intake and expressing it as a percentage of the gross intake that was digested. Metabolisable energy was determined according to NRC (2000).

2.5. Carcass characteristics

The bulls were slaughtered at a commercial slaughterhouse 10 km from Ponta Grossa Research Farm, following the industrial practices of Brazil, when they reached a final BW of 468 kg (SD 31.5). After slaughter, the carcasses were labelled and chilled for 24 h at 4°C. After chilling, the right part of the carcass was used to determine the quantitative characteristics.

The hot carcass weight (HCW) was determined before chilling. The hot carcass dressing (HCD) percentage of the individual animal dressing was defined as the ratio of the hot carcass weight to the live weight. The cold carcass weight (CCW) was determined after the carcass had chilled for 24 h. The cold carcass dressing (HCD) percentage of the individual animal was defined by the ratio of the hot carcass weight and the live weight.

2.6. Statistical analysis

The data for animal performance, feed intake, feed efficiency and carcass characteristics were analysed with an ANOVA using the GLM procedure (SAS, 2002) to examine the effect of the diets. When necessary, the variance of the data was stabilised using the natural logarithmic or square transformation. The mean values were compared using Tukey's test at 10%.

3 Results

3.1. Animal performance and feed intake

Partial corn grain replacement by glycerine and FO addition did not change the FBW ($P > 0.53$) or the ADG ($P > 0.49$; Table 4). However, the HCW was enhanced in the animals fed the FOL, GLY and GFO (+ 5.14%) diets in comparison to those fed the CON ($P < 0.0001$) diet (Table 4). Likewise, the HCD was higher ($P < 0.02$) for the animals fed the FOL, GLY and GFO (+ 2.76%) diets than fed the CON diet.

The inclusion of glycerine and FO in the diets did not change the ratio of corn silage to concentrate (%), or the corn silage (%/BW) and concentrate (kg/day) in the

feed (P > 0.53; P > 0.47; P > 0.31 and P > 0.17, respectively; Table 5). However, the mean daily concentrate (%/BW) was higher in the CON, FOL and GLY diets (+ 4.23%) than in the GFO diet (P < 0.0003). A higher corn silage (kg/day) intake was observed for the animals fed the FOL diet (+ 13.4%) in comparison to those fed the CON diet, which was similar to that of the animals fed the GLY and GFO diet (P < 0.09). Similar effects were observed for the DM (kg/day) intake, the GLY and GFO results were similar to the CON and FOL result, whereas the FOL result was higher (+ 7.76%) than the CON value (P < 0.0001). Likewise, the DM intake (%/BW) was higher with the FOL diet (+ 6.36%) compared to the CON diet; whereas the GLY and GFO values were similar (P < 0.08). The DM conversion improved with the GLY and GFO diets (+ 9.42%) than with the FOL diet; whereas CON was similar (P < 0.02). Similar effects for the DM efficiency were observed for the GLY diet, which was higher (+ 13.3%) compared to the value for the FOL diet, whereas the CON and GFO values were similar (P < 0.02).

In general, the nutrients intake for bulls finished with the FOL diet was higher (P < 0.01) than with other diets (Table 6). However, diets containing glycerine increased the DE and ME intake (P < 0.0001). An increase of 7.14% was observed for the OM in the FOL diet compared to that of the CON, GLY and GFO diets (P < 0.0001). Likewise, the CP intake was higher with the FOL diet compared to the GLY diet (+ 4.15%) or the CON diet (+ 5.44%). The mean daily EE intake was higher (P < 0.05) with the FOL diet than with the CON (+ 6.12%) or the GLY and GFO (+ 33.8%) diets (P < 0.0001). An increase of 7.97% in the TC was observed with the FOL diet in comparison with the GLY and GFO diet; whereas the CON diet reduced the TC by 6.18%. The mean NFC was higher for the FOL, GLY and GFO (P < 0.0001) diets compared to the CON (+ 7.13%) diet. Large differences in the NDF intake were observed; the FOL diet increased

the NDF intake by 18.9% in comparison to the values for the GLY and GFO diets; whereas the NDF intake for the CON diet was reduced by 10.0% ($P < 0.0001$). The mean daily ADF intake was higher for the animals fed the FOL diet than those fed the GLY and GFO diets (+ 9.72%) whereas those fed the CON diet reduced their intake by 11.7% ($P < 0.0001$). The mean daily CF intake displayed behaviour similar to that of the ADF; the animals fed the FOL diet had a 9.72% than those fed the GLY and GFO diets, and 11.8% in the CON diet. The TDN intake was higher for the FOL diet group than for the GLY (+ 6.05%) and the CON (+ 6.92%) diet groups ($P < 0.0001$). The digestible energy and metabolisable energy demonstrated similar behaviours. The mean daily DE and ME intakes were higher in the animals fed diets containing glycerine than in those fed diets without glycerine (22.6% for DE and 22.6% for ME).

3.2 Apparent digestibility in the total tract

The apparent digestibility of DM, OM, CP, TC, NFC and NDF in the entire digestive tract was higher ($P < 0.05$) when glycerine was added to the diets; whereas no change was observed in the apparent digestibility of EE, ADF and CF with the addition of glycerine or FO to the diets (Table 7). Diets with glycerine showed higher apparent digestibility's of DM and OM (GLY and GFO) than did the CON or FOL diets (15.4% for DM ($P < 0.0001$) and 15.4% for OM, respectively ($P < 0.0001$). The apparent digestibility of CP was higher with the GFO diet compared to the FOL diet (+ 20.9%), and the CON and GLY diets showed similar values ($P < 0.001$). In addition, a similar effect of the apparent digestibility of TC and NFC was observed; the values attained with diets containing glycerine (GLY and GFO diets) were higher in comparison those attained with diets lacking glycerine (CON and FOL, 16.2% for TC ($P < 0.0001$) and 19.3% for NFC ($P < 0.0001$). The inclusion of FO in the diet containing glycerine

improved the apparent digestibility of NDF (+ 18.0%) compared to diets without glycerine but with FO (FOL); whereas the CON and GLY diets showed similar values.

4. Discussion

The initial BWs ranged from 177 to 261 kg, which is consistent with those of production bulls slaughtered at 16 to 18 months of age (Eiras et al., 2013b; Ito et al., 2010). In contrast, using the feedlot practices of Brazil, the initial BW of beef cattle generally ranges from 350 to 380 kg at approximately120 days prior to slaughter (Prado et al., 2008b; Rotta et al., 2009; Zawadzki et al., 2011a; Zawadzki et al., 2011b). The FBW and ADG were unaffected by replacing corn grain with glycerine and by the addition of FO. The FBW value agrees with that of industrial practices in Brazil (Rotta et al., 2009). The low ADG of the bulls in this study could be due to the low level of CP in the DM (10.6% for diets without glycerine and 10.7% for diets with glycerine (NRC, 2000). Eiras et al. (2013b) reported that replacing corn grain with glycerine at 6, 12 or 18% had no effect on the FBW (471 kg) or the ADG (1.1 kg). In addition, studies conducted by Schneider (2010) evaluated the effect of 0, 4 and 8% glycerine in the diet of heifers, and Mach et al. (2009) examined the effect of 0, 4, 8 and 12% glycerine in the diet of steers, and both reported no effect on the ADG. However, others (Farias et al., 2012; Parsons et al., 2009) have reported a reduction in animal performance.

Feed containing glycerine and FO improved the HWC and HCD. Similar values were obtained in experiments with Purunã bulls finished in feedlot that were similar in age, weight and carcass dressing (Eiras et al., 2013b; Ito et al., 2010; Prado et al., 2009b). The HWC was increased in the FOL diet group, most likely due to their higher feed intake. In contrast, Coneglian (2009) reported no change in the DM intake in response to FO at a dose of 1, 2, 4 or 8 g/animal/day for cannulated steers fed a high-

grain diet, whereas Benchaar et al. (2006) observed an increase in DM intake in response to increasing doses of a mixture of FO (2 or 4 g/day). The compounds in cashew nut shell liquid and castor oil confer antibacterial activities (Himejima and Kubo, 1991; Kubo et al., 2003; Muroi et al., 1993; Novak et al., 1961; Shin et al., 2004); therefore, synergism between the compounds may improve fermentation in the rumen (Benchaar et al., 2008; Burt, 2004). Likewise, the higher dietary fibre content assists fermentation, improves ruminal motility and maintains an adequate pH. The nutrient intakes of the animals fed the GLY and GFO diets were lower, but these animals had a higher digestible energy intake and metabolisable intake and a high ability to digest nutrients. Rémond et al. (1993) reported that crude glycerine is 100% digestible within 4 h *in vivo*. Thus, the inclusion of glycerine provided higher available energy for the animal's metabolism, which improved the HWC. It is important to note that the diets containing glycerine included 164 g of glycerol/kg of DM. The corn grain was replaced by 16.5% of glycerol/kg of DM as an energy source. Glycerol is the principal component in crude glycerine, and it is involved in the process of gluconeogenesis, in cellular metabolism, and in the synthesis of phospholipids and triglycerides (Chung et al., 2007; Goff and Horst, 2001; Krehbiel, 2008). According to Zawadzki and Prado (2013), the HWC increase observed in this study would be due a higher fat depot in the carcass. In contrast, Eiras et al. (2013a) reported no effect on the fat depot.

In the current study, the intake of concentrate was a fixed %/BW; whereas the corn silage intake was *ad libitum*. A reduction in the concentrate intake was observed for animals in all of the treatments groups compared to the value that had been estimated (1.4%/BW). The concentrate intake was higher in animals fed the CON, FOL and GLY diets, whereas the corn silage intake was higher only for those in the FOL

diet. The addition of FO stimulated the fibre intake. According to Wallace et al. (2008), essential oils are particularly appealing and palatable, which can stimulate food intake. The bulls finished with the FOL diet had a high intake of NDF compared to the CON group, and to an extent, the GLY and GFO diet groups. Coneglian (2009) reported no change in the NDF intake. However, the fibre content was reduced when we replaced corn grain with glycerine. The glycerine used in this study is classified as medium purity (812 g of glycerol molecule /kg of glycerine and absent of fibre). Therefore, the GLY and GFO diets showed decreased fibre content (12.8, 2.72 and 2.15%, respectively for NDF, ADF and CF). A previous study conducted by AbuGhazaleh et al. (2011) reported a decrease in the feed intake of NDF when corn grain was replaced by glycerine (15, 30 or 45%). The ADF and CF intake were higher for the bulls fed the FOL diet; whereas these values were reduced for the bulls fed the GFO diet and reduced to a lesser extent for the bulls fed the CON or GLY diets. A similar effect was observed for the FOL diet, and when glycerine was combined with FO, the fibre intake improved.

The inclusion of glycerine and FO in the diets did not affect the DMI compared to the CON diet. However, in the current study, a reduction in the DMI (2.4%/BW) was observed relative to what was estimated at the beginning of the experiment and what is recommended by the NRC (2000). Eiras et al. (2013b) reported a reduction of 9.6% in the DMI with the inclusion of 18% glycerine in the diet of Purunã bulls with similar ages, final weights and ADG that were finished in feedlot. Previous studies reported a 0.7 kg/day reduction in the starch intake when fed a diet with 15% glycerine (Schröder and Südekum, 1999), a 13% reduction in the DMI when at 16% glycerine (Parsons et al., 2009) and 5% reduction in the DMI at 15% (Elam et al., 2008). The bulls fed the FOL diet had improved DM efficiency. The best DM efficiency was associated with a higher DMI. The diet containing glycerine improves the DM conversion. The bulls

finished with the GFO diet had a higher fibre intake in comparison to those fed the CON or GLY diets; the CON and GLY diets did not significantly differ in their DM efficiency. Elam et al. (2008) reported no difference in feed efficiency in response to glycerine contents of 7.5 and 15% in the diet. Parsons et al. (2009) observed that feed efficiency improved by 10.8, 10.0, 7.2 and 3.1% in response to the addition of 2, 4, 8 and 12% glycerine, respectively; however, 16% glycerine reduced the feed efficiency by 2.8%.

The apparent digestibility improved when corn was replaced by glycerine and FO in the diets. According to Coneglian (2009), the inclusion of 2 or 4 g/day of FO improves the digestion of steers on a high-grain diet. In contrast, Coneglian (2009) reported no difference in the total apparent digestion of steers on a high-forage diet in response to FO at a dose of 1, 2, 4 or 8 g/day. The increased digestibility of nutrients in diets containing glycerine may be explained by the metabolism of glycerol to volatile acids in the rumen. Glycerol affects rumen fermentation by increasing the volatile fatty acids (propionate and butyrate) and decreasing the ratio of acetate to propionate (Abo El-Nor et al., 2010; AbuGhazaleh et al., 2011; Lee et al., 2011; Rico et al., 2012). Wang et al. (2009) reported increased digestibility of OM, CP and NDF in the total digestive tract in response to increasing glycerol levels. Eiras et al. (2013b) observed improvements in the digestibility of DM, OM, CP, NFC and TC in response to glycerine at 6, 12 and 18%.

In the current study, the addition of functional oils improved the digestibility of the dietary NDF and CP in the diet containing glycerine. The lower digestibility of the diet containing FOL is most likely associated with the higher intake of CP and NDF. It is important to note that diets containing glycerine have a reduced level of fibre. Rico et al. (2012) reported that replacing corn starch by glycerol increased the digestibility of

NDF. Eiras et al. (2013b) and Krueger et al. (2010) reported that there were no negative effects on NDF digestibility. Previous studies have reported reductions in the activity of cellulolytic bacteria in response to glycerol addition (Paggi et al., 2004). Shin et al. (2012) reported a 30% reduction in the apparent total-tract digestion of dietary NDF. Abo El-Nor et al. (2010) and AbuGhazaleh et al. (2011) reported a decrease in the concentration of DNA obtained from *Butyrivibrion fibrisolvens* and *Selenomonas ruminatium* in response to increasing levels of glycerine (fibrolytic and non-fibrolytic bacteria, respectively). Other authors have also reported a reduction in proteolytic activity (Paggi et al., 1999) and a decrease in the concentration of DNA from *Clostridium proteoclasticum* (proteolytic bacteria) in response to glycerol addition (Abo El-Nor et al., 2010). Coneglian (2009) observed an increase in the allantoin content and the microbial protein synthesis of steers fed a high-grain diet. Likewise, Coneglian (2009) reported that there was no change in the pH ruminal, total fatty acid content, ratio of acetic acid to propionic acid, ruminal ammonia production or plasma urea nitrogen in steers fed high-forage diets with FO at doses of 1, 2, 4 or 8 g/day. Likewise, the same authors reported no change in the levels of propionic acid and butyric acid and the ratio of acetic acid to propionic acid in steers fed high-grain diets with FO at doses of 1, 2, 4 or 8 g/day.

5. Conclusion

Feed in which corn grain is replaced by glycerine can be fed to finishing bulls in feedlot because it did not change the animal performance. Furthermore, the inclusion of glycerine in the diets improved carcass weight and the dressing weight. Glycerine inclusion in the diets increased the digestible and the metabolisable energy intake and improved the apparent digestibility of the diet.

Functional oils could be added to the diet of bulls being finished in feedlots because they increased the carcass weight and dressing weight, although they no had effect on the feed intake and feed efficiency.

Acknowledgements

This project was funded by the Araucaria Foundation as well as the state of Paraná and the Brazilian Council for Research and Technological Development (CNPq). The authors would like to thank Processing Inc. (Biopar Bioenergia do Paraná, Rolândia, PR, Brazil) for providing the glycerine and Oligo Basics Agroindustrial Ltd. (Cascavel, PR, Brazil) for providing financial resources, the cashew nut shell liquid and castor oil used in this research. The trade names and commercial products named in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, State University of Maringá, Maringá PR Brazil.

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Table 1. Chemical composition of the glycerine

Parameters	Results
Water*	23.2 g/kg
Ashes	47.6 g/kg
Glycerol	812 g/kg
Methanol	3.32 mg/kg
Sodium	11.6 g/kg
Potassium	79.1 mg/kg
Chloride	35.8 mg/kg
Magnesium	16.3 mg/kg
Phosphorus	239 mg/kg
Crude energy	3.65 Mcal/kg

Realized by Institute of Technology of Paraná – TECPAR, Biofuels division, in Curitiba, Paraná.*Karl Fischer method.

Table 2. Percentage of the ingredients in the treatment diets

Ingredients, %	Diets, g/kg of DM			
	CON[1]	FOL[2]	GLY[3]	GFO[4]
Corn silage	420	420	420	420
Soybean meal	103	103	150	150
Corn grain	477	477	227	227
Glycerine	-	-	203	203

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.

Table 3. Chemical composition of the treatment diets [1]

Ingredients	DM[2]	%/DM												
		OM[3]	Ash	CP[4]	EE[5]	TFC[6]	NFC[7]	NDF[8]	ADF[9]	CF[10]	NNE[11]	TDN[12]	CE[13*]	DE[14*]	ME[15*]
Corn silage	28.9	99.0	0.97	5.79	2.07	91.1	48.3	42.8	21.6	17.2	73.8	64.7	3.96	2.85	2.34
Corn grains	89.4	99.0	0.99	7.43	4.66	86.9	69.6	17.3	2.28	1.82	85.1	83.0	3.97	3.66	3.00
Soybean meal	91.6	99.0	0.94	45.0	2.63	51.3	33.3	18.0	8.23	6.58	44.8	86.4	4.29	3.81	3.13
Glycerin	94.2	95.2	4.76	0.07	0.12	95.0	95.0	-	-	-	95.0	80.6	3.65	3.55	2.91
Total diet															
CON[16]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
FOL[17]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
GLY[18]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72
GFO[19]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72

[1]Analyses conducted by the Chemical Laboratory of the State University of Maringá. [2]Dry matter. [3]Organic matter. [4]Crude protein. [5]Ether extract. [6]Total fibre carbohydrate. [7]Non-fibre carbohydrate. [8]Neutral detergent fibre. [9]Acid detergent fibre. [10]Crude fibre. [11]Non-nitrogenous extract. [12]Total digestible nutrients. [13]Crude energy. [14]Digestible energy. [15]Metabolisable energy. *Mcal/kg. [16]Without glycerine or functional oils. [17]Functional oils. [18]Glycerine. [19]Glycerine and functional oils.

Table 4. Replacement of corn by glycerine as energy source and functional oils as additive on performance of Purunã bulls finished in a feed-lot

Item	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Initial body weight, kg	202	209	203	208	4.15	0.53
Final body weight[7], kg	460	467	471	473	6.86	0.49
Average daily gain, kg	1.02	1.02	1.06	1.05	0.09	0.85
Hot carcass weight[8], kg	258b	271a	269a	276a	0.07	<0.01
Hot carcass dressing[8], %	56.2b	58.0a	57.2a	58.4a	0.02	0.02

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.[5]Standard error. [6]Probability, n = 8 per treatment. [7]Characteristic adjusted for initial BW, kg and [8]final BW, kg.

Table 5. Replacement of corn by glycerine as energy source and functional oils as additive on feed intake of Purunã bulls finished in a feed-lot

Item	Diets				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Ratio corn silage, %	42.8	45.7	43.3	44.5	0.05	0.53
Ratio concentrate, %	57.2	54.2	56.6	55.4	0.04	0.47
Corn silage, % BW	0.89	1.01	0.90	0.92	0.01	0.31
Concentrate, % BW	1.18a	1.19a	1.17a	1.13b	0.02	<0.03
Corn silage, kg/day	2.96b	3.42a	3.04ab	3.13ab	0.02	0.09
Concentrate, kg/day	3.93	4.05	3.96	3.87	0.01	0.17
Dry matter, kg/day	6.89b	7.47a	7.00ab	7.00ab	0.02	<0.01
Dry matter, % BW	2.07b	2.20a	2.07b	2.05b	0.08	0.08
Dry matter conversion[7]	6.75ab	7.32b	6.60a	6.66a	0.02	0.02
Dry matter efficiency[8]	0.14ab	0.13b	0.15a	0.15ab	0.03	0.02

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.[5]Standard error. [6]Probability, n = 8 per treatment. [7]kg of DM intake/kg of ADG.[8]kg of ADG/kg of DM intake.

Table 6. Replacement of corn by glycerine as energy source and functional oils as additive on nutrients intake of Purunã bulls finished in a feed-lot

Item	Diets, kg/day				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Organic matter	6.835b	7.399a	6.886b	6.890b	0.07	<0.01
Crude protein	0.729c	0.771a	0.739b	0.732cb	0.07	<0.01
Ether extract	0.230b	0.245a	0.163c	0.162c	0.01	<0.01
Total carbohydrates	5.874c	6.383a	5.983b	5.994b	0.07	<0.01
Non fibre carbohydrate	3.919b	4.210a	4.234a	4.216a	0.07	<0.01
Neutral detergent fibre	1.955b	2.173a	1.749c	1.778c	0.09	<0.01
Acid detergent fibre	0.770c	0.874a	0.772c	0.789b	0.08	<0.01
Crude fibre	0.615c	0.699a	0.618c	0.631b	0.07	<0.01
Total digestible nutrients	5.213c	5.601a	5.262b	5.248cb	0.07	<0.01
Digestible energy*	12.12b	11.89b	14.74a	16.30a	0.01	<0.01
Metabolisable energy*	9.938b	9.749b	12.08a	13.36a	0.01	<0.01

[1]Without glycerine or functional oils.[2]Glycerine. [3]Glycerine and functional oils.[4]Functional oils.[5]Standard error. [6]Probability, n = 8 per treatment. *Mcal/kg day of DM intake.

Table 7. Replacement of corn by glycerine as energy source and functional oils as additive on apparent digestibility of Purunã bulls finished in a feed-lot

Parameters, %	Diets				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Dry matter	67.69b	62.96b	75.98a	78.61a	1.46	<0.01
Organic matter	67.60b	62.68b	75.60a	78.43a	1.45	<0.01
Crude protein	66.25bc	59.45c	70.09ab	75.16a	1.50	<0.04
Ether extract	78.40	72.93	74.37	79.34	1.31	0.24
Total carbohydrate	67.33b	62.72b	76.37a	78.85a	1.49	<0.01
Non fibre carbohydrate	68.15b	63.71b	81.49a	81.94a	1.84	<0.01
Neutral detergent fibre	66.17bc	61.41c	69.56ab	74.92a	1.35	<0.03
Acid detergent fibre	61.80	58.48	60.58	68.02	1.67	0.38
Crude fibre	61.80	58.48	60.58	68.02	1.67	0.38

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.[5]Standard error.[6]Probability, n = 8 per treatment.

III – Replacing of corn by glycerine and functional oils (cashew nut shell liquid and castor oil) as alternative additives in the diets of crossbred bulls finished in feedlot: carcass characteristics and *Longissimus* composition

ABSTRACT

This work was conducted to study the effect of replacing corn grain by glycerine (812 g of glycerol per kg/DM) and functional oil (cashew nut shell liquid and castor oil) extracted in northern Brazil on the carcass characteristics and the meat quality of Purunã bulls finished in a feedlot. Corn grain was replaced by glycerine at 203 g/kg of DM and functional oil at 3 g/animal/day. Each treatment group was composed of eight 8-month-old Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim), weighing 206.1 kg (SD 20.02), that were finished in a feedlot in individual pens for 252 days. The treatment diets were as follows: without glycerine (CON), with functional oils (FOL), with glycerine (GLY) and with glycerine and functional oils (GFO). Adding the functional oils to the diet with glycerine (GFO) improved the carcass conformation (12.8%) compared with those CON or GLY diets. Likewise, the fat thickness and the proportion of fat in the carcass were both higher for the GFO diet (25.64% and 14.3%, respectively) compared with those CON diet. Diets containing glycerine and functional oils increased the hot (+ 5.14%) and cold (+ 5.22%) carcass weight compared to those obtained in the CON diet. Whereas the diets containing functional oils (FOL and GFO) improved the carcass dressing weight by 3.66% compared with those CON diet. Feeding glycerine and functional oils did not change the mean area of the *Longissimus muscle* (68.0), the texture (4.24) and marbling (6.68) or the subjective colour (3.51) at 24 h *post mortem* ($P > 0.43$, $P > 0.44$, $P > 0.86$ and $P > 0.44$, respectively). Furthermore, the instrumentally obtained colour values indicated that the lightness (32.4), redness

(13.9), yellowness (4.94), Chroma (14.8) and angle hue (19.1) at 24 h *post mortem* were unaffected by the feeding treatments (P > 0.48; P > 0.97; P > 0.92; P > 0.97 and P > 0.84, respectively). Likewise, the mean values for the moisture content (74.4%), ash content (1.05%), crude protein content (21.6%), total lipid content (2.21%), WBS score (3.07 kgf), TBARS content (0.64 mg malonaldehyde (MDA) per kg of meat) and calorie content (225 kcal/100 g of meat) also were not affected by the diets (P > 0.89, P > 0.33, P > 0.73, P > 0.89, P > 0.49 P > 0.69 and P > 0.54). Thus, up to 20% of the DM of the diet can be replaced by glycerine, and functional oils from cashew nut shell liquid and castor plants can be added to the diets of bulls being finished in a feedlot for 252 days and will provide a high-density energetic diet.

Keywords: *anacardic* acid, bio-fuels, corn grain, colour, energy, glycerol, *ricinoleic* acid, tenderness

1. Introduction

Several factors that influence carcass characteristics and meat quality can cause important economic losses for the meat industry (Mach, Bach, Velarde & Devant, 2008). Brazil is a significant producer and exporter of beef meat (FAPRI, 2012). However, in recent years, beef production has operated within a narrow profit margin because of the high demand for investments. New products and co-products are being studied, such as compounds from plant extracts (Valero et al., 2011; Zawadzki et al., 2011a; Zawadzki et al., 2011b), essential oils (Benchaar et al., 2007) and glycerine as alternative energy sources to replace cereals (Françozo et al., 2013; Zawadzki & Prado, 2013).

The biodiesel industry expanded rapidly worldwide in the last few years, which has increased the availability of glycerine (FAPRI, 2012). Research has demonstrated that glycerine is an excellent energy source to partially replace the cereals in the diet of ruminants (Eiras, Marques, Zawadzki, Perotto & Prado, 2013b; Françozo et al., 2013; Mach, Bach & Devant, 2009; Zawadzki & Prado, 2013). In 2012, the biodiesel industry produced approximately 2 billion L of glycerine worldwide, and the Brazilian market produced approximately 2.6 million L (FAPRI, 2012). Glycerine is produced as a result of the transesterification of triglycerides by alcohol during the production of biodiesel (Ooi, Yong, Hazimah, Dzulkefly & Yunus, 2004). The glycerol contained in crude glycerine improves the synthesis of glucose in the liver (Chung et al., 2007), assists in the process of gluconeogenesis (Krehbiel, 2008), provides energy for cellular metabolism (Goff & Horst, 2001) and improves fat deposition (Mach et al., 2009). In the ruminal metabolism, glycerol is used by microorganism for the synthesis of volatile fatty, acid and it increases the propionic acid content (Abo El-Nor, AbuGhazaleh, Potu, Hastings & Khattab, 2010; Rémond, Souday & Jouany, 1993), which directly assists in gluconeogenesis. Propionate derived from glycerol affects the performance of animals because most of the propionate is used for gluconeogenesis in the liver (Bradford & Allen, 2007; Forbes, 1988b).

Other products are being investigated throughout the world to improve ruminal metabolism. Functional oils contain secondary metabolites (terpenoids and phenolic compounds) that have antimicrobial activity on gram-positive and gram-negative bacteria (Benchaar et al., 2008; Burt, 2004). Cashew nut shell liquid and castor oil contain a high percentage of compounds with characteristics that confer their antimicrobial activity. Previous studies reported that the antimicrobial activity is due to these compounds interacting with the bacterial cell membrane, and thereby affecting ion

gradients, electron transport, protein translocation, phosphorylation and enzyme-dependent reactions (Benchaar et al., 2008; Dorman & Deans, 2000; Ultee, Kets, Alberda, Hoekstra & Smid, 2000). Castor oil contains a high percentage of ricinoleic acid (Naughton, 2000), which has antimicrobial properties (Novak, Clark & Dupuy, 1961; Shin, Kim & Kang, 2004), as do their analogous derivatives (Ahmed, Ahmad & Osman, 1985; Narasimhan, Belsare, Pharande, Mourya & Dhake, 2004). Cashew nut shell liquid contain a high percentage of anacardic acid and a smaller percentage of cardol and cardanol (Gedam & Sampathkumaran, 1986; Patel, Bandyopadhyay & Ganesh, 2006). Various compounds in cashew nut shell liquid exhibit antibacterial (Himejima & Kubo, 1991; Muroi, Kubo & Kubo, 1993), antioxidant (Andrade et al., 2011; Kubo, Masuoka, Ha & Tsujimoto, 2006) activities.

Previous studies reported (Gill, Delaquis, Russo & Holley, 2002; Mourey & Canillac, 2002; Ultee et al., 2000) that synergism between the compounds extracted from different products increase their antibacterial activity. Thus, the synergism between the compounds in cashew nut shell liquid (anacardic acid, cardol, and cardonal) and castor oil (ricinoleic acid) may improve their antimicrobial effect. According to Coneglian (2009), the combination of compounds extracted from cashew nut shell liquid and castor oil had effects similar to those exhibited by sodium monensin. However, the effects of on the carcass characteristics and meat quality are unclear. Thus, we investigated the effects of replacing the corn grain in the diet with glycerine at a dose of 812 g of glycerol per kg of DM and functional oils from cashew nut shell liquid and castor oil from northern Brazil on the carcass characteristics and the meat quality of Purunã bulls finished in a feedlot.

2. Material and Methods

The experiment was conducted at the Experimental Farm of the Agronomic Institute of Paraná, in the city of Ponta Grossa, Paraná, south Brazil, and followed the guidelines for biomedical research with animals (CIOMS/OMS, 1985).

2.1 Experimental design, animals and diets

Thirty-two young Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were selected at birth and assigned to individual pens (8 m^2 for each animal) on May 13, 2009; their birth weight (BW) and live weight (LW) were 39.3 kg (SD 5.19) and 191 kg (SD 11.3), respectively. The young Purunã bulls were kept in a pasture of *Hemarthria altissima* from birth until they were allocated to the feedlot (264 days). The experimental design involved four treatments: CON – a diet without glycerol; FOL – a diet with functional oils (3 g/animal/day); GLY – a diet with glycerol (containing 20.1 % glycerine on a DM basis), and GFO – a diet with glycerol (containing 20.1 % glycerine in DM basis) and functional oils (3g/animal/day). Each experimental treatment group was composed of 8 young Purunã bulls. An adaption period of at least 21 days before the start of the experiment was chosen, during which the bulls were fed corn silage and concentrate in separate troughs (40:60 ratio, respectively), and provided a concentrate containing soybean meal, corn grain and mineral salts *ad libitum*. At the start of the experiment, the young Purunã bulls were assigned a birth BW and a LW of 206 kg (SD 20.0). They were given access to a diet formulated to provide a weight gain of 1.2 kg/day that met the requirements for fattening beef cattle (NRC, 2000).

Half of the feed was offered at 08:00 h, and half was offered in the afternoon at 16:00 h. The concentrate intake was fixed at 1.4% of the BW and was adjusted at 28-d intervals. Samples of corn silage and concentrate were collected twice weekly for

estimations of the DM%. The bulls were fed concentrate and corn silage in separate troughs, with *ad libitum* corn silage intake. The glycerine used was produced in a soy-diesel facility, and its chemical composition was determined at the Institute of Technology of Paraná. The glycerine contained (g/kg, as-fed) 23.2 water, 4.76 ashes, 812 glycerol, 11.6 Na and (mg/kg, as-fed) 3.32 methanol, 79.1 K, 35.8 Cl, 16.3 Mg, 239 P, and 3.65 Mcal/kg of crude energy. The glycerine fed in this study was an energetic ingredient; therefore, to obtain four isoenergetic diets, the glycerol level was counterbalanced mainly by decreasing the content of corn grain (Table 1). The FO contained ricinoleic acid, anacardic acid, cardanol and cardol. The ricinoleic acid was obtained from castor oil (extracted from castor seeds) and anacardic acid, cardanol and cardol from cashew nut shell liquid. Vermiculite was used for solidification of the FO. The mixture of the FO was produced in the Analysis Laboratory of Oligo Basics Agroindustrial Ltd. We used 3 g/animal day. All of the diets were formulated to be isonitrogenous (Table 2).Water and mineral salts were provided *ad libitum*. The mineral salts contained (g/kg; as-fed) 150 Ca, 88.0 P, 0.08 Co, 1.45 Cu, 10.0 S, 1.00 Fe, 0.88 F, 0.06 I, 10.0 Mg, 1.10 Mn, 0.02 Se, 120 Na and 3.40 Zn.

2.2. Animal performance

The animals were weighed at the beginning of the experiment and then every twenty-eight days after a 16-hours fast from solid food until the end of the experiment (252 days), to determine the animal performance (Zawadzki & Prado, 2013).

2.3. Slaughter

The bulls were slaughtered at a commercial slaughterhouse 10 km from the Ponta Grossa Research Farm, according to the industrial practices of Brazil, when the bulls reached a final BW of 468 kg (SD 31.53) and were approximately 19 months old.

2.4. Carcass characteristics

After slaughter, the carcasses were labelled and chilled for 24 h at 4^{o}C. After chilling, the right side of the carcass was used to determine the quantitative characteristics as follows:

The hot carcass weight (HCW) was determined before cooling.

The hot carcass dressing (HCD) of an individual animal was defined by the ratio of the hot carcass weight to the live weight, multiplied by 100.

The cold carcass weight (CCW) was determined after the carcass had chilled for 24 h.

The cold carcass dressing (CCD) was defined as the ratio of the cold carcass weight to the live weight, multiplied by 100.

The carcass conformation (CAC) was evaluated using the Müller (1980) point scale, in which the highest value indicated the best conformation; muscle development was calculated after the exclusion of the thickness fat. Carcass conformation was reported as superior, very good, good, regular, poor, and inferior; the ratings were also qualified as plus, average, and minus.

The carcass length (CAL) was evaluated by measurements taken from the anterior border of the pubic bone to the proximal side of the first rib using a tape line.

The cushion thickness (CUT) was evaluated by measurements taken with a wooden compass with metallic edges that determined the distance between the lateral

face and the median at the superior part of the cushion. The cushion is a flat muscle (*Biceps femoris* muscle).

The fat thickness (FAT) was determined using a calliper at three points on the *Longissimus* muscle between the 12th and 13th ribs.

The percentages of muscle (MUS), fat (FAT) and bone (BON). The muscle, fat and bone were physically separated from the section containing the *Longissimus*, which corresponded to the 9th, 10th and 11th ribs, and individually weighed according to Hankins and Howe (1946). The data were regressed to equations following Hankins and Howe (1946) to find the percentages of muscle (MUS), fat (FAT) and bone (BON). The ratios corresponding to the 9th, 10th and 11th ribs were regressed using the following equations:

MUS = (15.56 + 0.81) M%;

FAT = (3.06 + 0.82) F%; and

BON = (4.30 + 0.61) B%.

2.5. Longissimus muscle characteristics

After chilling (24 h at 4^{o}C), the right side of the carcass was used to determine the qualitative characteristics of the *Longissimus* muscle (LM). LM samples were taken by complete cross-section between the 12th and 13th ribs and were immediately taken to the laboratory. The subcutaneous fat and the muscle portion was frozen at -20^{o}C for later chemical analysis.

The temperature and pH were evaluated by measurements on the LM between the 12th and 13th ribs at 0 h (approximately 40 min. after slaughter, in the hot carcass) and 24 h (after chilling the carcass at 4°C). The temperature and pH values were

determined using a PCE-228M pH-meter portable equipped with a penetrating electrode metal probe.

Longissimus muscle area (LMA) was measured on right part of the carcass after a cross-section cut was made between the 12^{th} and 13^{th} ribs using a compensating planimeter that measures the areas of irregularly shaped objects.

Marbling (MAR) was evaluated subjectively in the LM between the 12^{th} and 13^{th} ribs, following the scores described by Müller (1980), using a point scale in which the highest value indicated very fine, as fine, slightly coarse, coarse and very coarse; ratings were also qualified as plus, average, and minus.

Texture (TEX) was determined by the size of the fascicles (muscle "grain" size) in the LM between the 12^{th} and 13^{th} ribs and evaluated subjectively with the same point scale used to evaluate marbling, as described by Müller (1980).

Colour (COR) was analysed 24 h after the carcass had chilled. Colour was analysed 30 min after a cross-sectional cut was made in the LM between 12^{th} and 13^{th} ribs according to a point scale in which the highest value indicated cherry red, as cherry red, red, slightly dark red, dark red and dark.

Instrumentally determined colour (I_{COL}) was analysed 24 h *post mortem*, 30 min after a cross-sectional cut was made in the LM between 12^{th} and 13^{th} ribs. The colour was evaluated using a Minolta CR-410 spectrophotometer (illuminant D65, observer angle 10°, Konica Minolta Holdings, Inc., Osaka, Japan) in the CIELAB space (CIE, 1986). The colour coordinates expressed as L*, a* and b* were recorded, where L* is lightness of colour, with values ranging from 0 for black to 100 for white; a* is redness, with values ranging from (+a*) for red to (–a*) for green; and b* is yellowness, with values ranging from (+b*) for yellow to (–b*) for blue. The Chroma (C*) and hue angle (H*) indexes were calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$ and $H^* = \tan^{-1}$ (b* / a*)*[360° /

(2*3.14)] and expressed in degrees. The colour difference between two stimuli (ΔE_{ab}*): ΔE_{ab}* was calculated as $((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$, where the various Δ quantities in the right-hand part of the formula represent the differences between the corresponding coordinates of the two stimuli (Brainard, 2003).

Wamer-Bratzler (WBSF) value. The mechanical properties of meats were obtained using Stable Micro Systems TAXT Plus (Texture Technologies Corp., UK) texture analyser with a 5.00 kg load cell and a Warner-Bratzler – WB (Stable Micro Systems Texure Analyser, model TA.XT Express Texture Analyser). The analysis was performed according to the methodology proposed by the Meat Animal Research Center of the USA (Wheeler et al., 1997).

Lipids oxidation analysis (TBARS). The samples for lipid oxidation analysis were individually packaged and frozen (-20ºC) for 6 month before analysis. The lipid oxidation was determined by the procedure described by (Botsoglou et al., 1994). The thiobarbituric acid-reactive substances (TBARS) rates were calculated from a standard curve and expressed as mg of malonaldehyde (MDA) per kg of meat.

2.6. Chemical composition

The analyses of the chemical composition of the *Longissimus* muscle were performed in a meat laboratory two months after sampling. The samples were defrosted at 4ºC (SD 1.5), ground, homogenised and analysed in triplicate. The moisture and ash contents of the meat were determined according to (ISO-R-1442, 1997), and the crude protein content was obtained following (ISO-R-937, 1978). The energy contained in the meat was determined by analysing fresh samples (in duplicate) using a bomb calorimeter Parr® 6200 (Parr Instrument Company – USA). The calculation for the gross heat of combustion is $H_c = (WT - e_1 - e_2 - e_3) / m$, where H_c = gross heat of

combustion; T = observed temperature rise; W = energy equivalent of the calorimeter being used; e_1 = heat produced by burning the nitrogen portion of the air trapped in the bomb to form nitric acid; e_2 = heat produced by the formation of sulphuric acid from the reaction of sulphur dioxide, water and oxygen; e_3 = heat produced by the heating wire and cotton thread; and m = mass of the sample.

2.7. Statistical analysis

The data for animal carcass characteristics, *Longissimus* muscle characteristics and composition were subjected to an ANOVA using the GLM procedure (SAS, 2002) to examine the effects of the feeding treatments. When necessary, the variance of the data was stabilised using the natural logarithmic transformation. The mean values were compared using Tukey´s test at 10%.

3. Results

3.1. Carcass characteristics

Corn grain replacement by glycerine and the addition of FO did not affect the FBW ($P > 0.49$, Table 3). However, the diets containing glycerine and FO (FOL, GLY and GFO) increased ($P < 0.05$) the HCW and the CCW by comparison to the value for the CON diet (5.14%, $P < 0.01$ and 5.22%, $P < 0.0001$, respectively, Table 3). The diets containing FO (FOL and GFO) improved the DCC by 3.66% relative to CON diet value. No difference among the groups was found for the chilling loss ($P > 0.57$), the carcass length ($P > 0.32$), the cushion thickness ($P > 0.20$), or the bone and muscle percentages of the carcass ($P > 0.60$ and $P > 0.88$, respectively, Table 3). However, the addition of FO to the diet containing glycerine (GFO) improved the carcass

conformation (12.8%) in comparison the values for the CON and GLY diets, whereas that of the FO was similar ($P < 0.001$, Table 3). Likewise, the fat thickness was greater for the GFO diet group (25.6%) *vs.* the CON diet group, whereas the FOL and GLY groups had similar values ($P < 0.03$, Table 3). Similarly, an increase in the carcass fat was observed in the GFO (14.3%) diet group in comparison to the CON diet group, whereas the values for the FOL and GLY diet groups were similar ($P < 0.0001$; Table 3).

3.2. Longissimus muscle (LM) characteristics

The diet did not effect on the mean temperature or the pH at slaughter (37.3 and 6.95, respectively) and at 24 h *post mortem* (5.93 and 5.76, respectively), $P > 0.53$ and $P > 0.74$, respectively at slaughter, and $P > 0.98$; $P > 0.99$, respectively at 24 h *post mortem*, (Table 4). Similarly, there was no dietary effect on the area of the LM (68.0), its texture (4.24) or marbling (6.68) and the subjective colour (3.51) values ($P > 0.43$, $P > 0.44$, $P > 0.86$ and $P > 0.44$, respectively; Table 4). Likewise, the instrumentally determined colour was unaffected at 24 h *post mortem*. The values for lightness (32.4), redness (13.9), yellowness (4.94), Chroma (14.8) and angle hue (19.11) were similar among the samples from the different treatment groups ($P > 0.48$; $P > 0.97$; $P > 0.92$; $P > 0.97$ and $P > 0.84$, respectively, Table 4). The values for the ΔE_{ab}* of the LM were not perceptible to the naked eye. The LM ΔE_{ab}* values for the comparison between the CON group and the FOL, GLY and GFO groups were 2.43, 1.14 and 0.83, respectively, at 24 h *post mortem*.

3.3. Longissimus muscle (LM) chemical composition

The diets had no effect on the chemical composition of the LM (Table 5). The mean moisture content (74.4%), ash content (1.05%), crude protein content (21.6%), total lipid content (2.21%), the WBS value (3.07 kg), the TBARS value (0.64 mg malonaldehyde (MDA) per kg of meat) and the calorie content (225 kcal/100 g of meat) of the muscle were not changed by the diets ($P > 0.89$, $P > 0.33$, $P > 0.73$, $P > 0.89$, $P > 0.49$ $P > 0.69$ and $P > 0.54$; Table 5).

4. Discussion

4.1 Carcass characteristics

The experimental design supported the production of young bulls and allowed for an examination of the effects of corn grain replacement by glycerine as an energy source and of FO as an additive on the carcass characteristics and the meat quality. The diets did not affect the FBW. The FBW values were in accordance with the industrial practices of Brazil (Rotta et al., 2009a). However, the HCW and CCW were affected by the substitution of corn grain by glycerine and the addition of FO. Previous studies reported similar values from experiments with young Purunã bulls finished in feedlots (Eiras, Marques, Zawadzki, Perotto & Prado, 2013a; Eiras et al., 2013b; Ito et al., 2012; Prado et al., 2009). The increased HCW and CCW observed in the current study were explained in a previous report by (Zawadzki & Prado, 2013). According to Zawadzki and Prado (2013), the feeding treatments improved the carcass weight owing to the higher nutrient intake in the FOL diet group, whereas young bulls fed diets containing glycerine had a higher digestible and metabolisable energy intake. In addition, these authors reported an improvement in the apparent digestibility of the diets containing glycerine. In this study, the inclusion of FO stimulated feed intake and the glycerine

provided more available energy for the animals' metabolism. In contrast to the results in the present study, Eiras et al. (2013a) reported that there was no difference in the HCW and DCW of young Purunã bulls in response to corn grain replacement by glycerine at the levels of 6, 12 or 18%.

The FO diet improved the DCC, whereas the diet containing glycerine and FO improved the conformation, fat thickness and proportion of fat in the carcass. The increased DCC resulted from the increased weights of the HCW, which are explained by feeding treatments (Zawadzki & Prado, 2013). The carcass conformation was considered adequate to meet the standards of the Brazilian market (Rotta et al., 2009b). However, young bulls finished with CON and GLY diets had a lower quality carcass conformation, which was considered "good to very good", whereas the young bulls finished with the GFO diet exhibited a carcass conformation that was considered "very good" (Müller, 1980). Eiras et al. (2013a) reported that there was no effect on the carcass conformation, which was considered very good (13.4 points). However, Mach et al. (2009) reported a 63% satisfactory rate for the carcass conformation obtained with glycerine levels of 4.8 and 12%. The higher CCW and conformation values most likely are associated with the higher fat deposition. The fat thickness and the percentage of fat in the carcass were increased in the GFO diet group. As was the case for the carcass conformation, the fat thickness obtained is considered adequate according to the practice guidelines of the Brazilian market (Rotta et al., 2009b), which require the carcass to have between 3 and 6 mm of fat thickness. A previous study reported a similar effect on the fat thickness of young bulls finished with feed containing glycerine levels of 6, 12 and 18% (Eiras et al., 2013a). In contrast, Parsons, Shelor and Drouillard (2009) observed a linear reduction in the subcutaneous fat (15.7%) with increasing amounts of glycerine in diets containing 2, 4, 8, 12 and 16% glycerine.

The percentage of fat on the carcass increased because of the higher digestible and metabolisable energy intake of the diets containing glycerine, which also improved the apparent digestibility by young bulls finished with a diet containing glycerine and FO (Zawadzki & Prado, 2013). Glycerol is a principal component of glycerine, which assists the processes of gluconeogenesis and the synthesis of triglycerides (Chung et al., 2007; Krehbiel, 2008). Moreover, eating glycerol modulates rumen fermentation to increase the content of volatile fatty acids (Abo El-Nor et al., 2010; AbuGhazaleh, Abo El Nor & Ibrahim, 2011; Rico et al., 2012). In contrast, FO improves ruminal fermentation (Coneglian, 2009) because of its content of compounds with antibacterial activity (Himejima & Kubo, 1991; Muroi et al., 1993; Novak et al., 1961). Thus, the higher propionate content available in the rumen improves gluconeogenesis in the liver (Forbes, 1988a). Considering the various mechanisms in which glycerine can participate, other nutrients or the same glycerol molecules can be targeted for fat synthesis and not affect the animal performance, which most likely occurred in this study.

The temperature and pH of the LM were measured at 0 h and 24 h *post mortem.* The mean temperature in ºC and the pH at 0 h (37.3 and 6.95, respectively) and 24 h (5.93 and 5.76, respectively), the area of the LM (68.0 cm^2), the texture (4.24 points) and the marbling (6.68 points) were similar to those found by Eiras et al. (2013a) in experiments with animals of a similar age, FBW and HCW in response to glycerine levels of 6, 12 and 18% in their diets. The LM area value indicated muscle development had occurred and therefore was directly correlated with the hot carcass weight and the comestible portion of the carcass and, consequently, with the higher weight of the commercial cuts. Mach et al. (2009) reported no effect on the area of the LM in response to glycerine levels of 4, 8 and 12%. In contrast, Parsons et al. (2009) observed

a linear reduction in the LM area (2%) with increasing amounts of glycerine, up to 16%. Moreover, Eiras et al. (2013a) reported that feeding glycerine did not affect the LM area. According to Müller (1980), the meat texture is classified by the granulation of the LM surface, which in present study was defined as “thin” or “very thin” (4.2 points). Similar results for the LM were observed by Eiras et al. (2013a), who reported that feeding glycerine had no effect on the marbling. The marbling was classified as “light” or “small” (6.7 points). A medium marbling is well accepted within the domestic market; however, to reach foreign markets, beef should feature more accentuated marbling. Parsons et al. (2009) observed a linear reduction in the LM area (2.0%) and the marbling (8.7%) with increasing amounts of glycerine, up to 16%, in the diet.

4.2 *Longissimus* muscle characteristics

Many factors influence the quality of meat (Wood et al., 1999). The high pH of the LM observed in this study resulted from glycogen depletion during the pre-slaughter period, during which the muscles were unable to accumulate an adequate lactic acid concentration (Immonen, Ruusunen & Puolanne, 2000). According to (Page, Wulf & Schwotzer, 2001), a higher muscle pH is associated with beef that is more green (a*) and more blue (b*), whereas a lower muscle pH is associated with beef that is more red (a*) and more yellow (b*). We also observed a high pH in the 24 h *post mortem* LM.

Colour is an important factor in meat quality and the purchase decisions of beef consumers; this characteristic is perceived by consumers as denoting the "food freshness". In the current study, the mean subjective colour (3.51), and the instrumentally that determined colour for lightness (32.5), redness (13.9), yellowness (4.9), Chroma (14.8) and hue angle (19.1) were unaffected by the diet treatments;

however, these values are considered to be high. We observed high values for lightness and redness (Page et al., 2001), whereas the yellowness value was lower.

The low yellowness value (4.9) can be explained by the long period that the bulls spent in the feedlot (252 days) eating diets with a low pigment carotenoid content (Dunne, Monahan, O'Mara & Moloney, 2009). Eiras et al. (2013a) reported a higher muscle pH (6.2) at 24 h *post mortem* and higher values for the colour characteristics of L*, a* and b* in the LM when glycerine was included at 0, 6, 12 or 18% with handling practices, experimental facilities and place of slaughter similar to those of the present study. However, when we assessed subjective colour of the LM at 24 h *post mortem*, the meat appeared to have good colour, ranging from "red" to "slightly dark red" (Müller, 1980). According to Renerre and Labas (1987), adequate nutrition and a younger age can be cause this meat colour.

4.3 *Longissimus* muscle chemical composition

Diets generally have little influence on the gross nutrient composition of the LM (Webb, 2006). The inclusion of glycerine and addition of FO to the diets did not change the mean moisture content (74.4%), ash content (1.05%), CP content (21.6%), total lipid content (2.21%), the WBS value (3.07 kg) or the calorie content (225.5 kcal/100 g of meat) of the LM. Reports in the literature (Ito et al., 2012; Ito et al., 2010; Prado et al., 2008) include similar values for the chemical composition of the LM from different genetic groups of bulls finished in feedlots. Furthermore, Eiras et al. (2013a) reported no differences in the moisture (74.2%), ash (1.0%), CP (21.3%) and total lipid (2.0%) contents of the LM from cattle of similar genetic group and age fed diets with different glycerine levels.

A previous study reported no effect on the total lipids content of the LM (3.8) in response to diets with glycerine levels of 4, 8 and 12% (Mach et al., 2009). In contrast, Françozo et al. (2013 in press) observed a decrease the total lipid content of the LM of bulls fed different amounts of glycerine (0, 5 and 12%). Moreover, the total lipid levels obtained with all of the treatments are acceptable for the prevention of diseases related to the fat content of beef (HMSO, 1994).

According (Jeleníková, Pipek & Staruch, 2008; Koohmaraie, 1996), meat tenderness is associated with rate of glycolysis, the decrease in the post-mortem temperature and the ultimate pH of the LM. Furthermore (Purchas, Burnham & Morris, 2002) associated tenderness with the intramuscular fat content. In the present study, the total lipids (2.21%) and the tenderness value obtained using the WBS device (3.07 kg) of the LM were similar. Eiras et al. (2013a) also reported no difference in the total lipids and the WBS value in response to different levels of glycerine levels. In contrast, Mach et al. (2009) reported that total lipids decreased in response to the level of glycerine, but the LM tenderness was not changed. Previous studies (Robbins et al., 2003; Shackelford, Wheeler & Koohmaraie, 1999) reported that WBSF results of < 4.0 kg ensure a tenderness that should be more acceptable to consumers. In addition, the mean caloric value (225 kcal/100 g of meat *in nature*) of the LM was not affected by the feeding treatments (Table 4), most likely because of the similar chemical composition of the LM from all of the treatment groups.

One of the most important causes of meat deterioration is lipid oxidation, which is affected by the intramuscular fatty acid composition, particularly the content of polyunsaturated fatty acids (Faustman, Sun, Mancini & Suman, 2010; Wood et al., 2008). Table 5 shows the lipid oxidation values that were determined by the TBARS (mg MDA/kg meat). Glycerine and FO did not affect ($P > 0.05$) the TBARS values

during the evaluation period (48 h *post-mortem*). It has been reported that lipid oxidation may lead to drip losses, the development of off-odour and off-flavour, the production of potentially toxic compounds and the oxidation of myoglobin (Faustman et al., 2010). The oxidation levels found in present study (0.64 mg MDA/kg meat) are above the values common for animals fed silage and concentrate for 100 days before slaughter (Descalzo & Sancho, 2008; Realini, Duckett, Brito, Dalla Rizza & Mattos, 2004). These elevated values could be explained by the long duration of frozen storage (6 mo), as has been observed by (Muela, Sañudo, Campo, Medel & Beltrán, 2010) in lamb meat.

Thus, the glycerine and FO in the bulls' diet did not affect the lipid oxidation of the meat. In fact, the fatty acid composition of the meat from the different treatment groups was similar. In any case, the MDA levels were below the acceptance limit, which is 2 mg of MDA/kg of fresh tissue (Campo et al., 2006). The diets tested in the current study were prepared with corn silage and cereal, which would contain vitamin E, rendering the samples oxidatively stable (Campo et al., 2006; Richardson, Nute, Wood, Scollan & Warren, 2004).

Red meat consumption contributes many minerals and vitamins to the diet (McAfee et al., 2010). The inclusion of glycerine and FO in the bulls' diet did not alter the calorie content of their meat (Table 5). The calorie content found in present study (225.2 Kcal per 100 g of meat) is consistent with that of adequate food. According (HMSO, 1991), adequate food consists of a daily calorie intake of 1.940 Cal/day and for women and 2.550 for men, and fat should not exceed 35% of the total diet. Previous studies suggest that lean red meat is part of an energy-controlled diet (McAfee et al., 2010; Spencer, Appleby, Davey & Key, 2003).

5. Conclusion

A diet in which 81.2% of the corn grain is replaced by glycerine containing glycerol and FO (cashew nut shell liquid and castor oil) may be fed to bulls in feedlots without negatively affecting the carcass characteristics or the characteristics the LM or its chemical composition. Diets containing glycerol and/or FO improved the cold carcass weight. Diets containing FO improved the dressed cold carcass weight. Glycerine combined with FO improved the conformation, fat thickness and proportion of fat of the carcass. Thus, a glycerine content of up to 20% on a DM basis and functional oils from cashew nut shell liquid and castor oil could be added to the diets for bulls being finished in feedlots for 250 days and fed a diet of high-density energy.

Acknowledgements

This project was funded by the Araucaria Foundation, funds from the state of Paraná and the Brazilian Council for Research and Technological Development (CNPq). The authors would like to thank Processing Inc. (Biopar Bioenergia do Paraná, Rolândia PR Brazil) for providing the glycerine and Oligo Basics Agroindustrial Ltd., (Cascavel PR Brazil) in providing financial resources, cashew nut shell liquid and castor oil used in this research. Trade names or commercial products mentioned in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, State University of Maringá, Maringá, Paraná, Brazil South.

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Table 1. Percentage of the ingredients in the treatment diets

Ingredients, %	Treatments, g/kg of DM			
	CON[1]	FOL[2]	GLY[3]	GFO[4]
Corn silage	420	420	420	420
Soybean meal	103	103	150	150
Corn grain	477	477	227	227
Glycerine	-	-	203	203

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.

Table 2. Chemical composition of the treatment diets[1]

Ingredients	DM[2]	%/DM												
		OM[3]	Ash	CP[4]	EE[5]	TFC[6]	NFC[7]	NDF[8]	ADF[9]	CF[10]	NNE[11]	TDN[12]	CE[13*]	DE[14*]	ME[15*]
Corn silage	28.9	99.0	0.97	5.79	2.07	91.1	48.3	42.8	21.6	17.2	73.8	64.7	3.96	2.85	2.34
Corn grains	89.4	99.0	0.99	7.43	4.66	86.9	69.6	17.3	2.28	1.82	85.1	83.0	3.97	3.66	3.00
Soybean meal	91.6	99.0	0.94	45.0	2.63	51.3	33.3	18.0	8.23	6.58	44.8	86.4	4.29	3.81	3.13
Glycerol	94.2	95.2	4.76	0.07	0.12	95.0	95.0	-	-	-	95.0	80.6	3.65	3.55	2.91
Total diet															
CON[16]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
FOL[17]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
GLY[18]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72
GFO[19]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72

[1]Analyses conducted by Chemical Laboratory of State University of Maringá. [2]Dry matter.[3]Orgnic matter.[4]Crude protein.[5]Ether extract. [6]Total fibre carbohydrate.[7]Non-fibre carbohydrate.[8]Neutral detergent fibre.[9]Acid detergent fibre.[10]Crude fibre. [11]Non-nitrogenousextract. [12]Total digestible nutrients. [13]Crude energy. [14]Digestible energy.[15]Metabolisable energy. *Mcal/kg. [16]Without glycerine or functional oils.[17]Functional oils.[18]Glycerine.[19]Glycerine and functional oils.

Table 3. Replacement of corn by glycerin as energy source and functional oils as additive on the characteristics of the carcass and the *Longissimus* dorsi muscle of Purunã bulls finished in a feed-lot

Item	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GOF[4]		
Final body weight, kg	460	467	471	473	6.86	0.49
Hot carcass weight, kg	258b	271a	269a	276a	0.07	0.01
Cold carcass weight, kg	254b	267a	265a	272a	4.78	<0.01
Dressing cold carcass, %	55.2b	57.1a	56.3ab	57.5a	0.02	<0.07
Chilling loss, %	1.54	1.58	1.48	1.65	0.04	0.57
Conformation[7], points	13.0b	13.8ab	12.8b	14.8a	0.01	<0.01
Carcass length, cm	128	130	130	132	0.02	0.32
Cushion thickness, cm	25.5	26.1	25.4	25.3	0.02	0.20
Fat thickness, cm	3.45b	4.00ab	3.81ab	4.64a	0.01	0.03
Bone, kg	35.0	35.9	37.8	37.5	0.77	0.60
Fat, kg	54.4b	59.5ab	57.1ab	63.5a	1.67	<0.01
Muscle, kg	165	172	170	171	3.33	0.88

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.[5]Standard error.[6]Probability, n = 8 per treatment. [7]Müller (1980)' scale.

Table 4. Replacement of corn by glycerin as energy source and functional oils as additive on characteristics and chemical composition of the *Longissimus* dorsi of Purunã bulls finished in a feed-lot

Item	Treatments				SEM[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Characteristics mensured[7] at 0 h[8]						
Temperature, °C	37.1	37.6	37.3	37.3	0.14	0.53
pH	6.97	6.94	7.01	6.88	0.04	0.74
Characteristics measured[7] at 24 h						
Temperature, °C	5.95	5.93	5.98	5.88	0.14	0.98
pH at 24 h	5.78	5.74	5.77	5.78	0.06	0.99
Longissimus area, cm^2	63.3	68.5	69.9	70.5	0,09	0.43
Texture[9], points	4.00	4.37	4.15	4.47	0.10	0.41
Marbling[9], points	6.00	6.87	6.75	7.12	0.47	0.86
Colour[9], points	3.62	3.25	3.40	3.78	0.03	0.44
Colour characteristics[7]						
L*	33.5	31.1	32.4	32.8	1.14	0.48
a*	13.8	14.0	14.1	13.7	0.69	0.97
b*	5.17	4.78	5.11	4.72	0.49	0.92
C*	14.7	14.8	15.4	14.5	0.80	0.97
H*	20.0	18.6	19.3	18.4	1.05	0.84
Visual						
$\Delta E_{ab}*_{COF}$[10]	2.43	0.00	1.34	1.72	-	-
$\Delta E_{ab}*_{GLY}$[10]	1.14	1.34	0.00	0.68	-	-
$\Delta E_{ab}*_{GOF}$[10]	0.83	1.72	0.68	0.00	-	-

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. [7]*Longissimus* muscle. [8]Approximately 60 min. after slaughter. [9]Müller (1980)' scale.

Table 5. Replacement of corn by glycerin as energy source and functional oils as additive on chemical composition of the *Longissimus* dorsi and fat thickness of Purunã bulls finished in a feed-lot

Item	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Moisture, %	74.6	74.4	74.1	74.4	0.21	0.89
Ash, %	1.04	1.02	1.09	1.07	0.01	0.33
Crude protein, %	21.9	21.6	21.4	21.7	0.15	0.73
Total lipids, %	2.03	2.28	2.25	2.30	0.22	0.89
Braztler shear, kgf[7]	3.32	2.80	2.98	3.21	0.12	0.49
TBARS[8]	0.72	0.53	0.67	0.64	0.05	0.69
Calories, kcal/100g of meat[9]	227	221	217	235	4.76	0.54

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. [7]Waner-Braztler shear measured by kilograms of force (kgf).[8]2-Thiobarbituric acid reacting substances (mg malonaldehyde (MDA) per kg of meat). [9]Gross energy determinate by bomb calorimeter Parr® 6200.

IV – Replacing of corn by glycerine and functional oils (cashew nut shell liquid and castor oil) as alternative additives in the diets of crossbred bulls finished in feedlot: fatty acid composition in perirenal fat, subcutaneous fat and *Longissimus* muscle

ABSTRACT

This work was conducted to study the effect of replacing corn grain by glycerine (812 g of glycerol per kg/DM) and functional oils (extracted in northern Brazil from cashew nut shell liquid and castor oil) on the fatty acid composition of perirenal fat, subcutaneous fat and *Longissimus* muscle of Purunã bulls finished in a feedlot. Corn grain was replaced by glycerine at 203 g/kg of DM and functional oils at 3 g/animal/day. Each treatment group was composed of eight 8-month-old Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim), weighing an average of 206 kg (SD 20.0), that were finished in a feedlot in individual pens for 252 days. The treatment diets were as follows: without glycerine (CON), with functional oils (FOL), with glycerine (GLY) and with glycerine and functional oils (GFO). The CON and FOL diets increased PUFA (+ 27.9%), total *n*-6 (+ 33.5%) and *n*-3 (+ 7.29%) levels. The PUFA:SFA ratio (+ 33.3%) increased in perirenal fat with the GLY and GFO diets, and the *n*-6:*n*-3 ratio increased in perirenal fat in the CON diet (+ 8.31% *vs.* GFO). The different diets did not affect the levels of lauric, myristic and palmitic acids in *Longissimus* muscle and subcutaneous fat. The FOL diet increased stearic acid (+ 15.3% *vs.* GLY and GFO), α-linolenic acid (+ 27.7% *vs.* GFO), SFA (+ 6.78% *vs.* GLY), and PUFA (+ 34.4% *vs.* GFO), and decreased MUFA (- 10.0% *vs.* GLY and GFO) levels in *Longissimus* muscle. Diets with glycerine has reduced linoleic acid (- 21.6% *vs.* CON and FOL) *Longissimus* muscle. The GLY diet has reduced stearic acid (- 21.9% *vs.* CON) subcutaneous fat. Linoleic acid levels were reduced subcutaneous fat

in the GLY and GFO diets (- 32.6% *vs.* FOL), and γ-linolenic acid was increased (+ 8.31% *vs.* CON and GLY). FO addition improves PUFA (+ 16.8% *vs.* GLY and GFO), total *n*-6 (+ 18.3% *vs.* GLY and GFO), and the PUFA:SFA (+ 20.2% *vs.* GFO) and *n*-6:*n*-3 (+ 24.2% *vs.* GFO) ratios subcutaneous fat. In general, diets without glycerine and with FO improve fatty acid composition in all tissues.

Keywords: bio-fuels, energy, fatty acids, meat quality, plants oil

1. Introduction

Fat is a key nutrient in the diet that improves food palatability, assists in the absorption of vitamins and carotenoids and serves as an energy reserve during caloric deficits (Abdel-Hamid, 2002; Goodman, Blomstrand, Werner, Huang & Shiratori, 1966; Kayden & Traber, 1993). However, high intakes of saturated fats are a risk factor for several chronic diseases (McAfee et al., 2010), such as cardiovascular disease (Kontogianni, Panagiotakos, Pitsavos, Chrysohoou & Stefanadis, 2007), obesity, diabetes and cancer (Alaejos, González & Afonso, 2007; Larsson & Wolk, 2006). According to HMSO (1991), the daily intake of fat should be provided by different food sources and should not exceed 35% of the total diet. In Brazil, red meat has an important nutritional value in the human diet. In 2012, about 7.82 million tons of carcasses were traded on the domestic market, the equivalent of 37.2 kg per habitant per year (about 102 g/d). Furthermore, 1.38 million tons of carcasses were exported to several countries around the world (ANUALPEC, 2012).

Recent research on livestock production has been focused on improving meat quality. In intensive livestock production system improves performance and meat quality (Fugita et al., 2012; Prado et al., 2008b; Prado et al., 2009a; Zawadzki et al.,

2011), but demands high investment, technology and alternative feeds (Capper & Hayes, 2012). Crude glycerine is an excellent energy source in the diet of ruminants for partial replacement of corn grain (Donkin, 2008; Eiras, Marques, Zawadzki, Perotto & Prado, 2013a, 2013b; Lee et al., 2011; Zawadzki & Prado, 2013a, 2013b). Animal glycerol assists in gluconeogenesis (Krehbiel, 2008), which improves the synthesis of glucose in the liver (Chung et al., 2007), provides energy for cellular metabolism (Goff & Horst, 2001) and increases fat deposition (Mach, Bach & Devant, 2009; Zawadzki & Prado, 2013b). Ruminal glycerol also improves the synthesis of volatile fatty acids (Abo El-Nor, AbuGhazaleh, Potu, Hastings & Khattab, 2010). Red meat has a high saturated fatty acid content (Rotta et al., 2009). In general, food containing polyunsaturated fatty acids is extensively metabolised by microorganisms and biohydrogenated, which results in the production of stearic acid (Kepler, Tucker & Tove, 1970; Wu & Palmquist, 1991) and its deposition in the muscle.

To improve ruminal parameters and decrease saturated fatty acid production, other feed substitutes are being researched around the world. According to Coneglian (2009), compounds extracted from cashew nut shell liquid and castor oils in the high-grain diet of steers (4 g/day) produced similar effects to sodium monensin. Functional oils contain secondary metabolites (terpenoids and phenolic compounds) that confer antimicrobial activity on gram-positive and gram-negative bacteria (Benchaar et al., 2008; Burt, 2004). Cashew nut shell liquid and castor oils contain a high percentage of compounds with characteristics that confer antimicrobial properties. Previous studies reported that the antimicrobial property results from compounds interacting with the bacterial cell membrane, affecting ion gradients, electron transport, protein translocation, phosphorylation and enzyme-dependent reactions (Benchaar et al., 2008; Dorman & Deans, 2000; Ultee, Kets, Alberda, Hoekstra & Smid, 2000). Castor oil

contains a high percentage of ricinoleic acid (James, Hadaway & Webb, 1965; Morris, 1967; Naughton, 2000) which confer antimicrobial properties (Novak, Clark & Dupuy, 1961; Shin, Kim & Kang, 2004). Cashew nut shell liquid contains a high percentage of anacardic acid and a low percentage of cardol and cardanol acids (Nagabhushana & Ravindranath, 1995; Patel, Bandyopadhyay & Ganesh, 2006; Trevisan et al., 2006). Compounds in cashew nut shell liquid confer antibacterial (Himejima & Kubo, 1991; Kubo, Nihei & Tsujimoto, 2003; Muroi, Kubo & Kubo, 1993), antioxidant (Andrade et al., 2011; Kubo, Masuoka, Ha & Tsujimoto, 2006). Previous studies reported that synergisms between compounds extracted from different products increase antibacterial activity (Gill, Delaquis, Russo & Holley, 2002; Mourey & Canillac, 2002; Ultee et al., 2000). Thus, the synergism between compounds in cashew nut shell liquid (anacardic, cardol, and cardanol acids) and castor oil (ricinoleic acid) may improve the antimicrobial effect on microorganisms. However, their effect on fatty acid content in meat is unclear.

The purpose of this study was to replace corn grain by glycerine (812 g of glycerol per kg/DM) and functional oils (cashew nut shell liquid and castor oil extracts of products produced in northern Brazil) and study the effect on carcass characteristics and meat quality in *Longissimus* muscle of young bulls finished in feedlot.

2. Materials and Methods

The experiments were conducted at the Experimental Farm of the Agronomic Institute of Paraná, in the city of Ponta Grossa, Paraná, south Brazil, and followed the guidelines for biomedical research with animals (CIOMS/OMS, 1985).

2.1 Experimental design, animals and diets

Thirty-two young Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) were selected at birth and assigned to individual pens (8 m^2 for each animal) on May 13, 2009. The average birth weight (BW) and live weight (LW) were 39.3 kg (SD 5.19) and 191 kg (SD 11.3), respectively. The young Purunã bulls were kept in a pasture of *Hemarthria altissima* for 264 days, from birth until being placed in the feedlot. The experimental design involved four diet treatments: CON – without glycerol; FOL – with functional oils (3 g/animal/day); GLY – with glycerol (containing 20.1 % glycerine on a DM basis), and GFO – with glycerol (containing 20.1 % glycerine on a DM basis) and functional oils (3g/animal/day) for 252 days before slaughter. Each experimental treatment group was composed of 8 young Purunã bulls. An adaption period of at least 21 days before the start of the experiment was used, during which the bulls were fed corn silage and concentrate (40:60 ratio) in separate troughs and provided a concentrate containing soybean meal, corn grain and mineral salts *ad libitum*. At the start of the experiment, the young Purunã bulls were assigned an average BW and LW of 206 kg (SD 20.0). They were given access to a diet formulated to provide a weight gain of 1.2 kg/day that met the requirements for fattening beef cattle (NRC, 2000).

Half of the feed was offered at 08:00 h, and half was offered in the afternoon at 16:00 h. The concentrate intake was fixed at 1.4% of the BW and was adjusted at 28-d intervals. Samples of corn silage and concentrate were collected twice weekly for estimations of the DM. The bulls were fed concentrate and corn silage in separate troughs, with *ad libitum* corn silage intake. All of the diets were formulated to be isonitrogenous (Table 2).Water and mineral salts were provided *ad libitum*. The mineral salts contained (g/kg; as-fed) 150 Ca, 88.0 P, 0.08 Co, 1.45 Cu, 10.0 S, 1.00 Fe, 0.88 F, 0.06 I, 10.0 Mg, 1.1 Mn, 0.02 Se, 120 Na and 3.40 Zn.

The glycerine used was produced in a soy-diesel facility, and its chemical composition was determined at the Institute of Technology of Paraná. The glycerine contained (g/kg, as-fed) 23.2 water, 4.76 ashes, 812.0 glycerol, 11.63 Na and (mg/kg, as-fed) 3.32 methanol, 79.1 K, 35.8 Cl, 16.3 Mg, 239.8 P, and 3.65 Mcal/kg of crude energy. The glycerine fed in this study was an energetic ingredient; therefore, to obtain four isoenergetic diets, the glycerol level was counterbalanced mainly by decreasing the content of corn grain (Table 1). The functional oils (FO) contained ricinoleic acid, anacardic acid, cardanol and cardol. The ricinoleic acid was obtained from castor oil (extracted from castor seed) and the anacardic acid, cardanol and cardol derived from the cashew nut shell liquid; both were produced in northern Brazil. Vermiculite was used for solidification of the FO. In the FO contained 9% of ricinoleic acid, 4% of cardol and 20% of cardanol of level guarantee. The mixture of the FO was produced in the Analysis Laboratory of Oligo Basics Agroindustrial Ltd.

2.2 Performance and carcass characteristics

The animals were weighed at the beginning of the experiment and then every twenty-eight days after a 16-hour fast from solid food until the end of the experiment (252 days) to determine animal performance (Zawadzki & Prado, 2013a) and carcass characteristics (Zawadzki & Prado, 2013b).

2.3 Samples

The bulls were slaughtered at a commercial slaughterhouse 10 km from the Ponta Grossa Research Farm according to the industrial practices of Brazil when the bulls reached a final LW of 468 kg (SD 31.5) and were approximately 19 months old. At slaughter, perirenal fat samples were separated and frozen at -20^{o}C for analysis.

After chilling for 24h at 4°C, the right part of the carcass was used to determine the quantitative and qualitative characteristics of *Longissimus* muscle (LM) (Zawadzki & Prado, 2013b). Perirenal fat, subcutaneous fat and LM samples were taken by complete cross-section between the 12th and 13th ribs and were immediately taken to the laboratory. In the LM samples, subcutaneous fat was separated and the muscle and fat portions were frozen at -20°C for later analysis.

2.4 Extraction and preparation of Fatty acid Methyl Ester (FAME)

Total lipids were extracted using the Bligh and Dyer (1959) method with a chloroform/methanol mixture. Fatty acid methyl esters (FAME) were prepared by triacylglycerol methylation according to (ISO-R-5509, 1978). Then, the esters were extracted with 2 mL of n-heptane and stored at -18 °C for later chromatographic analysis.

2.5 Chromatographic analysis

Methyl ester was separated by gas chromatography using a Thermo 3300 gas chromatograph fitted with a flame ionisation detector (FID) and a CP-7420 fused-silica capillary column (100 m x 0.25 mm i.d. x 0.25 μm of cyanopropyl, SELECT FAME). The operation parameters were as follows: detector temperature 240°C, injection port temperature 230°C, column temperature 165°C for 18 min, programmed to increase at 4 $^{o}Cmin^{-1}$ up to 235°C, with a final holding time of 14.5 min, hydrogen carrier gas at 1.2 mL min^{-1}, nitrogen make up gas at 30 mL min^{-1}, and split injection at a 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO). Retention times and peak area percentages were

automatically computed with Chronquest 5.0 software. The fatty acid compositions of perirenal fat, *Longissimus* muscle and subcutaneous fat were expressed as a percentage.

2.6 Enzyme activities of Δ*9 desaturase*

The Δ*9-desaturase* index for C16 and C18 was calculated as C16 = 100[16:1Δ^9 / (16:0 + 16:1Δ^9)] and C18 = 100[18:1Δ^9 / (18:0 + 18:1Δ^9)] (Malau-Aduli, Siebert, Bottema & Pitchford, 1997). The Δ*9-desaturase* index for C16 calculates the proportion of palmitate (C16:0) that is converted to palmitoleate (16:1Δ9) when a double bond is inserted by a Δ*9-desaturase* enzyme, and the proportion of stearate (C18:0) that is converted to oleate (18:1Δ9) for C18. The index elongase enzyme activity (EA) was calculated as EA = 100(18:0 + 18:1Δ^9 / 16:0 + 16:1Δ^9 + 18:0 + 18:1Δ^9) (Malau-Aduli et al., 1997). The index EA accounts for all of the non-essential C18 fatty acids as a proportion of the C16 and C18 fatty acids and is expressed as a percentage.

2.7 Statistical analysis

The data for animal fatty acid composition on perirenal fat, LM and subcutaneous fat underwent ANOVA using the GLM procedure (SAS, 2002) to examine the effect of the feeding treatment. The mean values were compared using Tukey's test at 10%.

3 Results

3.1 Fatty acid composition in the perirenal fat

The levels of lauric (0.04%, P > 0.42), myristic (3.29%, P >0.20), palmitoleic (1.71%, P > 0.15), trans-vaccenic (2.60%, P > 0.27), oleic (28.4%, P > 0.37), cis-

vaccenic (0.59%, P > 0.45), γ-linolenic (0.12%, P > 0.15), conjugated linoleic acid (0.07%, P > 0.13), arachidonic (0.13%, P > 0.21), docosapentaenoic (0.06%, P > 0.11), docosanoic (0.11%, P > 0.11), and docosahexaenoic (0.11%, P > 0.74) acid levels did not change by feeding treatments (Table 4). However, higher values for 7-tetradecenoicacid were observed with the GLY and GFO diets (+ 45.8%, P < 0.01) compared to the FOL diet. Likewise, an increase in pentadecanoic acid (+ 28.1%, P < 0.002) was observed in diets with glycerine (GLY and GFO) compared to the CON and FOL diets.

The 15:1 *n*-9 acid level was higher in CON diet (+ 23.3%, P < 0.03) compared to the GLY diet (Table 4), whereas palmitic acid was higher with the GFO diet (+ 10.3%, P < 0.09) compared to the FOL diet. Glycerine inclusion (GLY) increased margaric acid (+ 37.3%, P < 0.002) compared to the CON diet, and the GLY and GFO diets increased *cis*-10-heptadecaenoicacid (+ 36.4%, P < 0.002) compared to diets without glycerine. The levels of stearic acid increased 12.3% in FOL diet (P < 0.007) compared to the glycerine diets (GLY and GFO), whereas the CON diet maintained similar levels. Diets without glycerine (CON and FOL) showed increased linoleic acid levels (+ 37.2%, P < 0.01) compared to the GLY and GFO diets. Glycerine inclusion (GLY) decreased α-linolenic acid (- 38.4%) compared to the CON diet. The eicosapentaenoic acid level increased by 13.3% in FOL diet compared to the GFO diet (P < 0.05). The activity of the *Δ9-desaturase* (C16) enzyme did not change in the feeding treatments (P > 0.68). However, we observed increased *Δ9-desaturase* (C18) enzyme activity in the GLY and GFO diets (+ 9.70%) compared to the FOL diet (P < 0.04). Likewise, elongase enzyme activity was 5.5% higher in FOL diet compared to the GFO diet (P < 0.07).

The mean SFA (63.3%, P > 0.72) and MUFA (34.2%, P > 0.49) did not change with the feeding treatments (Table 5). Bulls fed diets with glycerine (GLY and GFO)

showed decreased PUFA (- 27.9%), total *n*-6 (- 33.7%) and *n*-3 (- 7.29%), and PUFA:SFA ratio (- 33.3%) levels ($P < 0.001$, $P < 0.001$, $P < 0.007$ and $P < 0.003$, respectively) Table 5. However, the *n*-6/*n*-3 ratio level increased in CON diet compared to the GFO diet (+ 33.2%), while bulls fed the FOL and GLY diets maintained similar levels ($P < 0.007$) Table 5.

3.2 Fatty acid composition in subcutaneous fat

The lauric (0.03%, $P > 0.15$), myristic (3.35%, $P > 0.79$), 7-tetradecenoic (1.02%, $P > 0.17$), palmitic (26.8%, $P > 0.80$), palmitoleic (4.73%, $P > 0.44$), *trans*-vaccenic (1.77%, $P > 0.46$), oleic (39.41%, $P > 0.46$), *cis*-vaccenic (1.16%, $P > 0.75$), α-linolenic (0.10%, $P > 0.29$), conjugated linoleic (0.11%, $P > 0.64$), arachidonic (1.03%, $P > 0.21$), eicosapentaenoic (0.12%, $P > 0.16$), docosanoic (0.18%, $P > 0.44$), docosapentaenoic (0.11%, $P > 0.17$) and docosaheaenoic (0.13%, $P > 0.50$) acid levels did not change with the feeding treatments (Table 6). However, bulls fed diets with glycerine (GLY and GFO) showed increased pentadecanoic acid (+ 37.3%, $P < 0.008$) compared to those fed the FOL diet and decreased pentadecanoic acid (- 23.3%, $P < 0.01$) compared to those fed the CON diet. Bulls fed the GFO diet had increased margaric acid (+ 44.0%, $P < 0.001$) compared to those fed the FOL diet.

Glycerine in the diet (GLY and GFO) increased *cis*-10-heptadecaenoic acid (42.3%, $P < 0.0003$) compared to diets without glycerine (Table 6). The stearic acid level was higher with the CON diet (+ 21.9%, $P < 0.07$) compared to the GLY diet. Bulls fed the FOL diet showed increased linoleic acid (+ 32.6%, $P > 0.0004$) compared to those fed diets with glycerine (GLY and GFO). In contrast γ-linolenic acid increased (+ 8.33%, $P < 0.004$) to the diets with glycerine (GLY and GFO) compared to the CON and FOL diets. The activities of Δ9-desaturase enzymes (C16 and C18) and elongase

did not change by feeding treatments (P > 0.44, P > 0.11 and P > 0.91, respectively), Table 6.

The levels of SFA (47.3%, P > 0.25), MUFA (49.2%, P > 0.22) and total *n*-3 (0.45%; P > 0.73) acids did not change with the diets (Table 7). However, bulls fed the FOL diet demonstrated increased PUFA (+ 16.8% *vs*. GLY and GFO diets), total *n*-6 (+ 19.8% *vs*. GLY and GFO diets), and PUFA:SFA (+ 20.2% *vs*. GFO diet) and *n*-6:*n*-3 ratios (+ 24.2% *vs*. GFO diet) (P < 0.01, P< 0.05, P < 0.006 and P < 0.04, respectively; Table 7).

3.3 Fatty acid composition in the *Longissimus* muscle

Corn grains replaced by glycerine and FO did not change the levels of lauric (0.03%, P > 0.16), myristic (2.63%, P > 0.98), 7-tetradecenoic (0.48%, P > 0.77), 15:1 *n*-9 (0.15%, P > 0.32), palmitoleic (3.30%, P > 0.69), *cis*-vaccenic (1.14%, P > 0.24), γ-linolenic (0.11%, P > 0.11), conjugated linoleic (0.18%, P > 0.18), docosanoic (0.13%, P > 0.73), docosapentaenoic (0.15%, P > 0.44) and docosahexaenoic (1.29%, P > 0.38) acids (Table 8). Glycerine inclusion (GLY) increased (+ 38.0%, P < 0.01) pentadecanoic acid compared to diets without glycerine (CON and FOL). Likewise, we observed an increase in margaric acid and *cis*-10-heptadecaenoic acid with the GLY diet (+ 42.1%, P < 0.04 and + 55.4%, P < 0.002, respectively) compared to the FOL diet. In contrast, stearic acid decreased (- 15.3%, P < 0.02) in bulls fed diets with glycerine (GLY and GFO) compared to the FOL diet, Table 8.

Bulls fed the GFO diet showed increased *trans*-vaccenic acid (+ 23.7%, P < 0.09) compared to those fed the CON and GLY diets (Table 8). The oleic acid level was reduced with the FOL diet (- 8.66%,P < 0.01) compared to the GLY and GOF diets, Table 8. The linoleic acid level increased (+ 21.6%, P < 0.05) with diets without

glycerine (CON and FOL) compared to the GLY diet, while γ-linolenic acid was higher with the FOL diet (+ 27.7%, $P < 0.09$) compared to the GFO diet, Table 8. The arachidonic acid level increased (+ 6.32%, $P < 0.05$) with diets with glycerine (GLY and GFO) compared to the CON and FOL diets, Table 8. Docosapentaenoic acid decreased in GFO diet (- 13.7%, $P < 0.01$) compared to the CON and FOL diets, Table 8.

Feeding treatments did not change the activities of *Δ9-desaturase* – C16 (10.6%, $P > 0.21$) and elongase (63.6%, $P > 0.89$), while Δ9-desaturase (C18) activity increased in diets with glycerine (+ 8.60%, $P < 0.003$) compared to the FOL diet (Table 8).

The FOL diet changed the SFA (+ 6.78%, $P < 0.09$ *vs.* GLY diet) and PUFA (+ 34.4%, $P < 0.08$ *vs.* GFO diet) levels (Table 9). The levels of MUFA increased 10.0% in diets with glycerine (GLY and GFO) compared to the FOL diet. Bulls fed diets without glycerine (CON and FOL) improved total *n*-6 (+ 35.5%, $P < 0.02$ *vs.* GFO diet), Table 9. Whereas mean of total *n*-3 (1.73%, $P > 0.37$), and the PUFA/SFA (0.15%, $P > 0.13$) and *n*-6/*n*-3 ratios (3.33%,$P > 0.38$) did not change with the feeding treatments (Table 9).

4. Discussion

4.1 Diets and animals

The experimental design allowed for the examination of the effects of glycerine as alternate energy sources and FO as additive on fatty acid composition in perirenal fat, *Longissimus* muscle and subcutaneous fat in young bulls. At the beginning of the experiment, we observed changes in fatty acid composition when glycerine replaced

corn grain in diets (Table 3). Diets with glycerine decreased SFA (- 13.0%), MUFA (- 26.1%) and PUFA (- 20.2%), Table 3.

The animals were slaughtered at about 19 months finished in a feedlot, 252 days. Feedlot practices in Brazil usually range from 24 to 28 months with a period of 120 days in a feedlot (Françozo et al., 2013; Fugita et al., 2012; Prado et al., 2011; Valero et al., 2011; Zawadzki et al., 2011). Previous studies reported that the fatty acid composition in meat is influenced by genetic groups (Ito et al., 2012; Moreira, Souza, Matsushita, Prado & Nascimento, 2003; Prado et al., 2008c; Rotta et al., 2009) and dietary factors (De Smet, Raes & Demeyer, 2004; Padre et al., 2007; Padre et al., 2006; Smet, Webb, Claeys, Uytterhaegen & Demeyer, 2000; Webb, 2006). Likewise, De Smet et al. (2004) observed that SFA and MUFA acid levels increased faster than PUPA with increasing fat. Thus, we selected animals from the same genetic group (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼ Canchim) to reduce the hereditary effect on fat deposition (Marshall, 1999; Pas, Everts & Haagsman, 2004).

4.2 Fatty acid composition in perirenal fat

Manipulation of the fatty acid composition through feeding is usually more practical and cost effective compared to new breeding strategies (Webb, 2006). Fat deposition occurs at different stages of growth (Berg, Andersen & Liboriussen, 1978; Hood & Allen, 1973) and is characterized by large rates of deposition in internal fat followed by intramuscular, subcutaneous and intramuscular fat deposition. Previous study reported increased intramuscular fat and subcutaneous fat in response to glycerine and FO inclusion in diets, whereas any effect on total lipids in the LM (Zawadzki & Prado, 2013b).

Free glycerol in the blood stream is metabolised in the liver to glycerol-3-phosphate by glycerol-kinase, which is used to synthesise triacylglycerol and phospholipids (Nelson & Cox, 2004). According to De Smet et al. (2004), the phospholipid content is independent of total fat content, while triacylglycerol content is strongly correlated with total fat content. Likewise, Aldai, Nájera, Dugan, Celaya and Osoro (2007) reported that intramuscular fat constituted the most homogenous adipose tissue in different genotypes of Asturiana bulls. Glycerine (GLY and GFO) diets increased 14:1 *n*-7, 15:0 and 17:1 *n*-9 fatty acids, whereas 17:0 fatty acids increased with the GLY diet. In contrast, the levels of 18:2 *n*-6 fatty acids decreased in diets with glycerine. Furthermore, 15:1 *n*-9 and 18:3 *n*-3 fatty acid levels were higher in CON diet. The levels of 20:5*n*-3 fatty acids were higher in FOL diet, whereas 22:5 *n*-3 fatty acids decreased.

In general, ruminant meat contains a high percentage of palmitic and stearic acids (Aricetti et al., 2008; Ducatti et al., 2009; Prado et al., 2008a; Prado, Moreira, Matsushita & Souza, 2003; Prado et al., 2009b). The glycerine inclusion with FO (GFO) diet increased the levels of palmitic acid, while diets with glycerine (GLY and GFO) decreased stearic acid compared to the FOL diet. In ruminants, 18:2 *n*-6 and 18:3 *n*-6 fatty acids in the diets are intensively biohydrogenated in the rumen, resulting in stearic acid (Polan, McNeill & Tove, 1964; Van de Vossenberg & Joblin, 2003). These variations are correlated with the fatty acid content in diets (Table 3).

The reduction of stearic acid in perirenal fat probably occurs due to a lower proportion of total fatty acids in diets with glycerine. We observed higher Δ9-desaturase (C18) activity in bulls fed diets with glycerine (GLY and GFO) and lower activity in bulls fed the FOL diet. The Δ9-desaturase enzyme converts stearic fatty acid into the Δ9 monounsaturated fatty acid oleic acid (Malau-Aduli et al., 1997). In contrast, 18:1 *n*-9

fatty acid did not change by feeding treatment. However, we observed an increase in elongase enzyme activity in FOL diet, and a decrease with the GFO diet. The levels of stearic acid in perirenal fat can be explained by elongase enzyme activity.

An increased proportion of SFA was observed in perirenal fat, which is probably due to the increased metabolism of glycerol to triacylglycerol. However, in this study, the phospholipid and triacylglycerol content in perirenal fat was not determined. Triacylglycerols contain lower amounts of PUFA, whereas phospholipids are rich in PUFA (De Smet et al., 2004) as constituents of the cell membrane (Nelson & Cox, 2004). Diets in which corn grain was replaced by glycerine (GLY and GFO) decreased PUFA, *n*-6, *n*-3 and the PUFA/SFA ratio, whereas diets without glycerine increased PUFA. The *n*-3/*n*-6 ratio decreased with glycerine and FO inclusion and increased in CON diet, whereas the FOL and GLY diets obtained similar results. In contrast, lower levels of SFA (54.4%) and UFA (44.4%) and higher levels of PUFA (4.4%) in different genotypes of Asturiana bulls were reported (Aldai et al., 2007). Likewise, the reduction of PUFA, total *n*-6 and *n*-3, the PUFA:SFA ratio and the *n*-6:*n*-3 ratio can be explained by the fatty acid content in diets.

4.3 Fatty acid composition in subcutaneous fat and *Longissimus* muscle

The diversity of fatty acids in the LM and subcutaneous fat is partly explained by biohydrogenation reactions in the rumen (Tamminga & Doreau, 1991). Coneglian (2009) reported changes in ruminal fermentation in response to FO inclusion in the diet. However, these authors did not evaluate fatty acid composition in LM. Glycerine and FO inclusion did not change the levels of lauric, myristic and palmitic acids in the LM and subcutaneous fat. Lauric, myristic and palmitic fatty acids raise serum levels of total cholesterol and low-density lipoprotein, whereas stearic acid, *cis*-18:1 and C18:2

decrease these serum levels (Zock, Vries & Katan, 1994). Likewise, Bonanome and Grundy (1988) reported no effect on triglycerides or high-density protein by stearic acid.

The levels of stearic fatty acid have different effects on LM and subcutaneous fat. FO addition increased stearic acid in the LM, whereas stearic acid was increased in subcutaneous fat with the CON diet. Eiras et al. (2013a) did not report a difference in stearic fatty acid levels in LM in response to glycerine levels at 6, 12 and 18%. A previous study reported elongase enzyme activity on C16:0 to C18:0 (Malau-Aduli et al., 1997), but no difference was observed on C16:0 and elongase activity for LM and subcutaneous fat. Diets with glycerine (GLY and GFO) increased C18:1 *n*-9 in LM, which can be explained by the activity of Δ9-desaturase (18) enzyme (Table 6), an important enzyme in the synthesis of monounsaturated fatty acids in bovine tissue (Chang, Lunt & Smith, 1992; St John, Lunt & Smith, 1991). In contrast, Eiras et al. (2013a) reported no difference on C18:1 *n*-9 acid in the LM in response to glycerine levels in the diet.

The levels of C15:0, C17:0 and C17:1 *n*-9 fatty acids were higher in LM with diets with glycerine (GLY) (Table 6). In subcutaneous fat, C15:0 and C17:1 *n*-9 fatty acids increased with diets with glycerine (GLY and GFO), whereas C17 fatty acid increased with the GFO diet. Furthermore, we observed decreased 15:1 *n*-9 fatty acid in subcutaneous fat with diets with glycerine. In general, these fatty acids have a low concentration of 15:1 *n*-9 fatty acid in LM (Padre et al., 2007; Padre et al., 2006; Prado et al., 2008a; Prado et al., 2009b). Similarly, Eiras et al. (2013a) reported no difference for C15:0 (0.39%), C17:0 (1.33%) and C17:1 *n*-9 (0.80%) fatty acid levels in LM in response to glycerine levels. A previous study reported that C18:1 *n*-9 fatty acid was the most prominent MUFA, occurring mainly as cis and *trans* isomers (Scollan et al.,

2006). The levels of *trans*-vaccenic fatty acid increased in LM when FO was added to a glycerine diet (GFO) (Table 6), whereas levels in subcutaneous fat did not change in the feeding treatments. Eiras et al. (2013a) reported a quadratic effect of trans-vaccenic fatty acid in LM in response to glycerine levels, with the maximum point at 10.5% of glycerine inclusion.

Previous studies reported trans-vaccenic fatty acid, which is a precursor of conjugated linoleic acid (CLA), as an important intermediate produced by microorganisms in the biohydrogenation of fatty acids in the rumen (Griinari & Bauman, 1999; Knight et al., 2003). Likewise, others showed beneficial health effects of CLA in humans (Kritchevsky, 2000; Whigham, Cook & Atkinson, 2000). In LM and subcutaneous fat, C18:2 *cis*-9, *trans*-11 (CLA) did not change with feeding treatments. These results were similar in animals of the same breed and with similar feedlot practices (Eiras et al., 2013a). However, these values are low when compared with animals finished in pasture (Aricetti et al., 2008; Padre et al., 2007; Padre et al., 2006; Prado et al., 2003). Previous studies reported the health benefits of CLA, but meats from ruminants only have a small contribution in the human diet. According to Schmid, Collomb, Sieber and Bee (2006), beef contributes 1.2 to 10.0 mg/g lipid, on the other hand 25-30% of the CLA intake in Western populations is from meat and meat products.

Diets with glycerine (GLY and GFO) decreased levels of linoleic fatty acid (LA) in subcutaneous fat and to the diet GLY in LM, whereas α-linoleic fatty acid (LNA) increased in FOL diet in LM (Table 6 and 8). Diets in this study contained high values of LA and LNA fatty acid; however, these fatty acids are intensively biohydrogenated by microorganisms to stearic fatty acid in the rumen (Griinari & Bauman, 1999). Previous studies with different compounds extracted from plants reported modification

to ruminal fermentation (Benchaar et al., 2007; Yang, Benchaar, Ametaj & Beauchemin, 2010). Likewise, Coneglian (2009) reported FO diets (cashew nut shell liquid and castor oil) with similar characteristics to ionophores on ruminal fermentation.

Previous studies reported that LA and LNA, which are elongated and desaturated to long-chain PUFAs, docosahexaenoic (DHA), arachidonic (AA) and eicosapentaenoic acid (EPA), are essential in the human diet (Burdge, Jones & Scholz-Ahrens, 2002; Burdge & Nagura, 2002; Spector, 1999). Results from the current study showed higher levels of AA with diets with glycerine (GLY and GFO) and higher levels of EPA with the CON and FOL diets in LM, and no difference in DHA was observed. However, these values are below the levels in a normal diet. The recommended daily intake of EPA and DHA for adults in several countries ranges from 200 to 680 mg/d (Givens & Gibbs, 2008). These fatty acids are important to maintain cell membrane structure and physiological function (Spector, 1999).

The SFA and PUFA acids levels were higher in LM with the FOL diet. Likewise, we observed higher values for total *n*-6 with diets without glycerine (CON and FOL), whereas MUFA had higher levels in LM with diets with glycerine (GLY and GFO). In subcutaneous fat, the FOL diet influenced PUFA, total *n*-6, the PUFA:SFA ratio and the *n*-6:*n*-3 ratio, which showed higher values. A previous study by Eiras et al. (2013a) reported decreased SFA, total *n*-6 and *n*-3, and PUFA:SFA and *n*-6/*n*3 ratios with increased MUFA and PUFA in response to glycerine levels. Prado et al. (2008d) reported similar values for SFA, PUFA and the PUFA:SFA ratio to the values in this study, while lower values for SFA and the *n*-6:*n*-3 ratio were observed by Eiras et al. (2013a). Fatty acids (UFA, SFA, MUFA, PUFA, total *n*-6 and *n*-3) have a higher dispersion on intramuscular fat between genotype, whereas internal and subcutaneous adipose tissues are more homogeneous (Aldai et al., 2007). Likewise, Aldai et al. (2007)

reported similar values for the *n*-6:*n*-3 ratio in subcutaneous tissue, but higher values for PUFA and the PUFA:SFA ratio.

Results from the current study showed lower values of the PUFA:SFA ratio (0.07%) and higher levels of the *n*-6:*n*-3 ratio (6.01) in subcutaneous fat. In contrast, LM had higher levels of the PUFA:SFA ratio (0.15) and lower levels of the *n*-6:*n*-3 ratio (3.33). However, the PUFA:SFA ratio is lower than what is recommended for an adequate diet, while the *n*-6:*n*-3 ratio was adequate. According to HMSO (1994), the PUFA:SFA ratio should be above 0.45 and the *n*-6:*n*-3 ratio should be lower than 4.0. Likewise, results from this study indicated that essential fatty acids in the human diet were observed in LM for diets without glycerine (LA) and the FOL diet (LNA). Similarly, LA increased in subcutaneous fat in FOL diet, while both tissues had similar CLA levels.

5. Conclusions

A corn grain replacement by glycerine with 81.2% glycerol might be fed to finishing bulls in feedlots to change the fatty acid composition in perirenal fat, *Longissimus* muscle and subcutaneous fat. Glycerine inclusion reduces SFA, MUFA and PUFA levels with the diet, while lauric, myristic and palmitic acids in *Longissimus* muscle and subcutaneous fat did not change. Diets with glycerine reduced levels of stearic acid in perirenal fat, subcutaneous fat and *Longissimus* muscle. Likewise, linoleic acid was reduced in perirenal fat, *Longissimus* muscle and subcutaneous fat with diets with glycerine inclusion. Diets without glycerine or with FO led to increased linoleic, α-linolenic and eicosapentaenoic acid levels in *Longissimus* muscle and subcutaneous fat. Total PUFA, *n*-6 and the PUFA:SFA and *n*-6:*n*-3 ratios increased in subcutaneous fat with FO addition to diets. Diets without glycerine and FO increased

SFA, PUFA and n-6 levels in *Longissimus* muscle. In general, diets with FO and diets without glycerine increased fatty acid levels in all tissues.

Acknowledgements

This project was funded by the Araucaria Foundation and funds of the state of Paraná and the Brazilian Council for Research and Technological Development (CNPq). The authors would like to thank Processing Inc. (Biopar Bioenergia do Paraná, Rolândia PR Brazil) for providing the glycerine and Oligo Basics Agroindustrial Ltda. (Cascavel PR Brazil) for providing financial resources and cashew nut shell liquid and castor oil used in this research. Trade names or commercial products used in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, State University of Maringá, Maringá PR Brazil.

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Table 1. Percentage of the ingredients in the treatment diets

Ingredients, %	Diets, g/kg of DM			
	CON[1]	FOL[2]	GLY[3]	GFO[4]
Corn silage	420	420	420	420
Soybean meal	103	103	150	150
Corn grain	477	477	227	227
Glycerine	-	-	203	203

[1]Without glycerine or functional oils.[2]Functional oils.[3]Glycerine. [4]Glycerine and functional oils.

Table 2. Chemical composition of the treatment diets [1]

Ingredients	DM[2]	%/DM												
		OM[3]	Ash	CP[4]	EE[5]	TFC[6]	NFC[7]	NDF[8]	ADF[9]	CF[10]	NNE[11]	TDN[12]	CE[13*]	DE[14*]	ME[15*]
Corn silage	28.9	99.0	0.97	5.79	2.07	91.1	48.3	42.8	21.6	17.2	73.8	64.7	3.96	2.85	2.34
Corn grains	89.4	99.0	0.99	7.43	4.66	86.9	69.6	17.3	2.28	1.82	85.1	83.0	3.97	3.66	3.00
Soybean meal	91.6	99.0	0.94	45.0	2.63	51.3	33.3	18.0	8.23	6.58	44.8	86.4	4.29	3.81	3.13
Glycerin	94.2	95.2	4.76	0.07	0.12	95.0	95.0	-	-	-	95.0	80.6	3.65	3.55	2.91
Total diet															
CON[16]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
FOL[17]	52.1	99.0	0.98	10.6	3.36	85.0	56.9	28.1	11.0	8.81	76.2	75.6	3.99	3.34	2.74
GLY[18]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72
GFO[19]	51.8	98.2	1.76	10.6	2.33	85.2	60.6	24.5	10.7	8.62	76.6	75.3	3.95	3.32	2.72

[1]Analyses conducted by the Chemical Laboratory of the State University of Maringá. [2]Dry matter. [3]Organic matter. [4]Crude protein. [5]Ether extract. [6]Total fibre carbohydrate. [7]Non-fibre carbohydrate. [8]Neutral detergent fibre. [9]Acid detergent fibre. [10]Crude fibre. [11]Non-nitrogenous extract. [12]Total digestible nutrients. [13]Crude energy. [14]Digestible energy. [15]Metabolisable energy. *Mcal/kg. [16]Without glycerine or functional oils. [17]Functional oils. [18]Glycerine. [19]Glycerine and functional oils.

Table 3. Fatty acid composition of the treatment diets

Item	Fatty acid, %									
	14:0	16:0	16:1 *n*-9	18:0	18:1 *n*-9c	18:2 *n*-6c	18:3 *n*-3	AGS	AGMI	AGPI
Corn silage	0.86	18.8	0.39	0.62	29.6	43.9	5.66	20.3	30.1	49.5
Soybean oil	0.11	21.1	0.10	4.28	15.1	53.7	5.40	25.5	15.2	59.2
Corn grain	0.04	11.6	0.15	1.95	34.6	50.6	0.97	13.6	34.7	51.6
Glycerin	-	-	-	-	-	-	-	-	-	-
CON[1]	0.39	15.6	0.25	1.63	30.5	48.1	3.40	17.6	30.7	51.5
FOL[2]	0.39	15.6	0.25	1.63	30.5	48.1	3.40	17.6	30.7	51.5
GLY[3]	0.39	13.6	0.21	1.32	22.5	37.7	3.38	15.3	22.7	41.1
GFO[4]	0.39	13.6	0.21	1.32	22.5	37.7	3.38	15.3	22.7	41.1

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils.

Table 4. Fatty acid compostion of the perirenal fat of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
12:0, lauric	0.03	0.04	0.03	0.03	0.02	0.42
14:0, myristic	3.18	2.92	3.47	3.60	0.12	0.20
14:1 *n*-7, 7-Tetradecenoic	0.21ab	0.13b	0.24a	0.24a	0.01	0.01
15:0, pentadecanoic	0.40b	0.39b	0.55a	0.55a	0.02	<0.02
15:1 *n*-9, pentadecanoic	0.30a	0.28ab	0.23b	0.24ab	0.09	0.03
16:0, palmitic	26.1ab	25.2b	26.4ab	28.1a	0.43	0.09
16:1 *n*-9, palmitoleic	1.69	1.54	1.76	1.83	0.04	0.15
17:0, margaric	1.04c	1.17bc	1.66a	1.51ab	0.06	<0.01
17:1 *n*-9, *cis*-10-heptadecaenoic	0.30b	0.31b	0.51a	0.45a	0.02	<0.02
18:0, stearic	32.1ba	34.3a	30.6b	29.5b	0.54	<0.07
18:1 *n*-11t, *trans*-vaccenic	2.60	2.85	2.21	2.79	0.12	0.27
18:1 *n*-9c, oleic	28.6	27.3	29.5	28.4	0.42	0.37
18:1 *n*-7c, *cis*-vaccenic	0.49	0.59	0.64	0.64	0.03	0.45
18:2 *n*-6, linoleic, LA	2.06a	2.01a	1.35b	1.20b	0.09	0.01
18:3 *n*-6, γ-linolenic	0.13	0.12	0.12	0.11	0.02	0.15
18:3 *n*-3, α-linolenic, LNA	0.13a	0.11ab	0.08b	0.09ab	0.05	0.05
18:2 c9, t11 - CLA	0.07	0.07	0.08	0.08	0.02	0.13
20:4 *n*-6, arachidonic, AA	0.12	0.12	0.12	0.14	0.01	0.21
20:5 *n*-3, eicosapentaenoic, EPA	0.14ab	0.15a	0.14ab	0.13b	0.01	0.05
22:0, docosanoic	0.05	0.06	0.05	0.07	0.05	0.11
22:5 *n*-3, docosapentaenoic, DPA	0.11	0.11	0.11	0.11	0.06	0.11
22:6 *n*-3, docosahexaenoic, DHA	0.11	0.11	0.11	0.11	0.09	0.74

Δ9-desaturase (16)[A]	6.09	5.79	6.27	6.10	0.13	0.68
Δ9-desaturase (18)[B]	47.1ab	44.2b	48.9a	49.0a	0.69	0.04
Elongase[C]	68.5ab	69.7a	68.1ab	65.9b	0.53	0.07

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. [A]Δ9-desaturase (16) = index of desaturase enzyme activity in C16 fatty acids = 100 (16:1 Δ^9 / 16:0 + 16:1 Δ^9). [B]Δ9-desaturase (16) = index of desaturase enzyme activity in C18 fatty acids = 100 (18:1 Δ^9 / 18:0 + 18:1 Δ^9). [C]Index of elongase enzyme activity in the chain-lengthening of C16-C18 fatty acids = 100 (18:0 + 18:1 Δ^9 / 16:0 + 16:1 Δ^9 + 18:0 + 18:1 Δ^9).

Table 5. Total fatty acid composition of the perirenal fat of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Saturated, SFA	62.9	64.1	62.8	63.4	0.43	0.72
Monounsaturated. MUFA	34.1	33.0	35.1	34.5	0.45	0.49
Polyunsaturated. PUFA	2.87a	2.80a	2.11b	1.97b	0.09	<0.01
n-6. omega – 6	2.31a	2.25a	1.59b	1.44b	0.09	<0.01
n-3. omega – 3	0.48a	0.48a	0.44b	0.45b	0.06	<0.07
PUFA:SFA	0.05a	0.04a	0.03b	0.03b	0.01	<0.03
n-6:*n*-3	4.78a	4.67ab	3.59bc	3.19c	0.34	<0.07

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. Total SFA sum of (C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0). Total MUFA sum of (C14:1 *n*-7 + C15: *n*-9 + C16:1 *n*-9 +C16:1 *n*-7 + 17:1 *n*-9 +C18:1 *n*-11t + C18:1 *n*-9c + C18:1 *n*-7c). Total *n*-6 sum of (C18:2 *n*-6 + C18:3 *n*-6 + C20:4 *n*-6). Total *n*-3 sum of (C18:3 *n*-3 + C20:5 *n*-3 + C22:5 *n*-3 + C22:6 *n*-3).

Table 6. Fatty acid composition of the subcutaneous fat of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
12:0, lauric	0.03	0.04	0.03	0.03	0.01	0.15
14:0, myristic	3.24	3.39	3.52	3.26	0.10	0.79
14:1 *n*-7, 7-Tetradecenoic	0.96	0.94	1.29	0.91	0.06	0.17
15:0, pentadecanoic	0.51ab	0.42b	0.65a	0.69a	0.03	<0.08
15:1 *n*-9, pentadecanoic	0.30a	0.26ab	0.24b	0.22b	0.09	0.01
16:0, palmitic	27.3	26.9	26.4	26.7	0.30	0.80
16:1 *n*-9, palmitoleic	4.69	4.63	5.21	4.37	0.18	0.44
17:0, margaric	0.89bc	0.80c	1.20ab	1.43a	0.06	<0.01
17:1 *n*-9, *cis*-10-heptadecaenoic	0.71b	0.64b	1.15a	1.19a	0.07	<0.03
18:0, stearic	16.4a	15.9ab	12.8b	15.9ab	0.55	0.07
18:1 *n*-11t, *trans*-vaccenic	1.81	1.90	1.51	1.85	0.12	0.46
18:1 *n*-9c, oleic	38.4	38.9	41.2	39.1	0.56	0.32
18:1 *n*-7c, *cis*-vaccenic	1.05	1.20	1.24	1.13	0.06	0.75
18:2 *n*-6, linoleic, LA	1.67ab	1.99a	1.45b	1.23b	0.08	<0.04
18:3 *n*-6, γ-linolenic	0.16b	0.17b	0.20a	0.16a	0.04	<0.04
18:3 *n*-3, α-linolenic, LNA	0.10	0.10	0.10	0.09	0.03	0.29
18:2 c9, t11 - CLA	0.11	0.11	0.12	0.10	0.05	0.64
20:4 *n*-6, arachidonic, AA	1.03	1.02	1.03	1.03	0.01	0.21
20:5 *n*-3, eicosapentaenoic, EPA	0.12	0.13	0.12	0.12	0.01	0.16
22:0, docosanoic	0.16	0.14	0.21	0.19	0.01	0.44
22:5 *n*-3, docosapentaenoic, DPA	0.11	0.11	0.11	0.11	0.06	0.17

22:6 *n*-3, docosahexaenoic, DHA	0.12	0.13	0.12	0.14	0.08	0.50
Δ9-desaturase (16)[A]	14.5	14.7	16.4	14.1	0.52	0.44
Δ9-desaturase (18)[B]	70.1	70.9	76.2	70.9	0.97	0.11
Elongase[C]	63.1	63.4	63.1	63.8	0.39	0.91

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. [A]Δ9-desaturase (16) = index of desaturase enzyme activity in C16 fatty acids = 100 (16:1 Δ^9 / 16:0 + 16:1 Δ^9). [B]Δ9-desaturase (16) = index of desaturase enzyme activity in C18 fatty acids = 100 (18:1 Δ^9 / 18:0 + 18:1 Δ^9). [C]Index of elongase enzyme activity in the chain-lengthening of C16-C18 fatty acids = 100 (18:0 + 18:1 Δ^9 / 16:0 + 16:1 Δ^9 + 18:0 + 18:1 Δ^9).

Table 7. Total fatty acid composition of the subcutaneous fat of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Saturated, SFA	48.5	47.7	44.8	48.3	0.73	0.25
Monounsaturated, MUFA	48.1	48.5	51.8	48.6	0.73	0.22
Polyunsaturated, PUFA	3.42ab	3.76a	3.25b	3.00b	0.08	0.01
n-6, omega – 6	2.86ab	3.18a	2.67b	2.43b	0.08	0.05
n-3, omega – 3	0.45	0.46	0.45	0.47	0.09	0.73
PUFA:SFA	0.071ab	0.079a	0.073ab	0.063b	0.02	<0.06
n-6:*n*-3	6.34ab	6.89a	5.90ab	5.22b	0.83	0.04

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. Total SFA sum of (C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0). Total MUFA sum of (C14:1 *n*-7 + C15: *n*-9 + C16:1 *n*-9 +C16:1 *n*-7 + 17:1 *n*-9 +C18:1 *n*-11t + C18:1 *n*-9c + C18:1 *n*-7c). Total *n*-6 sum of (C18:2 *n*-6 + C18:3 *n*-6 + C20:4 *n*-6). Total *n*-3 sum of (C18:3 *n*-3 + C20:5 *n*-3 + C22:5 *n*-3 + C22:6 *n*-3).

Table 8. Fatty acid composition of the *Longissimus* muscle of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
12:0, lauric	0.03	0.03	0.03	0.03	0.01	0.16
14:0, myristic	2.60	2.69	2.60	2.66	0.10	0.98
14:1 *n*-7, 7-Tetradecenoic	0.45	0.40	0.52	0.53	0.04	0.77
15:0, pentadecanoic	0.32b	0.30b	0.50a	0.44ab	0.02	0.01
15:1 *n*-9, pentadecanoic	0.16	0.17	0.15	0.15	0.05	0.32
16:0, palmitic	27.7	27.4	26.5	27.7	0.37	0.69
16:1 *n*-9, palmitoleic	3.16	2.91	3.43	3.72	0.16	0.33
17:0, margaric	0.84ab	0.77b	1.33a	1.23ab	0.08	0.04
17:1 *n*-9, *cis*-10-heptadecaenoic	0.56bc	0.45c	1.01a	0.89ab	0.06	<0.02
18:0, stearic	17.0ab	18.6a	15.4b	16.1b	0.39	0.02
18:1 *n*-11t, *trans*-vaccenic	1.26b	1.59ab	1.24b	1.64a	0.07	0.09
18:1 *n*-9c, oleic	37.1ab	34.8b	38.5a	37.7a	0.45	0.01
18:1 *n*-7c, *cis*-vaccenic	0.93	1.18	1.11	1.35	0.07	0.24
18:2 *n*-6, linoleic, LA	4.69a	5.29a	3.91b	2.71ab	0.30	0.05
18:3 *n*-6, γ-linolenic	0.11	0.11	0.12	0.11	0.03	0.11
18:3 *n*-3, α-linolenic, LNA	0.17ab	0.18a	0.17ab	0.13b	0.08	0.09
18:2 c9, t11 - CLA	0.18	0.17	0.18	0.18	0.01	0.18
20:4 *n*-6, arachidonic, AA	1.11b	1.11b	1.19a	1.18a	0.03	0.05
20:5 *n*-3, eicosapentaenoic, EPA	0.14a	0.15a	0.13ab	0.12b	0.03	0.01
22:0, docosanoic	0.12	0.11	0.15	0.13	0.01	0.73
22:5 *n*-3, docosapentaenoic, DPA	0.14	0.16	0.15	0.14	0.03	0.44

22:6 *n*-3, docosahexaenoic, DHA	1.18	1.34	1.59	1.04	0.11	0.38
Δ9-desaturase (16)[A]	10.1	9.54	11.3	11.6	0.39	0.21
Δ9-desaturase (18)[B]	68.4ab	65.1b	71.3a	70.1a	0.67	<0.03
Elongase[C]	63.5	63.7	64.1	63.1	0.48	0.89

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. [A]Δ9-desaturase (16) = index of desaturase enzyme activity in C16 fatty acids = 100 (16:1 Δ^9 / 16:0 + 16:1 Δ^9). [B]Δ9-desaturase (16) = index of desaturase enzyme activity in C18 fatty acids = 100 (18:1 Δ^9 / 18:0 + 18:1 Δ^9). [C]Index of elongase enzyme activity in the chain-lengthening of C16-C18 fatty acids = 100 (18:0 + 18:1 Δ^9 / 16:0 + 16:1 Δ^9 + 18:0 + 18:1 Δ^9).

Table 9. Total fatty acid composition of the *Longissimus* muscle of Purunã bulls finished in a feed-lot

Fatty acid, %	Treatments				STD[5]	P[6]
	CON[1]	FOL[2]	GLY[3]	GFO[4]		
Saturated, SFA	48.8ab	50.1a	46.7b	48.4ab	0.48	0.09
Monounsaturated, MUFA	43.5ab	41.4b	45.9a	46.1a	0.57	<0.07
Polyunsaturated, PUFA	7.61ab	8.41a	7.33ab	5.51b	0.41	0.08
n-6, omega – 6	5.90a	6.51a	5.21ab	4.00b	0.30	0.02
n-3, omega – 3	1.63	1.83	2.03	1.43	0.12	0.37
PUFA:SFA	0.16	0.17	0.16	0.11	0.08	0.13
n-6:*n*-3	3.60	3.70	2.77	3.26	0.22	0.38

[1]Without glycerine or functional oils. [2]Functional oils. [3]Glycerine. [4]Glycerine and functional oils. [5]Standard error. [6]Probability, n = 8 per treatment. Total SFA sum of (C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0). Total MUFA sum of (C14:1 *n*-7 + C15: *n*-9 + C16:1 *n*-9 +C16:1 *n*-7 + 17:1 *n*-9 +C18:1 *n*-11t + C18:1 *n*-9c + C18:1 *n*-7c). Total *n*-6 sum of (C18:2 *n*-6 + C18:3 *n*-6 + C20:4 *n*-6). Total *n*-3 sum of (C18:3 *n*-3 + C20:5 *n*-3 + C22:5 *n*-3 + C22:6 *n*-3).

V – Influence of level of barley supplementation on plasma carotenoid content and fat spectrocolorimetric characteristics in lambs fed a carotenoid-rich diet

ABSTRACT

This study investigated changes in plasma carotenoid concentration and fat reflectance spectrum characteristics and color in lambs fed a carotenoid-rich diet with low-level (L, 100 g/lamb/day) or high-level (H, 400 g/lamb/day) of barley supplementation for 75 days before slaughter. Each treatment used 24 Romane male lambs that were individually penned indoors. Plasma carotenoid concentration at slaughter was 16% lower in H lambs than L lambs. H lambs had heavier and fatter carcasses than L lambs. Yellowness and redness of perirenal fat were slightly lower in H lambs than in L lambs. The absolute value of the mean integral (AVMI) calculated from the reflectance spectrum of the fat in the 450–510 nm band was not affected by the treatment. Yellowness, chroma and AVMI of subcutaneous fat were not affected by the treatment but decreased with initial animal's liveweight.

Keywords: carcass; carotenoid; fat color; reflectance; sheep

1. Introduction

Carotenoid pigments contribute to color and nutritional characteristics of herbivore meat products (Dian, Chauveau-Duriot, Prado & Prache, 2007b; Dunne, Monahan, O'Mara & Moloney, 2009; Prache, Aurousseau, Theriez & Renerre, 1990a; Priolo, Prache, Micol & Agabriel, 2002; Ripoll, Albertí & Joy, 2012; Röhrle et al., 2011) and are potential

biomarkers for authenticating carcasses produced from animals fed green forage-based diets, both in sheep (Dian et al., 2007a; Prache & Theriez, 1999; Priolo et al., 2002) and cattle (Röhrle et al., 2011; Serrano, Prache, Chauveau-Duriot, Agabriel & Micol, 2006). Prache and Theriez (1999) pioneered a mathematical analysis of fat reflectance spectrum to provide a traceability index that discriminates pasture-fed lambs from lambs fed a concentrate-based diet; this method was further improved by (Dian et al., 2007a). However, as farmers frequently supplement green forage-based diets with grain, we undertook this study to gauge how far different levels of barley supplementation affect fat color, fat reflectance spectrum characteristics and index of traceability in lambs fed green forage-based diets. Our hypothesis was that lambs supplemented with a higher level of barley will accrete more adipose tissue than their counterparts fed a lower level of barley but a similar level of carotenoids, causing a 'dilution' of the carotenoids stored in the fat.

2. Materials and Methods

The experiment took place at the Herbivore Research Unit at the INRA's Clermont-Ferrand–Theix Research Centre, France. The animals were handled by specialized staff who ensured their welfare in accordance with EU Directive 609/1986.

2.1 *Experimental design, animals and diets*

We compared two barley supplementation levels in lambs fed a carotenoid-rich diet for 75 days before slaughter. Each experimental treatment used 24 male Romane lambs individually penned indoors and fed a carotenoid-rich diet that was supplemented for 75 days with 100 g barley (low-level treatment, L) or 400 g barley (high-level treatment, H).

In the pre-experimental period, the animals were housed in a sheepfold and managed uniformly. The lambs were born between 3 August and 10 August 2011. They were offered a commercial concentrate containing no green vegetative matter *ad libitum* from 3 wk age until weaning on 24 October 2011. The dams, which were also kept indoors, received a commercial concentrate containing no green vegetative matter and *ad libitum* access to hay.

The 48 lambs were assigned to 24 blocks according to birth weight and live weight (LW) on 2 November. Mean lamb birth weight and LW on 2 November was 4.14 kg (SD 0.980) and 25.15 kg (SD 4.85), respectively.

To allow different slaughtering dates, the experiment began on 19 November, 26 November, 3 December and 10 December, with 8 and 12 animals balanced for treatments on the first two dates and 14 animals balanced for treatments on the lasts two dates. Lambs were individually penned and fed indoors from weaning until slaughter. We therefore implemented an adaptation period of at least 26 days before the start of the experiment and during which the lambs were fed a commercial concentrate containing no green vegetative matter plus 200 g straw. During the experimental period, lambs were fed a daily ration of 400 g dehydrated alfalfa supplemented with 100 g (L lambs) or 400 g barley (H lambs). Agglomerated straw was also offered in incremental amounts starting with 200 g/lamb/day up to 400 g/lamb/day. Alfalfa pellet feeding level was chosen to reach a similar plasma carotenoid concentration in L lambs to that obtained by Dian et al. (2007b) in their Romane pasture-fed lambs. The feeds were offered half in the morning at 9 a.m. and half in the afternoon at 4 p.m. Feed tubes were emptied twice a week, and refusals were weighed, recorded and discarded. Samples of offered and refused alfalfa pellets, barley and straw were collected twice weekly for estimations of DM. Samples of offered alfalfa pellets,

concentrate, barley and straw were collected twice weekly for estimations of carotenoid concentration.

Water and salt blocks were available *ad libitum*. The salt blocks contained (g/kg; as-fed) 60 Ca, 20 P, 10 Mg, 280 Na, 17.5 Zn, 1.5 Fe, 5.5 Mn, 0.03 Co, 0.03 I, and 0.01 Se.

2.2 *Slaughter procedures*

Lamb groups balanced for both treatments were slaughtered at the INRA Clermont-Ferrand Centre's experimental slaughterhouse according to EU animal welfare guidelines on the 2nd (n = 8), 9th (n = 12), 16th (n = 14) and 23rd (n = 14) February 2012. The lambs were slaughtered in the morning and were not fed on the day of slaughter. They were transported by truck to the slaughterhouse at less than 800 m from the stall. Immediately on arrival, the lambs were electrically stunned and slaughtered by throat cutting. Carcasses were placed in a refrigerated room at 4°C until 24 h *post mortem*, and were always kept in the dark.

2.3 *Measurements*

Lamb live weight. Lambs were weighed at 9 a.m. at the beginning of the experiment and then once per week thereafter.

Carotenoid concentration in the feed. The carotenoid content of the alfalfa, barley, concentrate and straw was extracted using the procedure described in (Cardinault, Doreau, Poncet & Nozière, 2006). Lipophilic components of 50 mg of lyophilized and ground food were first extracted with acetone then purified with diethyl ether containing echinenone, kindly donated by Hoffman La Roche (Basel, Switzerland), as internal standard. After

saponification and cleaning with water, carotenoid content was analyzed by HPLC using the method described by (Lyan et al., 2001). The HPLC apparatus consisted of a Waters Alliance 2996 HPLC system (Waters S.A., Saint-Quentin-en-Yvelines, France) with a photodiode array detector monitoring at between 280 and 600 nm. Carotenoids were separated on a 150 x 4.6 mm RP C18 3 µm column coupled with a 250 x 4.6 mm, RP C18 5 µm Vydac TP 54 column (Interchim, Montluçon, France). Waters SA (Saint-Quentin-en-Yvelines, France) Millennium 32 software was used for instrument control, data acquisition and data processing. Wavelength for carotenoid detection was 450 nm, and compounds were identified by comparing retention times and spectral analyses against those of pure standards (> 95% of zeaxanthin, 9-*cis* and 13-*cis* β-carotene), kindly donated by Hoffman La Roche, and all-*trans* β-carotene and lutein (Sigma Chemical Co., St. Louis, MO). Concentrations of each compound were calculated using external standard curves and then adjusted by percent recovery of the added internal standard.

Plasma carotenoid concentration. Plasma carotenoid concentration (PCC) was measured 10 days after beginning the experiment to assess whether level of alfalfa distribution was satisfactory, considering the pattern of PCC described by (Oliveira, Carvalho & Prache, 2012), and again at slaughter. Blood samples were taken from the jugular vein of each lamb at 8 a.m., i.e. before feed distribution, and collected into lithium heparin (Consortium de Matériel pour Laboratoires, Nemours, France). Plasma was stored at -20°C until required for assay. Carotenoid extraction from plasma was performed within 3 months post-collection.

Crude estimation of total carotenoid was obtained by a spectrophotometric procedure using the following method. Protein from 3 mL of plasma diluted with 2 mL of distilled water was precipitated with 4 mL of ethanol. Carotenoids were then extracted with 4 mL of

hexane. Absorption of the upper layer obtained after centrifugation at 5000 x g for 5 min was measured between 600 and 400 nm using a Kontron Uvikon 860 spectrophotometer (Kontron Instruments S.A., Montigny-le-Bretonneux, France). Concentration of total carotenoids was calculated from absorption maxima, assuming a value of 2,500 for the E1% extinction coefficient (Karijord, 1978; Patterson, 1965) and allowing for the dilution of the original sample. Care was taken throughout the experimental and analytical procedure to protect samples from natural light: samples and test tubes were wrapped in aluminum foil to keep light out and extraction was carried out under the dim artificial light.

Animal characteristics at slaughter. The lambs were weighed just before slaughter. Carcass weight, perirenal fat weight and subcutaneous fat thickness were measured after 24-h shrinkage. Perirenal fat together with kidneys was removed from the carcass. The fat was separated from the kidneys with a knife, and then weighed. The cold carcass was weighed before removal of these tissues. Subcutaneous fat thickness was then measured by making two incisions through the fat along lines extending 4 cm ventro-laterally from the dorsal midline at the last rib and, at the limit of that cut, extending 4 cm cranially. A flap of fat was raised, and subcutaneous fat thickness was measured at the intersection of the incisions (Fisher & de Boer, 1994).

Instrumental color and reflectance spectrum of perirenal fat and subcutaneous caudal fat. We measured the reflectance spectrum of subcutaneous caudal fat from the tail root and perirenal fat at wavelengths between 400 nm and 700 nm. Color coordinates were expressed as lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (h) in the CIELAB uniform color space, using a MINOLTA CM-700d spectrophotometer (illuminant: D65, observer angle: 10°; Minolta France S.A., Carrières-sur-Seine, France). The instrument was fitted with protective glass to shield the eye of the apparatus from the

fat sample. This apparatus measures the proportion of light reflected every 10 nm. Measurements were performed at slaughter and after 24-h shrinkage. For the measurements made on perirenal fat at 24-h post mortem, a plane surface was cut with a knife to allow the fat to adhere perfectly to the eye of the apparatus. For each tissue and each measurement timepoints, 5 measurements were done at 5 randomly-selected locations. We avoided blood spots, discolorations, and less covered areas.

2.4 *Data Analysis*

The reflectance spectrum of subcutaneous caudal and perirenal fat between 510 nm and 450 nm was translated to set reflectance value at 510 nm to zero. The translated reflectance values (TR_i) were calculated from the reflectance values (R_i) as follows : $TR_i = R_i - R_{510}$, with i = 400, 410, 420,…, 700. On the translated spectrum, the integral value ($I_{450-510}$, i.e. traceability index) was calculated from:

$$I_{450-510} = [(TR_{450} / 2) + TR_{460} + TR_{470} + TR_{480} + TR_{490} + TR_{500} + (TR_{510} / 2)] \times 10.$$

For each fat tissue and each measurement timepoints, the integral value was averaged over the 5 measurements. The mean integral values were all negative, prompting us to use the absolute value of the mean integral (AVMI).

The color 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).

The data on animal performances, carcass characteristics, daily alfalfa, barley and straw intake, plasma carotenoid content and the characteristics of each fat tissue were analyzed by ANOVA using the GLM procedure to examine the feeding treatment effect.

When necessary, data variance was stabilized using the natural logarithmic transformation, or otherwise the data was analyzed using non-parametric statistics (Mann and Whitney test).

As there were large between-animal differences in LW, therefore probably in the animal's degree of fatness, at the start of the experiment, we performed a variance-covariance analysis on fat color indices and AVMI to (i) examine whether LW at the start of the experiment had a significant effect on these variables, and (ii) to take this effect into account to refine the effect of the feeding treatment on fat color indices and AVMI.

3. Results

3.1 *Feed and carotenoid intake levels*

Alfalfa pellets, barley and straw contained 925.6, 902.0 and 910.6 g DM/kg and 1421.3, 10.6 and 11.9 µg total carotenoid pigments/g DM, respectively. Lutein was the predominant carotenoid in alfalfa pellets and represented 59.6% of the total carotenoid pigment content, the remainder being composed of β-carotene (32.0%), neoxanthin (4.5%), zeaxanthin (3.3%) and a trace amount of β-cryptoxanthin (0.06%) (Table 1).

Mean daily alfalfa intake was similar for both treatments (Table 2). Mean daily barley intake was higher for H lambs than L lambs (360.8 and 91.3 g DM, respectively; $P < 0.001$). Mean daily straw intake was higher for L lambs than H lambs (304.9 and 280.2 g DM respectively; $P < 0.001$). Mean daily alfalfa intake was very close for all lambs, ranging from 369.0 to 371.0 g DM for L lambs and from 368.0 to 372.0 g DM for H lambs. Mean daily carotenoid intake was therefore very close for all lambs, ranging from 529.0 to

532.1 mg for L lambs and from 530.7 to 535.8 mg for H lambs. Similarly, mean daily lutein intake was very close for all lambs, ranging from 315.8 to 317.7 mg for L lambs and from 316.9 to 319.8 mg for H lambs.

3.2 *Animal performances and carcass characteristics*

Mean LW at the beginning of the experiment was similar between both treatments (28.18 kg and 27.55 kg for L and H treatments, respectively; P = 0.54), ranging from 22.80 to 35.59 kg in the L treatment and from 20.10 to 34.49 kg in the H treatment.

Mean daily gain and total LW gain during the experiment were higher for H lambs than L lambs (P < 0.001; Table 3). LW gain was 18.13 kg for H lambs and 11.12 kg for L lambs. H lambs had heavier and fatter carcasses than L lambs. Carcass weight was 24% higher, perirenal fat weight was 104% higher, and subcutaneous fat was 49% thicker (P < 0.001) in H lambs than L lambs (Table 3).

3.3 *Plasma carotenoid concentration*

PCC did not differ between H and L lambs 10 days after the start of the experiment, although it tended to be higher in H lambs (P = 0.11). At slaughter, PCC differed between treatments (P < 0.01), being 16% lower in H lambs than L lambs (Table 4).

3.4 *Reflectance spectrum and color of the fat*

Mean fat reflectance spectrum and the significance of AVMI are given in Figures 1 and 2, respectively, for measurements made on perirenal fat at 24 h post mortem.

For measurements made at slaughter, proportion of light reflected by perirenal fat was higher in the 410–420 nm band ($P < 0.05$) and lower in the 660–700 nm band ($P < 0.025$ to 0.05) for H lambs than for L lambs. For measurements made at 24 h post mortem, proportion of light reflected by perirenal fat was lower for H lambs than for L lambs in the 400–470 nm band ($P < 0.05$ to $P < 0.001$) as well as in the 510–580 nm band ($P < 0.05$ to $P < 0.01$). The proportion of light reflected by caudal fat was not affected by feeding treatment for measurements made at slaughter; for measurements made at 24h post mortem, the proportion of light reflected was lower for H lambs than L lambs at 400 nm ($P < 0.025$) and in the 470–520 nm ($P < 0.05$), 550–560 ($P < 0.05$) and 590–650 nm ($P < 0.025$ to $P < 0.05$) bands.

At slaughter, subcutaneous caudal fat color parameters were unaffected by feeding treatment (Table 5). At 24 h post mortem, lightness of subcutaneous caudal fat was higher in L lambs than H lambs ($P < 0.05$), but the other color parameters were unaffected by feeding treatment (Table 6). The caudal fat ΔE_{ab}* value for the comparison between H and L lambs was 0.86 and 2.32 for measurements made at slaughter and 24 h post mortem, respectively. At slaughter, redness, yellowness and chroma of perirenal fat were lower ($P < 0.005$ to 0.001) and hue angle was higher ($P < 0.001$) in H lambs than in L lambs (Table 5). At 24 h post mortem, redness, yellowness and chroma of perirenal fat were lower ($P < 0.001$) and hue angle was higher ($P < 0.001$) in H lambs than in L lambs (Table 6). The perirenal fat ΔE_{ab}* value for the comparison between H and L lambs was 2.18 and 3.26 for measurements made at slaughter and 24 h post mortem, respectively.

For both subcutaneous caudal and perirenal fat and at both measurement timepoints (at slaughter and 24 h post mortem), AVMI was unaffected by feeding treatment (P ranging from 0.17 to 0.54; Table 4 and Figure 2). At slaughter, AVMI of subcutaneous caudal fat

ranged from 70.3 to 210.6 units for L lambs and from 35.6 to 216.2 units for H lambs, and AVMI of perirenal fat ranged from 98.1 to 256.2 units for L lambs and from 79.9 to 284.3 units for H lambs. At 24 h post mortem, AVMI of subcutaneous fat ranged from 105.0 to 381.0 units for L lambs and from 161.5 to 383.4 units for H lambs, and AVMI of perirenal fat ranged from 235.1 to 394.0 units for L lambs and from 212.7 to 472.7 units for H lambs.

Yellowness (b*), chroma (C) and AVMI of subcutaneous fat decreased significantly with LW at the start of the experiment ($P < 0.001$, $P < 0.001$ and $P < 0.01$, respectively, for measurements made at slaughter and $P < 0.05$, $P < 0.025$ and $P < 0.025$, respectively, for measurements made at 24 h post mortem). The decreases in yellowness (b*), chroma (C) and AVMI at 24 h post mortem were 0.27 units, 0.27 units and 5.7 units, respectively, per kg increase in initial LW. In perirenal fat, beyond the effect of feeding treatment ($P < 0.01$), similar effects of LW at the start of the experiment were observed for measurements made at slaughter, with significant decreases in yellowness ($P < 0.01$) and chroma ($P < 0.025$) (0.21 and 0.20 units less per kg increase in LW).

4. Discussion

Feeding animals individually penned indoors with dehydrated alfalfa is a good scenario for studying the effect of concentrate supplementation on the concentration of carotenoid pigments in animal tissues and their subsequent color. Both carotenoid intakes and supplement intakes were easily controlled. Setting up this feeding scenario achieved a greater degree of control over the experimental conditions than when using pasture-feeding conditions which are prone to high variability in both level of pasture intake and carotenoid

content of the herbage. Furthermore, supplement intake levels at pasture cannot be controlled individually when lambs are fed within groups.

All the lambs consumed all the alfalfa pellets and all the barley offered straight from the start of the experiment. The mean daily intakes of alfalfa, carotenoid and lutein were high and very close between both treatments, and the barley intake levels reached the assigned values, enabling sound comparisons between both treatments. As expected, the higher barley intake level in H lambs led to increased adipose tissue accretion (higher perirenal fat weight and dorsal fat thickness). It should be noted that although the feeding level of L lambs was lower, it was sufficient to avoid any fat mobilization and the corresponding risk of carotenoid pigments being released from the fat (Patterson, 1965).

The carotenoid concentration in alfalfa pellets was 1421.3 µg/g of DM, i.e. over two-fold higher than the maximum values observed by (Prache, Priolo & Grolier, 2003a) in pasture herbage. Lutein, which is the only carotenoid pigments stored in the fat of sheep (Prache, Priolo & Grolier, 2003b), accounted for 59.6% of total carotenoid pigments, i.e. a similar proportion to that observed by (Prache et al., 2003a) in pasture herbage.

We succeeded in our objective of reaching a similar PCC at slaughter in L lambs to that obtained by (Dian et al., 2007a) in their Romane lambs grazing green vegetative pastures. The mean PCC at slaughter in L lambs (129 µg/L) was actually close to the mean value obtained by (Dian et al., 2007b) (112 µg/L).

Although all lambs ate the same amount of alfalfa, PCC was lower in H lambs than L lambs at the start of the experiment. Though it was not possible to allocate animals to feeding treatments on the basis of their individual ability to absorb and store carotenoid pigments given that carotenoid intake started at the beginning of the experiment, it is most likely that this difference in PCC was not caused by high inter-animal variability given the

high number of animals used. Three reasons may be put forward to explain this result. First, higher stimulation of rumen degradation of carotenoids in H lambs due to the fact that they ate higher amounts of cereals (Larsen, Yang and Tume (1993). Note that cereals are rich in lipoxygenases that oxidize unsaturated fatty acids and other lipids containing a *cis*-*cis*-penta-1,4-diene unit, and the resulting hydroperoxide radicals oxidize the carotenoids (Kalač and McDonald (1981). Second, it has been shown that carotenoids absorption is greater when animals are fed polyunsaturated fatty acids (PUFA)-rich diets (Ashes, Burley, Sidhu & Sleigh, 1984), and it is likely that the diet of H lambs contained less PUFA/kg DM than the diet of L lambs. Third, the decrease in PCC in H lambs may also be attributed to the development of subclinical acidosis, which (Knight, Wyeth, Ridland & Death, 1994) claimed negatively affects carotenoids absorption and/or the ability of high-density lipoproteins to bind absorbed carotenoids. Here, the average proportion of concentrate feedstuffs (alfalfa pellets + barley) in the diet was 72.3% and 60.0% for H and L lambs, respectively, and the straw was offered in an agglomerate form to facilitate intake measurements.

The decreased perirenal fat yellowness in H lambs resulted from a likely combination of (i) lower carotenoid absorption relative to their L counterparts, as indicated by PCC, and (ii) higher adipose tissue accretion that 'diluted' the fat-stored carotenoid content (Prache et al., 2003b). This was confirmed by the increased proportion of light reflected by H lamb samples in the band of the reflectance spectrum where carotenoid pigments absorb light. The lower perirenal fat redness in H lambs was caused by increased fatness which 'diluted' fat-stored haeminic pigments (Dian et al., 2007a; Irie, 2001; Prache et al., 1990a; Swatland, 1989). This was confirmed by an increased proportion of light reflected for H lambs in the band of the reflectance spectrum where haeminic pigments absorb light. Absorption

maxima in the 570-580, 540-550 and 410-420 nm bands where differences were found between H and L lambs are characteristic of oxyhemoglobin in the visible range for the 570-580 and 540-550 bands and in the near ultraviolet for the 410-420 nm band (Prache et al., 1990a). The decrease in perirenal fat chroma in H lambs was due to reductions in both yellowness and redness. Perirenal fat hue angle was higher in H lambs than L lambs, indicating a shift from red to yellow. Note that although these differences in perirenal fat color between H and L lambs were significant, they were of low biological amplitude, as ΔE_{ab}* values were much lower than the threshold of 5.9 suggested by (Schwarz, Cowan & Beatty, 1987) as enabling visual appraisals on fat to find significant differences.

These differences in fat yellowness, redness, chroma and hue angle between H and L lambs were not observed in subcutaneous caudal fat. This may be partly explained by the fact that subcutaneous caudal fat contained much lower carotenoid pigment concentrations than perirenal fat (Kirton, Crane, Paterson & Clare, 1975). The lightness of subcutaneous caudal fat was slightly lower in H lambs than L lambs at 24 h post mortem, possibly due to a lower proportion of light reflected at a range of wavelengths across the fat reflectance spectrum. The reasons for this difference remain unclear, but the difference was of low biological amplitude, as ΔE_{ab}* value was 0.86, i.e. imperceptible to the naked eye.

Surprisingly, although both PCC and yellowness of perirenal fat were lower in H lambs than L lambs, there was no significant between-treatments difference in the AVMI of perirenal fat at slaughter or at 24 h post mortem. This result may be partly due to higher inter-animal variability for AVMI than for yellowness within the same feeding treatment. For example, for measurements made on perirenal fat at 24h post mortem, the coefficient of variation for yellowness was 13.8% and 15.7% in L and H lambs, respectively, whereas for AVMI it ranged from 16.5% to 23.8% in L and H lambs, respectively. Given the observed

mean difference and sample variance in AVMI of perirenal fat at 24 h post mortem, we would have to increase our sample size more than 10-fold to reach a probability of finding a significant difference. Second, the apparent discrepancy between a lack of treatment effect on perirenal fat AVMI despite a significant treatment effect on yellowness may be due to differences in the mode of calculation of these two variables. Yellowness is calculated from raw reflectance spectrum data whereas AVMI is calculated from translated reflectance spectrum data (making the reflectance value at 510 nm zero). In this regard, the outcome of the comparison of translated reflectance spectrum data between both treatments showed that there were no significant differences between H and L lambs in the 510-450 nm band where AVMI is calculated, except at 450 nm for which the value was lower for L lambs than for H lambs ($P < 0.025$). This resulted in very similar AVMI values between the two feeding treatments, as shown in Figure 2. Note that these observations are consistent with results obtained by (Oliveira et al., 2012) on their G75 lambs, where the coefficient of variation was higher for AVMI than for yellowness of perirenal fat (25.1% *vs* 19.2%, respectively). Moreover, some between-treatments differences in fat yellowness were significant in the study by Oliveira et al. (2012), whereas the between-treatments differences in AVMI were not.

The yellowness of the fat of L lambs was 11% (subcutaneous) and 2% (perirenal) lower than the values obtained by Dian et al. (2007b) in their pasture-fed Romane lambs, despite PCC being 15% higher. Similarly, the AVMI values we obtained for subcutaneous and perirenal fat of L lambs were 9% and 15% lower than those obtained by Dian et al. (2007b) in their pasture-fed lambs. Here, mean AVMI for L lambs at 24 h post mortem was 267 and 319 units for subcutaneous and perirenal fat, respectively, *versus* 293 and 374 units in Dian et al. (2007b). This difference may be due to differences in carotenoid pigments

source (dry alfalfa pellets *vs*. pasture) and to differences in LW and the corresponding animal's degree of fatness at the start of the carotenoid-rich diet, as lambs weighed 14.2 kg when they were turned out to pasture in Dian et al. (2007b) *versus* 27.8 kg at the beginning of alfalfa consumption here. An interesting new finding from the present study is actually that yellowness, chroma and AVMI of subcutaneous were negatively correlated to LW, i.e. to the animal's degree of fatness, at the beginning of alfalfa consumption.

Although there are inherent difficulties in managing such an experiment under pasture-feeding conditions, further investigation to test the generalization of these results to pasture-fed lambs is warranted. Compared to the present study, pasture-feeding conditions may add the phenomenon of substitution of forage by concentrate, i.e. the partial replacement of the pasture with the concentrate and a resulting decline in level of carotenoid intake. However, although concentrate intake level was not specified, studies with sheep found no difference in AVMI (Ripoll, Joy, Muñoz & Albertí, 2008) or fat color coordinates (Ripoll et al., 2012) in lambs pasture-fed with or without concentrate supplementation at pasture between birth and slaughter at 22-24 kg LW. Similarly, despite a 50% drop in pasture, carotenoid and lutein intake levels in pasture-fed heifers that were supplemented with 4.63 kg concentrate/day compared to their unsupplemented counterparts, (Röhrle et al., 2011) found no change in subcutaneous fat color coordinates or AVMI. It is therefore most likely that the changes in AVMI and fat color induced by concentrate supplementation at pasture are of low biological amplitude and are lower than the changes observed in animals finished indoors with a concentrate-based diet after a period of pasture-feeding (Prache et al., 2003a, 2003b).

5. Conclusions

This study demonstrates that there is a slight decrease in plasma carotenoid concentration and perirenal fat color when lambs fed a carotenoid-rich diet are supplemented with a high level of barley compared with counterparts supplemented with low level of barley. However, the level of barley supplementation did not significantly change the value of the traceability index, i.e. the absolute value of the mean integral (AVMI) calculated from the fat reflectance spectrum in the 450 nm–510 nm band where carotenoid pigments absorb light. Yellowness, chroma and AVMI of subcutaneous caudal az220d4xevtfee9vnxxezz0szdzel of barley supplementation, but were negatively correlated to animal's liveweight at the start of the carotenoid-rich diet.

Further work is required to evaluate the effect of concentrate supplementation on reflectance spectrum characteristics, fat color and traceability index under pasture-feeding conditions, where the phenomenon of the substitution of forage by concentrate may further decrease the pasture and carotenoid intake levels. Note that this decrease depends on the level of pasture availability (Prache, Bechet & Theriez, 1990b) and the level of concentrate supplementation.

Acknowledgements

The authors thank F. Anglard, P. Payard, C. Rougheol, D. Roux, C. Coustet, I. Constant, S. Faure and all the staff of the INRA-Theix experimental farm, abattoir and laboratory for their collaboration. We also thank B. Chauveau-Duriot for analyzing the carotenoid concentrations in the feed. F. Zawadzki was awarded financial support from the Brazilian Ministry of Science and Technology 'Conselho Nacional de Desemvolvimento Cientifíco e Tecnológico' (CNPq) via CNPq Project grant 201094/2011-8.

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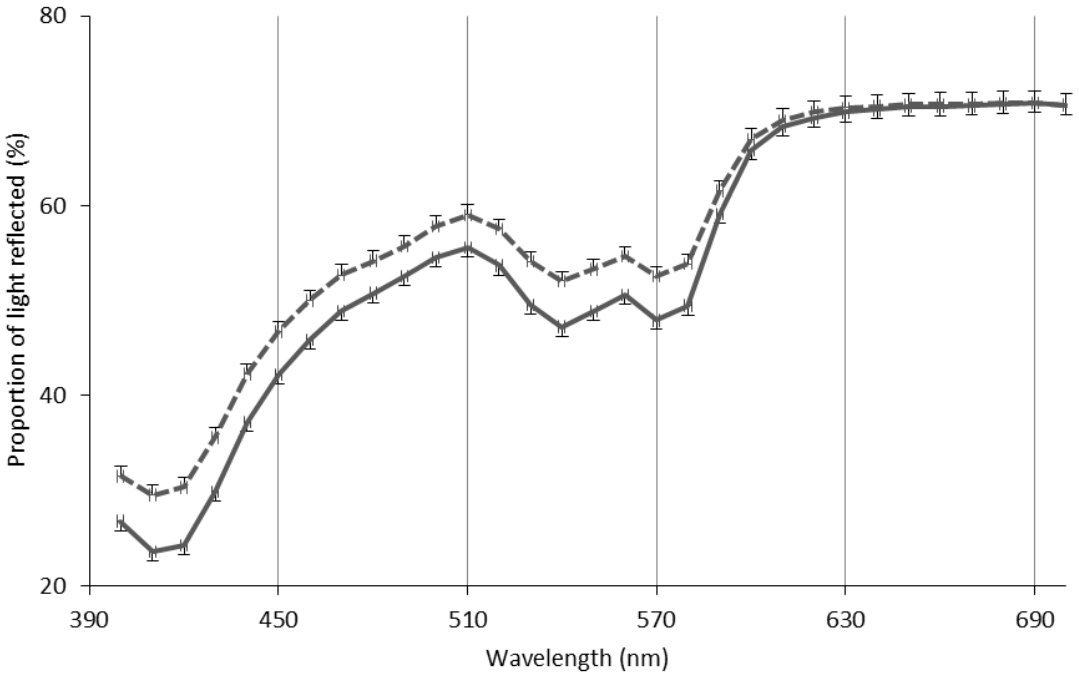

Fig. 1. Mean reflectance spectrum of perirenal fat at 24 h *post mortem* for lambs fed a carotenoid-rich diet and supplemented with a low (---) or a high (—) level of barley. Bars represent S.E.M.

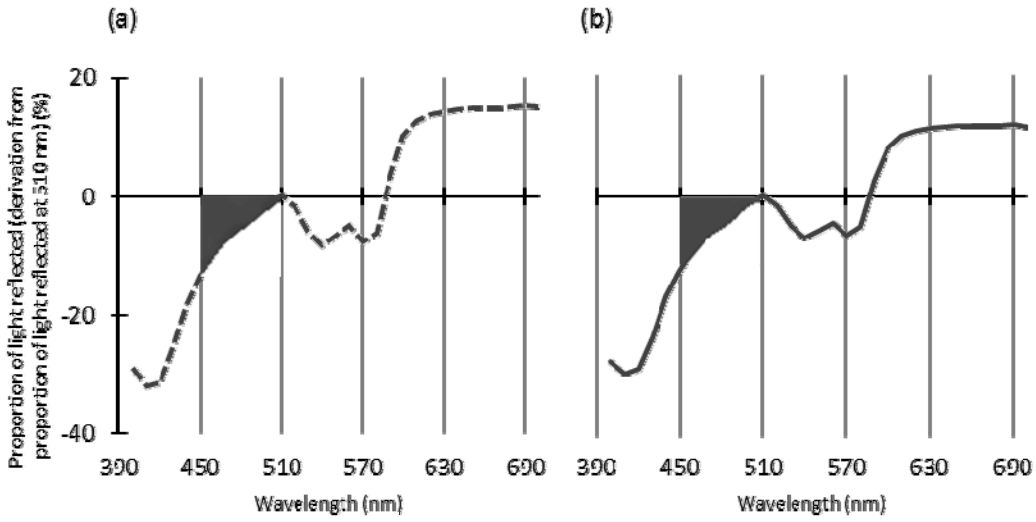

Fig. 2. Mean reflectance spectrum pattern of perirenal fat at 24 h *post mortem* for lambs fed a carotenoid-rich diet and supplemented with a low (a) or a high (b) level of barley. The AVMI is the shadow area comprised between the curve and the X-axis in the 450–510 nm band.

Table 1. Carotenoid concentration (µg/g DM) of the feed offered

Carotenoid (µg/g DM)	Dehydrated alfalfa	Barley	Straw
Lutein	846.6	6.3	9.5
β-Carotene	296.0	2.1	2.4
Zeaxanthin	47.5	2.2	-
13-*cis β*-Carotene	25.5	-	-
9-*cis β*-Carotene	133.5	-	-
Neoxanthin	63.8	-	-
β-Cryptoxanthine	8.41	-	-
Total carotenoids	1421.3	10.6	11.9

Table 2. Mean daily intakes of the different feeds offered

	Level of barley supplementation			
	Low	High	S.E.M	*P*
Mean daily alfalfa intake, g DM/d	370.5	370.6	0.001	0.56
Mean daily barley intake, g DM/d	91.3	360.8	0.003	< 0.001
Mean daily straw intake, g DM/d	304.9	280.2	0.021	< 0.001

Table 3. Animal performances and characteristics at slaughter

	Level of barley supplementation			
	Low	High	S.E.M	*P*
Live weight at beginning of experiment, kg	28.18	27.55	3.56	0.54
Average daily gain during the experiment, kg	0.149	0.243	0.030	<0.001
Live weight gain during the experiment, kg	11.12	18.13	2.19	<0.001
Slaughter age, d	189	188	7.3	0.42
Slaughter weight, kg	39.31	45.68	2.18	<0.001
Cold carcass weight, kg	17.04	20.72	1.43	<0.001
Perirenal fat weight, g	133	272	86.4	<0.001
Subcutaneous fat thickness, mm	2.5	3.7	0.68	<0.001

Table 4. Plasma carotenoid concentration and AVMI[1] of subcutaneous caudal and perirenal fat

	Level of barley supplementation			
	Low	High	S.E.M	*P*
Plasma carotenoid concentration 10 days after the start of the experiment (µg/L)	107.6	95.0	26.71	0.11
Plasma carotenoid concentration at slaughter (µg/L)	129.0	108.0	25.20	<0.01
Hot carcass				
AVMI of subcutaneous caudal fat, units	139.2	149.8	43.09	0.40
AVMI of perirenal fat, units	175.8	155.7	50.73	0.18
Cold carcass				
AVMI of subcutaneous caudal fat, units	266.8	254.4	60.65	0.48
AVMI of perirenal fat, units	319.3	308.1	63.95	0.55

[1]AVMI = absolute value of the mean integral of the translated spectrum between 450 and 510 nm

Table 5. Mean color parameters for subcutaneous caudal fat and perirenal fat at slaughter according to level of barley supplementation

	Level of barley supplementation			
	Low	High	S.E.M	*P*
Subcutaneous caudal fat				
*L**	70.67	69.99	3.53	0.51
*a**	0.308	0.457	0.878	0.56
*b**	9.53	10.04	3.09	0.57
*C**	9.61	10.08	3.11	0.60
h	88.50	87.16	4.73	0.33
Perirenal fat				
*L**	69.59	69.90	2.65	0.68
*a**	1.628	0.337	1.056	<0.001
*b**	13.06	11.33	1.98	<0.005
*C**	13.22	11.38	1.98	<0.005
h	83.05	88.33	5.05	<0.001

Table 6. Mean color parameters for subcutaneous caudal fat and perirenal fat after 24 h shrinkage, according to level of barley supplementation

	Level of barley supplementation			
	Low	High	S.E.M	*P*
Subcutaneous caudal fat				
*L**	73.99	71.81	3.59	<0.05
*a**	1.997	2.090	1.463	0.83
*b**	12.45	13.24	2.79	0.33
*C**	12.66	13.46	2.92	0.34
h	81.79	81.48	5.18	0.84
Perirenal fat				
*L**	78.09	80.17	3.59	0.051
*a**	4.050	2.484	1.235	<0.001
*b**	14.12	12.16	1.93	<0.001
*C**	14.74	12.43	2.10	<0.001
h	74.35	78.52	3.73	<0.001

CONSIDERAÇÕES FINAIS

No experimento 1, a substituição do milho grão por glicerina pode ser realizada para terminar touros em confinamento, sem afetar negativamente o desempenho animal, as características da carcaça, as características do músculo *Longissimus* ou a sua composição química. A inclusão de glicerina na dieta aumentou o consumo de energia digestível, metabolizável e melhorou a digestibilidade aparente da dieta. Óleos funcionais podem ser adicionados à dieta de touros terminados em confinamento, pois aumentam o peso e rendimento da carcaça, sem ocasionar efeito negativo sobre o consumo de ração e a eficiência alimentar. Dietas contendo glicerol e / ou FO melhorou o peso de carcaça fria. Dietas contendo óleos funcionais melhorou o rendimento de carcaça fria. A associação de glicerina e óleos funcionais melhora a conformação, espessura de gordura e percentagem de gordura da carcaça. Por outro lado, a substituição do milho grão por glicerina com 81,2% de glicerol pode alterar a composição de ácidos graxos na gordura perirrenal, godura subcutânea e no músculo *Longissimus*. A inclusão de glicerina reduz o somatório de ácidos graxos saturados, monoinsaturados e os níveis dos ácidos graxos poliinsaturados na dieta total. As dietas não alteram os ácidos graxos láurico, mirístico e palmítico no músculo *Longissimus* e na gordura subcutânea. Por outro lado, as dietas com glicerina reduz os níveis do ácido esteárico na gordura perirrenal, gordura subcutânea e no músculo *Longissimus*. Da mesma forma, o ácido linoléico na gordura perirrenal, gordura subcutânea e no músculo *Longissimus* foram reduzidos com a inclusão de glicerina na dieta. Dietas sem glicerina ou com óleos funcionais melhoram os ácidos linoléico, α-linolênico e eicosapentaenóico sobre a gordura subcutânea e no múculo *Longissimus*. Enquanto que o somatório dos ácidos graxos poli-insaturados, n-6 e as razões AGP:AGS e *n*-6:*n*-3 melhora com a adição de óleos funcionais na gordura subcutânea.

No experimento. 2, demonstrou-se que existe um ligeiro decréscimo na concentração plasmática de carotenóides e na cor da gordura perirenal de cordeiros

alimentados com uma dieta rica em carotenóides e suplementados com um elevado nível de cevada, em comparação com cordeiros suplementados com baixo nível de cevada. No entanto, o nível de suplementação de cevada não alterou de forma significativa o valor do índice de rastreabilidade, ou seja, o valor absoluto médio da integral (AVMI), calculado a partir do espectro de refletância do tecido adiposo na banda de 450 a 510 nm, onde absorvem luz pigmentos carotenóides. Os parâmetros de cor amarela, croma e AVMI no tecido adiposo subcutâneo caudal não se alteraram com o nível de suplementação de cevada, mas foram negativamente correlacionados ao peso vivo do animal no início da dieta rica em carotenóides. Mais estudos são necessários para avaliar o efeito da suplementação concentrada nas características do espectro de refletância, cor do tecido adiposo e índice de rastreabilidade sob condições de pastagem, onde o fenômeno da substituição do volumoso e concentrado pode diminuir ainda mais os níveis de ingestão de pasto e de carotenóides. Note-se que esta redução depende do nível de disponibilidade de pastagem e o nível de suplementação com concentrado.