UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ADITIVOS NATURAIS NA DIETA DE RUMINANTES E SEUS EFEITOS NA QUALIDADE DA CARNE

Autor: Dayane Cristina Rivaroli
Orientador: Prof. Dr. Ivanor Nunes do Prado

MARINGÁ
Estado do Paraná
Fevereiro – 2018

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ADITIVOS NATURAIS NA DIETA DE RUMINANTES E SEUS EFEITOS NA QUALIDADE DA CARNE

Autora:Dayane Cristina Rivaroli
Orientador: Prof. Dr. Ivanor Nunes do Prado

Tese apresentada como parte das exigências para obtenção do título de Doutora em Zootecnia, no Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá – Área de concentração Produção Animal

MARINGÁ
Estado do Paraná
Fevereiro – 2018

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

Rivaroli, Dayane Cristina
R618a Aditivos naturais na dieta de ruminantes e seus efeitos na qualidade da carne / Dayane Cristina Rivaroli. -- Maringá, PR, 2018.
xi, 92 f.: il.

Orientador: Prof. Dr. Ivanor Nunes do Prado.
Coorientador: Prof. Dr. André Mendes Jorge.
Tese (doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia, 2018.

1. Nutrição animal. 2. Antioxidantes. 3. Extratos de plantas. 4. Óleos essenciais - Nutrição - Ruminantes. I. Prado, Ivanor Nunes do, orient. II. Jorge, André Mendes, orient. III. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Zootecnia. IV. Título.

CDD 23.ed. 636.208

Márcia Regina Paiva de Brito – CRB-9/1267

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ADITIVOS NATURAIS NA DIETA DE RUMINANTES E SEUS EFEITOS NA QUALIDADE DA CARNE

Autora: Dayane Cristina Rivarolli
Orientador: Prof. Dr. Ivanor Nunes do Prado

TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 27 de fevereiro de 2018.

Profª Drª Ana Guerrero Barrado

Profª Drª Adriana Aparecida Pinto

Prof. Dr. Luiz Paulo Rigolon

Profª Drª Polyana Pizzi Rotta Costa e Silva

Prof. Dr. Ivanor Nunes do Prado
Orientador

“L'avenir a plusieurs noms: pour les faibles, il se nomme l'inaccessible. Pour les peureux, il se nomme l'inconnu. Pour les courageux, il se nomme opportunité." – **Victor Hugo**

Em tradução livre: “*O futuro tem muitos nomes. Para os fracos é o inalcançável. Para os temerosos, o desconhecido .Para os valentes é a oportunidade.”*

DEDICATÓRIA

Dedico à minha abençoada família, a qual sempre me apoiou de todas as formas no decorrer da minha vida, proporcionando-me, além de extenso carinho e amor, os conhecimentos da integridade, da perseverança e de procurar sempre em Deus a força maior para o meu desenvolvimento como ser humano. Por essa razão, gostaria de dedicar e reconhecer a vocês, com minha imensa gratidão e sempre amor. *In momoriam* de Catharina Galiano Rivaroli e Antônio Rivaroli, que mais do que avós, foram pais.

AGRADECIMENTOS

A Deus,

Ao meu pai, Miguel Carlos Rivaroli, minha mãe, Eliana Cláudia Silva, meus avós Catarina Galiano Rivaroli (*in memoriam*) e Antônio Rivaroli (*in memoriam*) que são tudo de mais precioso que tenho nessa vida: meus maiores incentivadores, minha maior inspiração, meus queridos e amados PAIS.

Aos meus avós, Hilda Gardiolo e Azaury Silva, e ao meu padrinho, Azaury Silva Junior, pelo carinho, ajuda e amor.

Ao meu querido Carlos Frederico Steiniger e sua filha Duda, por toda paciência, convívio, otimismo, dedicação e incentivo durante o período final e crítico do meu doutorado.

Ao meu orientador, Ivanor Nunes do Prado, pela paciência que teve comigo, pelos ensinamentos, dedicação, conselhos, amizade e acima de tudo, exemplo profissional e maior motivador cientifico. Obrigada pela confiança e oportunidade de aprender.

Ao meu professor coorientador, André Mendes Jorge, pelo acolhimento, ensinamento e dedicação.

À minha coorientadora francesa, Sophie Prache, pelo acolhimento, aprendizado e, principalmente, pelas palavras “duras” necessárias para minha formação como pesquisadora.

Ao meu professor Carlos Sanudo, pelo aprendizado, pelo otimismo e bom humor vinculado.

Ao Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá.

Ao conselho Nacional de desenvolvimento científico - CNPq, pelo financiamento do projeto de pesquisa e bolsa de estudo.

Ao grupo de pesquisa do professor Ivanor Nunes do Prado, especialmente José Carlos e os colegas da pós-graduação

Aos estagiários que me ajudaram durante o experimento.

À pós-doutoranda Ana Guerrero, da Universidad de Saragoza, pelos ensinamentos, amizade e conselhos.

Aos meus amigos brasileiros: Patrícia Granero, Déborah Tostes Martins, Fernanda Paiva, Bruna Paes, Aline Dalosse, Ana Cláudia Furlan (banana) e Gabriela Pessuto, pelo incentivo, por não me deixarem desistir, por me alegrarem, por escutarem diversas vezes meu choro e pela paciência, principalmente nos meus dias de "abandono".

Às minhas amigas que vivem na Espanha e que foram minha família durante o período em que habitei na europa: Marina Meneguetti (Nina), Karoline Grimmes (Karólis) e Mari Oliveira (minha "mamis") – muito obrigada pelo incentivo, momentos felizes, ajuda pessoal, pela estadia e pelo amor de família que encontrei em vocês.

Às minhas duas "mães francesas": Néna Echassoux e Pascale Beraud, por terem me acolhido como filha e terem me guiado e ajudado nos melhores e piores momentos da minha vida. Eu amo vocês.

Aos meus amigos franceses: Yohan Gaudron, Cyril Labonne, Lucas Ofeu, Luciano Barreto Mendes, Jose Pires, Celine Closier, Emma Labonne, Fred Says, Sébastien Halsberghe. Lucille Ferrero, Martine Tourret, Didier, Emelie Tixier, Hélene Fougére, Karol Pawlowski, Adeline Boubou, por terem me proporcionado alguns dos melhores momentos da minha vida e terem tornado minha caminhada na França mais doce.

Ao meus "colocataires": Clément, Benjamin Devoucoux e Elodie Alice, por além de terem paciência e me ensinarem o francês do dia a dia, terem sido minha família e repartido além da casa, todas as experiências nesse um ano de convívio diário.

A todos aqueles que não me deixaram "desanimar", meu MUITO OBRIGADA,

BIOGRAFIA

Dayane Cristina Rivaroli, filha de Miguel Carlos Rivaroli e Eliana Cláudia Silva, nasceu no município de Maringá, Estado do Paraná, Brasil, no dia 18 de março de 1989.

Em fevereiro de 2007, ingressou no curso de graduação em Zootecnia, pela Universidade Estadual de Maringá, concluindo o mesmo em dezembro de 2011.

Em março de 2012, ingressou no programa de Pós-Graduação em Zootecnia, em nível de mestrado, pela Universidade Estadual "Júlio de Mesquita Filho" (município de Botucatu, estado de São Paulo), concentrando seus estudos nas áreas de produção e nutrição animal. Obteve o título de mestre em Zootecnia, com ênfase em Produção Animal em fevereiro de 2014.

Em março de 2014, ingressou no programa de Pós-Graduação em Zootecnia, em nível de doutorado, pela Universidade Estadual de Maringá (Maringá, Paraná, Brasil), concentrando seus estudos nas áreas de produção animal, aditivos na alimentação de bovinos e qualidade de carne. Durante o período de maio a abril de 2017, realizou intercâmbio em nível de doutorado no Institut National de la Recherche Agronomique (Clermont-Ferrand,França)

Em junho de 2017, submeteu-se ao exame de qualificação do Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá e em fevereiro de 2018 submeteu – se à defesa da tese.

ÍNDICE

LISTA DE TABELAS

Página

II-Effects of essential oil on meat and fat qualities of crossbred heifers finished in feedlot

III-Effects of essential oil on meat and fat qualities of crossbred heifers finished in feedlot

IV-Effect of sainfoin supplementation on fat skatole and indolw concentration, meat quality, odour and flavor in lambs grazing alfafa pasture

RESUMO

O trabalho com novilhas mestiças terminadas em confinamento foi realizado para avaliar o efeito da inclusão do mix de óleos essenciais (orégano, alho, limão, alecrim, tomilho, eucalipto e laranja doce) na dieta e seus efeitos na qualidade da carne (composição química, coloração, capacidade de retenção de água e textura) e da gordura (oxidação lipídica e perfil de ácidos graxos). Foram utilizados 30 animais (12 meses; 219,8 kg) (½ Angus *vs.* ½ Nellore), alocados em baias individuais, recebendo uma dieta composta de 90% de concentrado e 10% de bagaço de cana peletizado durante 3 meses, e divididos em 3 tratamentos: sem adição de óleos essenciais (E0.0), com adição de 3.5 (E3.0) ou 7 (E7.0) g/animal/dia do mix de óleos essenciais. Em adição, foi avaliado o efeito da maturação (1, 7 e 14 dias) na capacidade de retenção de água, coloração, textura e oxidação lipídica. A adição de óleos essenciais não influenciou ($P > 0.05$) na composição química, coloração da carne, capacidade de retenção de água e textura, mas a inclusão de 7 g/animal/dia mostrou uma tendência ($P < 0.10$) a aumentar a oxidação lipídica tendo assim, um efeito pro-oxidante. A adição de 7 g/animal/dia também aumentou ($P < 0.05$) a luminosidade da carne durante a maturação aos 14 dias. A maturação da carne reduziu ($P < 0.05$) a textura da carne. O segundo trabalho foi realizado para avaliar a aceitabilidade sensorial da carne de 57 animais mestiços (½ Angus *vs.* ½ Nellore) de diferentes sexos (novilhas *n*=30, e tourinhos *n*=27) terminados em confinamento com uma dieta isoenergética e isoproteica, e com a inclusão de diferentes níveis (0.0, 3.5 ou 7.0 g/animal/dia) do mix de óleos essenciais (Mixoil®) constituído de 7 diferentes plantas: orégano, alho, limão, alecrim, tomilho, eucalipto e laranja doce). Cento e vinte consumidores classificaram, utilizando uma escala hedônica de 9 pontos, o músculo *Longissimus thoracis* com 24 horas de maturação, para os seguintes critérios: *aceitabilidade da maciez, aceitabilidade do flavor e aceitabilidade geral*. A inclusão de óleos essenciais afetou a aceitabilidade da maciez e a aceitabilidade do flavor ($P \leq 0.01$), com uma tendência ($P < 0.10$) para a aceitabilidade geral. Carne de novilhas foi mais apreciada pelos consumidores que a carne de tourinhos em todos os atributos ($P \leq 0.001$), entretanto, diferentes grupos de consumidores possuíram diferentes preferências. A inclusão de óleos essenciais na dieta de bovinos de corte foi bem aceita pelos consumidores e pode aumentar a aceitabilidade da carne de tourinhos quando é adicionada na concentração de 3.5 g/animal/dia durante os 4 meses finais da terminação. O terceiro trabalho foi realizado para avaliar o efeito da adição de pellets de sainfoin na dieta de cordeiros terminados em pastagem sobre a concentração de escatol e indol, coloração, odour e flavour atribuídos à carne e à gordura. Cinquenta e quatro cordeiros (Romano) foram separados em diferentes grupos pelos 87 dias antes do abate: animais confinados (SI), animais pastejando alfafa (*M. sativa*) (AF) e animais pastejando alfafa com a suplementação de pellets de sainfoin (*O. viciifolia*) (AS). O ganho médio de peso foi o mesmo para os três tratamentos. As concentrações de escatol da gordura perirenal e dorsal foram quantificadas com valores menores que os valores limites para todos os animais do grupo SI. A suplementação de sainfoin para cordeiros em pastagem, reduziu a concentração de escatol nas gorduras perirenal e dorsal. Os tratamentos não afetaram a coloração da gordura subcutânea caudal e do músculo *Longissimus thoracics et lumborum,* exceto para a luminosidade (L*) na gordura. A suplementação de pellets de sainfoin para a terminação de animais em pastagem pode ser uma estratégia para reduzir a concentração da concentração do escatol na gordura perirenal e dorsal.

Palavras-chave: anti-oxidanteantioxidante, escatol, extratos de plantas, taninos

ABSTRACT

The study with crossbred heifers, finished in feedlot, fed with diets containing essential oils mix (oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange) was carried out to evaluate the effect on meat quality (chemical composition, water holding capacity and texture) and fat (lipid oxidation and fatty acid profile). Thirty animals (12 months-old and 219.8 kg) (½ Angus *vs.* ½ Nellore) were allocated in individual pens for 3 months with one of the following diets (90% concentrate and 10% sugarcane bagasse pelletized): without addition of essential oils (E0.0), with 3.5 (E3.5) or 7 (E7.0) g/animal/day of essential oils' blend. Chemical composition, fatty acid profile on *Longissimus* muscle and meat color were evaluated. In addition, the effect of ageing (1, 7 and 14 days) was evaluated on water holding capacity, texture and lipid oxidation. Essential oils had no influence ($P > 0.05$) on chemical composition, meat colour, water holding capacity and texture, but the inclusion of 7.0 $g.d^{-1}$ had a tendency ($P < 0.10$) to increased lipid oxidation. The addition of 7.0 g/animal/day had a pro-oxidant effect ($P < 0.05$) on meat during the ageing and showed higher values for lightness at 14 days. Ageing reduced ($P < 0.05$) meat texture. The second study was to evaluate the sensory acceptability of meat from 57 crossbred (½ Angus - ½ Nellore) animals of different sexes [heifers n=30, and young bulls n=27], finished in feedlot with isoenergetic and isonitrogenous diets, in different levels of supplementation (0.0, 3.5 or 7.0 g/animal/day) of a commercial mix of essential oils (Mixoil®) from seven plants: oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange. 120 consumers who scored tenderness acceptability, flavor acceptability and overall acceptability using a hedonic nine-point scale evaluated the *Longissimus thoracis* muscle, with 24 hours of maturation. The essential oils inclusion affected tenderness acceptability and flavor acceptability (*P*

≤ 0.01), with a tendency ($P < 0.10$) for overall acceptability. Meat from heifers was more appreciated by consumers than that from young bulls for all attributes ($P \leq 0.001$), however, different groups of consumers had different preferences. The inclusion of essential oils mix in beef diets was well accepted by consumers and could improve the meat acceptability from young bulls, when added at a concentration of 3.5 g/animal/day during the last 4 months of fattening. The third work was carried out to investigate the influence of sainfoin pellets supplementation on fat skatole and indole concentration, fat and meat color and chop sensory attributes in grazing lambs. Fifty-four lambs (Romane) were separated in different groups for at least 87 days before slaughter: feedlot (SI), pasture-fed alfalfa (*M. sativa*) (AF) and pasture-fed alfalfa plus sainfoin (*O. viciifolia*) pellets (AS). Perirenal and dorsal fat skatole concentrations were below quantification limit for all SI lambs. Sainfoin pellet supplementation in grazing lambs reduced perirenal and dorsal fat skatole concentration. The treatments did not affect subcutaneous caudal fat and *Longissimus thoracics et lumborum* muscle color, except for lightness (L*) on fat. The sainfoin pellet supplementation for grazing lambs can be a strategy to reduce perirenal and dorsal fat skatole concentration.

Key words: anti-oxidant, eskatole, plants extract, tannins

CAPÍTULO 1.

CONSIDERAÇÕES INICIAIS

I INTRODUÇÃO

1) ADITIVOS NA ALIMENTAÇÃO DE RUMINANTES

No Brasil, segundo o Ministério da Agricultura Pecuária e Abastecimento (MAPA), os aditivos são substâncias que são intencionalmente adicionadas na dieta com a finalidade de intensificarem, conservarem, ou até mesmo modificarem suas propriedades, desde que não prejudiquem o valor nutritivo do alimento.

Sabendo que em um sistema de produção intensiva de bovinos, a alimentação representa aproximadamente 80% dos custos, precisa-se assegurar que os nutrientes oriundos da dieta sejam bem aproveitados pelo animal. Um dos métodos para reduzir o custo da alimentação e aumentar o desempenho animal é a utilização de aditivos como por exemplo, os ionóforos (BERGEN & BATES, 1984; GOODRICH et al., 1984.)

Os ionóforos são assim denominados pela sua capacidade em transportar íons, podendo formar complexos lipossolúveis com cátions e mediar seu transporte pelas membranas lipídicas (PRESSMAN, 1968). Existem mais de 120 ionóforos descritos, mas somente 3 são aproveitados em alguns países para o uso na dieta dos ruminantes, são eles: laidomicina, lasolacida e principalmente a monensina (NAGARAJA et al., 1997). No Brasil, apenas os dois últimos são liberados para o uso na produção de ruminantes, sendo os nomes comerciais mais conhecidos Taurotec e Rumensin, respectivamente.

No entanto, a utilização de ionóforos na alimentação animal foi banida da União Europeia desde 2006 pela EFSA (Autoridade Europeia da Segurança do Alimento), por meio da regulação 1831/2003/EC. A preocupação está relacionada com possível desenvolvimento de microrganismos resistentes, pelo uso inadequado dos ionóforos na dieta animal, que transmitiria e comprometeria a ação terapêutica dos antibióticos em humanos (RUSSELL & HOULIHAN, 2003; DEWULF et al., 2007). Neste sentido, é necessário o estudo dos efeitos de possíveis substituintes naturais, para que haja melhorias no sistema de produção de animais confinados, e para que a carne brasileira não tenha muitos entraves ao ser exportada para os países europeus (RIVAROLI et al., 2016; ORNAGHI et al., 2017; MONTESCHIO et al., 2017).

1.2. ÓLEOS ESSENCIAIS

A utilização dos óleos essenciais para o consumo animal e humano é permitida por serem substâncias reconhecidas como seguras, de acordo com o FDA - "Food and Drug Admnistration", órgão governamental dos Estados Unidos responsável pelo controle dos alimentos.

Óleos essenciais são moléculas hidrofóbicas que contêm compostos voláteis produzidos por plantas (LOSA, 2001) que podem ser extraídos de diversas partes morfológicas da mesma, tais como, das flores, cascas, folhas e sementes. A composição dos óleos essenciais não é considerada simples, mas sim uma mistura de vários compostos (principalmente terpenos e derivados de terpenos). A composição dos princípios ativos das moléculas de óleos essenciais, além de variarem conforme as regiões morfoanatômicas da planta que são extraídos, podem variar também com a metodologia utilizada para extração (YANG et al., 2010). Há várias formas de extração, sendo a destilação simples a forma mais utilizada na obtenção dos óleos essenciais comerciais, mas podendo também ser feita por meio da fermentação ou extração por solventes (GREATHEAD, 2003; YANG et al., 2010; ZHANG et al., 2010).

Como citado anteriormente, os extratos de plantas contêm uma ampla variedade de compostos com diferentes funções e mecanismos de ação (BURT et al., 2004). Dependendo do composto predominante e sua concentração, é que se determinará o modo de ação e a função de cada extrato vegetal (BENCHAAR et al., 2008). Alguns óleos essenciais possuem capacidade antimicrobiana, enquanto outros, capacidade antioxidante.

Algumas classes de compostos, como por exemplo, os compostos fenólicos (fenóis simples – cetocol, ácidos fenólicos – ácido anacárdico, cinâmico, cafeico e rícininoleico, quinonas – hipericina, flavonóis – totarol, taninos – Elagitanina, Cumarinas – Warfarin), terpenoides (Capsaicina, Thimol Mentol, Carvacrol, Cânfora, Eugenol) e fenóis (CHAO et al., 2000) conferem a alguns óleos essenciais capacidade antimicrobiana. O mecanismo de ação antibacteriana dos óleos essenciais no rúmen se dá uma vez que estes atuam na estrutura da parede celular bacteriana, desnaturando e coagulando as proteínas, alterando a permeabilidade da membrana citoplasmática (BENCHAAR et al., 2008). Com a permeabilidade da membrana citoplasmática alterada, há comprometimento no transporte de elétrons essenciais para a manutenção da célula, além da translocação de proteínas, etapas da fosforilação e outras reações dependentes de enzimas, resultando assim, em perda do controle quimiosmótico da célula e, consequentemente, sua morte (DORMAN & DEANS, 2000).

Já os compostos fenólicos, flavonoides e terpenoides conferem a alguns óleos essenciais capacidade antioxidante (OLDONI, 2007). Essas substâncias podem interceptar e neutralizar radicais livres, impedindo a propagação do processo oxidativo (HUI, 1996). Se ministrados em excesso, os óleos essenciais podem ter atividade pro-oxidante (BAKKALI et al., 2008, RIVAROLI et al., 2017).

Além de ter efeitos antimicrobianos e atividade antioxidante, alguns autores afirmam que os óleos essenciais também atuam melhorando a digestão, através do estímulo da atividade enzimática (MELLOR, 2000; BENCHAAR et al., 2008; PATRA, 2011).

Alguns pesquisadores acreditam que, para obtenção de melhores resultados, devem ser administradas combinações de óleos essenciais de diferentes plantas (LANGHOUT, 2000).

1.2.1. Orégano (Origanum vulgare)

O orégano (*Origanum vulgare*) é uma planta perene que pertence à família *Lamiacea*. Várias espécies do gênero *Origanum* são nativas do Mediterrâneo Europeu.

O óleo essencial de orégano vem despertando interesse por possuir atividade biológica (antibacteriana, antifúngica e antioxidante), combatendo alguns tipos de bactérias que costumam ser muito resistentes aos antibióticos.

Tem sido descrito que o óleo essencial de *Origanum vulgare* possui mais de 34 princípios ativos sendo os principais carvacrol, timol, gama terpeno e p-cimeno que representa aproximadamente 85% da composição total do óleo (ZHANG et al., 2010).

O modo de ação do carvacrol ainda não foi totalmente esclarecido, porém, sua ação pode ser atribuída principalmente à capacidade de tornar a membrana das bactérias permeáveis, sobretudo às bactérias gram-positivas (LAMBERT et al., 2001; LAMBERT et al., 2004), reagindo com os lipídeos da membrana e convertendo-os em produtos instáveis (YANISHLIEVA et al., 2001). Todavia, o carvacrol também parece ser capaz de desintegrar a membrana externa das bactérias gram-negativas, e aumentando a permeabilidade da membrana citoplasmática (ULTEE et al., 2000; BURT, 2004).

Na produção de ruminantes, Chaves et al. (2008); Chaves et al. (2011) verificaram que o carvacrol pode aumentar a proporção de propionato, sendo este, um precursor da glicose em ruminantes, que posteriormente poderá refletir em maiores ganhos de peso ao animal.

1.2.2. Alho (*Allium sativum*)

O alho tem sua origem na região da Sicília, Ásia Central e vários pontos da Europa e do Ocidente (CORRÊA, 1984). Pertence à família *Liliaceae*.

A principal substância que compõe o sabor característico do alho é a dialila dissulfeto que compõe aproximadamente 70% dos compostos voláteis deste produto, o que confere características antioxidantes e antimicrobianas (IVANOVA et al., 2009).

Outros compostos também são identificados nos óleos essenciais do alho, tais como a aliina, alicina e garlanicina. A aliina e alicina são compostos que agem como antioxidantes por terem efeito na enzima xantina-oxidase, inibindo assim a peroxidação lipídica além da alicina tem em sua composição o selênio que combate os radicais livres.

Em pesquisas conduzidas *in vivo* e *in vitro* foram identificados no alho dois princípios antibacterianos distintos: alicina (CAVALLITO et al., 1944) e garlicina ambos de ação contra bactérias tanto Gram-positivas quanto Gram-negativas.

1.2.3. Limão (*Citrus limonium*)

O gênero *Citrus* tem aproximadamente 16 espécies na família *Rutaceae* e são frequentemente cultivadas em regiões subtropicais.

A família *Rutaceae* é bastante conhecida por sua atividade antimicrobiana. O óleo essencial de Citrus limonum contém limoneno (90%), citral (3,5%), uma boa quantidade de pineno e citronela ambos com características antioxidantes e antimicrobianas (ALONSO, 1998).

1.2.4. Alecrim (*Rosmarinus officinalis*)

O alecrim é uma erva aromática pertencente à família *Lamiaceae*, comum na região mediterrânea. O óleo essencial é retirado das folhas e flores (CARVALHO & ALMANÇA, 2003).

Esse óleo essencial tem em sua composição compostos com atividade inibitória sobre as bactérias, tanto as Gram-negativas quanto as Gram-postivas, como por exemplo, hidrocarbonetos mono-terpênicos, ésteres terpênicos, linalol, verbinol, terpineol, 3-octanona e acetato de isobornila (BARATTA et al., 1998).

Segundo Silva et al., (2008), o óleo essencial de alecrim é também constituído por terpenoides (carnosol, ácidos carnosílico, oleânico, ursólico) que possuem atividade antioxidante.

1.2.5. Tomilho (*Thymus vulgaris*)

O tomilho é originário do Mediterrâneo. O óleo essencial é rico em timol, que tem ação antimicrobiana contra vários agentes, quer bactérias quer fungos, por vezes mesmo contra micróbios que adquiriram resistência aos antibióticos comuns (MATOS et al., 1999).

1.2.6. Eucalipto (*Eucalyptus saligna*)

Eucalyptus é um género de plantas com flor da família Myrtaceae originárias da Austrália.

Os óleos essenciais de eucalipto são compostos formados por uma complexa mistura de componentes orgânicos voláteis, apresentando grupos químicos como: hidrocarbonetos, álcoois, aldeídos, cetonas, ácidos e ésteres. Em geral, os óleos essenciais são constituídos de terpenos mais complexos, como o citronelal e o cineol. (DORMAN folhas (CHAIBI et al., 1997; OYEDEJI et al., 1999). Os terpenos conferem aos óleos essenciais propriedades antimicrobianas.

1.2.7. Laranja Doce (*Citrus sinensis*)

A laranja doce é originária da África do Sul e pertence à família Rutácea. Na maioria das vezes, o óleo essencial da laranja doce é extraído da casca da laranja pela metodologia de prensagem a frio. O óleo essencial de laranja doce possui capacidade antimicrobiana (DENS AND RITCHIE, 1987), graças ao limoneno, que corresponde a 95% do total de compostos voláteis encontrados no óleo essencial (MOUFIDA AND MAZOUK, 2003). Outros compostos como o linalool e citral também conferem ao óleo essencial de laranja doce capacidade antimicrobiana (CACCIONI et al., 1998)

1.3. TANINOS

Taninos são compostos fenólicos e, assim como os óleos essenciais, são formados a partir do metabolismo secundário das plantas. Os taninos podem ser divididos em dois grupos; taninos condensados e hidrolisados.

Os taninos condensados, são polímeros ou oligômeros formados pela ligação de dois ou mais monômeros de catequina (flavan-3-ol) ou flavan-3-4-diol (ZUANAZZI, 2000) e possuem como mais importante propriedade química, a habilidade de formar complexos com as macromoléculas como carboidratos e proteínas, sendo determinante para assim obter efeito nutricional, principalmente em pequenos ruminantes (DEVINCENZI et al., 2014).

Já os taninos hidrossolúveis, são compostos que, após interação com a água, formam carboidratos e ácidos fenólicos (HAGERMAN & BUTLER, 1978) e, por isso, são classificados em grupos de acordo com os produtos formados a partir desta hidrolise.

1.3.1. Efeito do tanino condensado na qualidade da carne de pequenos ruminantes

A rápida degradação da proteína no rumem de pequenos ruminantes, como os ovinos, origina a formação de compostos denominados odoríferos como o indol e o escatol (RIVAROLI et al., 2017). Esses compostos promovem na carne desses animais, um forte odour e sabor (GIRARD et al., 2015).

A adição de taninos condensados na dieta, faz com que a proteína proveniente da dieta seja degradada mais vagarosamente, reduzindo, assim, a formação dos compostos *off-flavours* (RIVAROLI et al., 2017) e aumentando a aceitabilidade do consumidor final.

2. Referências bibliográficas

ALONSO, J.R. **Tratado de fitomedicina: bases clínicas y farmacológicas**. Buenos Aires: Isis Ediciones Buenos Aires, 1998.

BAKKALI, F.; AVERBECK, S.; AVERBECK, D.; IDAOMAR, M. Biological effects of essential oils–a review. **Food and Chemical Toxicology,** v. 46, n. 2, p. 446-475, 2008.

BARATTA, M.T.; DORMAN, H.J.D.; DEANS, S.G.; BIONDI, D.M.; RUBERTO, G. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. **Journal of Essential Oil Research,** v. 10, n. 6, p. 618-627, 1998.

BERGEN, W. G.; BATES, D. B.. Ionophores: Their effect on production efficiency and mode of action. **Journal of Animal Scienc**, v. 58, n. 6, p. 1465-1483, 1984.

BENCHAAR, C.; CALSAMIGLIA, S.; CHAVES, A. V.; FRASER, G. R.; COLOMBATTO, D.; MCALLISTER, T. A.; BEAUCHEMIN, K. A. A review of plant-derived essential oils in ruminant nutrition and production. **Animal Feed Science and Technology,** v. 145, n. 1-4, p. 209-228, 2008.

BENCHAAR, C., GREATHEAD, H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. **Animal Feed Science and Technology,** v. 166, p. 338-355, 2011.

BURT, S. Essential oils: their antibacterial properties and potential applications in foods—a review. **International Journal of Food Microbiology,** v. 94, n. 3, p. 223-253, 2004.

CACCIONI, D. R. L.,GUIZZARDI, M.,BIONDI, D. M.,RENDA, A. AND RUBERTO, G. Relationship between volatile components of citrus essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. International Journal of Food Microbiology, v, **43**,p. 73–79, 1998.

CARVALHO, J.C.T., ALMANÇA, C.C.J. **Formulário de prescrição fitoterápica**. Ed. Atheneu, 2003.

CAVALLITO, C.J., BUCK, J.S., SUTER, C.M. Allicin, the antibacterial principle of Allium sativum. II. Determination of the chemical structure. **Journal of the American Chemical Society,** v. 66, n. 11, p. 1952-1954, 1944.

CHAIBI, A., ABABOUCH, L.H., BELASRI, K., BOUCETTA, S., BUSTA, F.F. Inhibition of germination and vegetative growth of (*Bacillus cereus*) T and (*Clostridium botulinum)* 62A spores by essential oils. **Food Microbiology,** v. 14, n. 2, p. 161-174, 1997.

CHAO, S. C., YOUNG, D. G., OBERG, C. J. Screening for inhibitory activity of essential opils on selected bacteria, fungi and viruses. **Journal of Essential Oil Research,** v. 12, n. 5, p. 639-649, 2000.

CHAVES, A. V., DUGAN, M. E. R., STANFORD, K., GIBSON, L. L., BYSTROM, J. M., MCALLISTER, T. A., VAN HERK, F., BENCHAAR, C. A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. **Livestock Science,** v. 141, n. 2-3, p. 213-220, 2011.

CHAVES, A. V., STANFORD, K., GIBSON, L. L., MCALLISTER, T. A., BENCHAAR, C. Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. **Animal Feed Science and Technology,** v. 145, n. 1-4, p. 396-408, 2008.

CORRÊA, M.P. Dicionário de plantas úteis do Brasil e das exóticas cultivadas. 1984.

DEANS, S. G. AND RITCHIE, G. Antibacterial properties of plant essential oils. International **Journal of food microbioly**, v. *5, p.* 165–180, 1987.

DEVINCENZI, T., PRUNIER, A., METEAU, K., & PRACHE, S. Influence of Fresh alfafa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture. **Meat science**, v. 98, p. 607-614, 2014.

DEWULF, J., CATRY, B.; TIMMERMAN, T., OPSOMER, G., DE KRUIF, A., MAES, D. Tetracycline-resistance in lactose-positive enteric coliforms originating from Belgian fattening pigs: Degree of resistance, multiple resistance and risk factors. **Preventive Veterinary Medicine,** v. 78, n. 3-4, p. 339-351, // 2007.

DORMAN, H. J. D., DEANS, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **Journal of Applied Microbiology,** v. 88, n. 2, p. 308-316, 2000.

GREATHEAD, H. Plants and plant extracts for improving animal productivity. **Proceedings of the Nutrition Society,** v. 62, n. 2, p. 279-290, 2003.

GOODRICH, R. D., GARRET, J. E., GAST, D. R., KIRICK, M. A., LARSON, D. A., MEISKE, J. C. Influence of monensin on the performance of cattle. **Journal of animal science,** v. 58, n. 6, p. 1484-1498, 1984

IVANOVA, A., MIKHOVA, B., NAJDENSKI, H., TSVETKOVA, I., KOSTOVA, I. Chemical composition and antimicrobial activity of wild garlic *Allium* ursinum of Bulgarian origin. **Natural Product Communications,** v. 4, n. 8, p. 1059-1062, 2009.

KAMEL, C. A novel look at a classic approach of plant extracts The focus on herbs and spices in modern animal feeding is too often forgotten. Since the prohibition of most of the anti-microbial growth promoters, plant extracts have gained interest in alternative feed strategies. **Feed Mix,** v. 8, n. 4, SPI/1, p. 19-23, 2000.

LAMBERT, C., APEL, K., BIESALSKI, H. K., FRANK, J. 2-Methoxyestradiol induces caspase-independent, mitochondria-centered apoptosis in DS-sarcoma cells. **International Journal of Cancer,** v. 108, n. 4, p. 493-501, // 2004.

LAMBERT, R.J.W., SKANDAMIS, P.N., COOTE, P.J., NYCHAS, G.J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. **Journal of Applied Microbiology,** v. 91, n. 3, p. 453-462, 2001.

LANGHOUT, P. New additives for broiler chickens. **World poultry,** v. 16, n. 3, p. 1-6, 2000.

LOSA, R. The use of essential oils in animal nutrition. **Cahiers Options Mediterraneennes,** v. 54, p. 39-44, 2001.

MATEU, E., MARTIN, M. Why is anti-microbial resistance a veterinary problem as well? **Journal of Veterinary Medicine, Series B,** v. 48, n. 8, p. 569-581, 2001.

MATOS, F.J.A, MACHADO, M.I.L., CRAVEIRO, A. A., ALENCAR, J.W., SILVA, M.G.V. Medicinal Plants of Northeast Brazil Containing Thymol and Carvacrol-Lippia sidoides Cham. and L. gracillis HBK (Verbenaceae). **Journal of Essential Oil Research,** v. 11, n. 6, p. 666-668, 1999.

MELLOR, S. Alternatives to antibiotics. **Feed Mix,** v. Special, p. 6-8, 2000.

MONTESCHIO, J. O., SOUZA, K. A., VITAL, A. C. P., GUERRERO, A., VALERO, M. V., KEMPINSKI, E. M. B. C., PRADO, I. N. Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers. **Meat Science**, v. 130, p. 50-57, 2017.

MOUFIDA, S. AND MARZOUK, B. Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. **Phytochemical,** v.62, p. 1283-1289, 2003.

NAGARAJA, T. G., NEWBOLD, C. J., VAN NEVEL, C. J. Manipulation of ruminal fermentation. **Black Academical and professional**, v. 46, p. 523-632, 1997.

ORNAGHI, M. G., PASSETTI, R. A. C., TORRECILHAS, J. A., MOTTIN, C., VITAL, A. C. P., GURERRERO, A., CAMPO, M. M. Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. **Animal Feed Science and Technology**, v. 234, p. 274-283, 2017.

OYEDEJI, A.O., EKUNDAYO, O., OLAWORE, O.N., ADENIYI, B.A., KOENIG, W.A. Antimicrobial activity of the essential oils of five (*Eucalyptus*) species growing in Nigeria. **Fitoterapia,** v. 70, n. 5, p. 526-528, 1999.

PATRA, A.K. Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. **Asian Journal of Animal Veterinary Advances,** v. 6, p. 416-428, 2011.

PRESSMAN, B. C. Ionophorus antibiotic as model for biological transport. **Feeding process, v. 27,** p. 1283-1288, 1968.

RIVAROLI, D. C., GUERRERO, A., VALERO, M. M., ZAWADZKI, F., EIRAS, C. E., CAMPO, M. M., PRADO, I. N. . Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. **Meat Science**, v. 121, p. 278-284, 20016

RUSSELL, J. B., HOULIHAN, A. J. Ionophore resistance of ruminal bacteria and its potential impact on human health. **FEMS Microbiology Reviews,** v. 27, n. 1, p. 65-74, 2003.

ULTEE, A., KETS, E. P. W., ALBERDA, M., HOEKSTRA, F. A., SMID, E. J. Adaptation of the food-borne pathogen Bacillus cereus to carvacrol. **Archives of Microbiology,** v. 174, n. 4, p. 233-238, 2000/10/01 2000.

YANG, W. Z., AMETAJ, B. N., BENCHAAR, C., HE, M. L., BEAUCHEMIN, K. A. Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics, and blood metabolites. **Journal of Animal Science,** v. 88, n. 3, p. 1082-1092, 2010.

YANISHLIEVA, N., POKORNÝ, J., GORDON, M.H. **Antioxidants in Foods: Practical Applications**. CRC Press, 2001.

ZHANG, W., XIAO, S., SAMARAWEERA, H., LEE, E.J., AHN, D.U. Improving functional value of meat products. **Meat Science,** v. 86, n. 1, p. 15-31, 2010.

2) OBJETIVOS GERAIS

Avaliar o efeito do mix de óleos essenciais e sua dosagem na qualidade da carne de novilhas terminadas em confinamento;

Avaliar a aceitabilidade dos consumidores da carne de tourinhos e novilhas terminados em confinamento com a adição de diferentes níveis do mix de óleos essenciais na dieta;

Avaliar o efeito da adição de tanino na dieta de cordeiros terminados em confinamento sob a concentração de escatol e indol e a coloração do músculo *Longissimus* e na gordura, além dos atributos sensoriais

CAPÍTULO 2.

Effect of essential oils on meat and fat qualities of crossbred heifers finished in feedlot

Effect of essential oils on meat and fat qualities of crossbred heifers finished in feedlot

Abstract

A blend of essential oils (oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange) on the diet was evaluated on meat and fat of crossbred heifers. Thirty animals (12 months, 219.8 kg, ½ Angus *vs.* ½ Nellore) were allocated in individual pens receiving 90% concentrate and 10% sugarcane bagasse pelletized for 3 months with one of the following diets: without addition of essential oils (E0.0), with 3.5 (E3.5) or 7 (E7.0) g/animal/day of essential oils' blend. Chemical composition, fatty acid profile on *Longissimus* muscle and meat colour were evaluated. In addition, the effect of ageing (1, 7 and 14 days) was evaluated on water holding capacity, texture and lipid oxidation. Essential oils had no effect ($P > 0.05$) on chemical composition, meat colour, water holding capacity and texture, but the inclusion of 7.0 g/d had a tendency ($P < 0.10$) to increase the lipid oxidation. The addition of 7.0 g/animal/day had a pro-oxidant effect on meat during the ageing and showed greater values for lightness at 14 days of ageing. Ageing reduced ($P < 0.05$) shear force.

Keywords: Antioxidant effect; ageing; beef; fatty acid composition; high-concentrate system; natural additives.

1. Introduction

The addition of natural additives has been studied in the last decade as a substitute for antibiotics used in intensive beef production systems to prevent diseases and metabolic disorders and increase animal performance and meat quality (Cruz et al., 2014; Valero et al., 2014; Rivaroli et., 2016).

Several studies have reported several activities of essential oils (Patra & Saxena, 2010; Jayasena & Jo, 2013;) as feed additives for livestock because they improve feed efficiency and animal productivity due to their antimicrobial, antioxidant (improving the food shelf life) (Chaves et al., 2008; Chaves et al., 2011; Rivaroli et al., 2017), and ruminal fermentation modulation (Bakkali et al., 2008; Benchaar, et al., 2008) thus reducing the byohidrogenation and changing fatty acids meat composition (Valero et al., 2014; Prado et al., 2016; Rivaroli et al., 2016).

Due to the possible synergy or interactions that exist between the different principal components of each plant, the combinations between species are numerous, as they are the ways to incorporate the essential oil in the diet, or the quantity (doses) utilized. Studies reported that high level of essential oils in the diet of bulls finished in feedlot could have effect pro-oxidant in meat (Chaves et al., 2011; Rivaroli et al., 2016).

This work was carried out to investigate the effect of meat (chemical composition, colour, water holding capacity and texture) and fat quality (lipid oxidation and fatty acid composition) supplemented with different levels of a blend of essential oils.

2. Materials and methods

2.1. Local, animals and diets

This experiment was approved (no. 185/2012-CEUA) by the ethical committee of São Paulo State University 'Julio de Mesquita Filho' (UNESP). This study was conducted at the Rosa & Pedro Sector of the Experimental Station at Iguatemi Farm, Maringá city, Paraná, Brazil.

Thirty 12 months-old half-sister crossbred heifers (F1 – ½ Angus *vs.* ½ Nellore) with an average weight of 219.8 ± 8.8 kg, were randomly assigned to one of three finishing diets (*n* = 10 per treatment). The heifers were allocated in individual pens.

The basal diet was the same for all animals (Table 1), and was formulated according to the NRC (2000) recommendations for a 1.50 kg average daily gain. The three experimental diets were as follows: E0.0 diet without the addition of the essential oils' blend or control diet; E3.5 diet with 3.5 g/animal/day of the essential oils' blend; and E7.0 diet with 7 g/animal/day of the essential oils' blend. The oils' blend (MixOil®) was produced by Animal Wellness Products (A.W.P.™), Oakland-Nebraska-USA, and was added directly into the concentrate. Components of the blend consisted of seven plant extracts: oregano (*Origanum vulgare*), garlic (*Allium sativum*), lemon (*Citrus limonium*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus saligna*), and sweet orange (*Citrus aurantium*).

Heifers were finished on their respective intensive diets (90:10 concentrate:sugarcane bagasse pelletized) for 3 months until they reached commercial weights (345.3 ± 9.8 kg), with 1.4 ± 0.10 kg of average daily gain. Afterwards, they were slaughtered at a commercial slaughterhouse placed 20 km far from the feedlot, after a solid fasting period of 12 hours according to the cattle finishing routine in Brazil. After slaughter, carcasses were divided medially through the sternum and vertebral column, identified, and chilled at a temperature below 4ºC for 24 h.

2.2. Nutrients and diet analyses

Dry matter (DM) contents of the ingredients (sugarcane bagasse pellets and concentrate mix) were determined by oven-drying at 65°C for 72 h (Table 1). Analytical DM content was determined by drying at 135 °C for 3 h by method 930.15 (AOAC, 2005). The organic matter content was calculated as the difference between DM and ash contents, with ash determined through combustion at 550°C for 5 h by method 936 (AOAC, 2005). The neutral detergent fiber and acid detergent fiber contents were determined through methods described by Mertens (2002). Nitrogen content in the samples was determined by method 976.05 (AOAC, 2005). The total digestible nutrient content was obtained by the methodology described by Kearl (1982). Samples were analyzed at the Laboratory of Feed Analyses and Animal Nutrition, of the State University of Maringá.

2.3. Sampling and meat quality

The 6th rib was frozen, being the *longissimus thoracis* (LT) of this rib previously separated, weighed, and divided into two parts to determine the chemical composition and fatty acids. The rest of the LT was excised from the left side carcass between the 7th and the 9th ribs, sliced into steaks (2.5 cm thick), weighed, vacuum-packed, and aged for either 24 h, 7 or 14 days before being frozen and stored (-20ºC) for one month for subsequent analyses.

2.4. pH measurements

At 24 h post-mortem, LT pH was measured by using a Meter Text Model (Tradelab, Contagem MG Brazil) pH-meter and a penetration electrode at the point of the 3rd lumbar vertebra (Young, West, Hart, & Van Otterdijk, 2004).

2.5. Chemical composition

The chemical composition (percentage of water, ash, crude protein, total lipids, and total collagen) was determined by the principle of Near Infrared Transmittance by using the equipment Food Scan Lab TM (Foss NIR Systems, Inc., USA). Total lipids were extracted by using the BlighDyer (1959) method with a chloroform/methanol mixture.

2.6. Meat colour

Meat colour in the CIE L*a*b* space was assessed in fresh meat before the steaks were frozen by using a Minolta CR-400 spectrophotometer with a 10º view angle and a D65 illuminant at 24h, and 7 and 14 days of ageing under vacuum package conditions, after blooming for 30 minutes. Five measurements were taken per sample.

2.7. Thawing losses and cooking losses

To perform thawing losses, steaks were thawed over the course of 24 hours under refrigeration conditions (4ºC); afterwards, they were weighed, and thawing losses were calculated as the difference between fresh and thawed weights in percentage.

To assess cooking losses, the steaks were weighed and wrapped in aluminum film. Each sample was cooked in a pre-heated grill at 200ºC until reaching an internal temperature of 75ºC, which was monitored with a penetration thermocouple. Afterwards, the sample was moved away from the grill and chilled at ambient temperature. Once it reached 20ºC, each steak was weighed in order to calculate the cooking losses as the difference in weight before and after cooking.

2.8. Lipid oxidation

At each ageing time, a small portion from a raw steak was cut and used to assess lipid oxidation (TBARS) through the procedure described by Pfalzgraf, FriggSteinhart (1995). Results were expressed as mg malonaldehyde/kg meat.

2.9. Texture measurements

For the texture analysis, the previously cooked steaks were analyzed by using a texture analyzer Stable Micro Systems TAXT Plus (Texture Technologies Corp., Serial Number 41288, Godalming, Surrey, UK) with a Warner-Bratzler cell, while following the principles proposed by Honikel (1998). The meat was cut into rectangular pieces of 1 cm^2 (8 pieces per animal) cross-section, which were cut perpendicularly to the direction of the muscle fibers.

2.10. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared by triacylglycerine methylation according to the ISO (1978) method. FAMEs were analyzed in a gas chromatograph (Varian, Palo Alto, CA, USA), equipped with a flame ionization detector and a fused silica capillary column CP-7420 (100 m, 0.25 mm and 0.39 mmo. diameter, Varian). The column temperature was programmed at 165° C for 18 minutes, 180°C (30° C min^{-1}) for 22 minutes, and 240° C (15° C min^{-1}) for 30 minutes with 45-psi pressure. The injector and detector were kept at 220°C and 245°C, respectively. Gas fluxes (White Martins, São Paulo, Brazil) were 1.4 mL min^{-1} for carrier gas (H_2); 30 ml min^{-1} for make-up gas (N_2); and 30 mL min^{-1} and 300 mL min^{-1} for H_2 and synthetic flame gas, respectively. The sample was injected by using a split mode 1/80. Fatty acids (FA) were identified by comparing the relative retention time of FAME peaks of the samples with standard FAMEs 189-19 from the Sigma Company, St Louis, MO, USA by spiking samples with the standard. The peak areas were determined by using Star software (Varian, Walnut Creek, CA, USA).

2.11. Statistical analysis

The experimental design was completely randomized with three treatments and nine replications. All characteristics that were under study were tested (Shapiro-Wilk test) for normality. Those that showed a normal distribution were analyzed through analysis of variance by using the procedure proc MIXED with SAS (2004) statistical package (Statistical Analysis System, version 8.1) with animal as a random effect. The experimental diet effect was evaluated (E0.0, E3.5 and E7.0) for all variables and the effect of ageing (24h, 7 and 14 days) for water holding capacity (WHC), lipid oxidation (TBARS) and Warner-Braztler Shear Force (WBSF) variables. Differences between group means were assessed by using the Tukey Test (P<0.05).

3. Results

Ultimate $pH_{(24h)}$ was unaffected by the addition of essential oils to the diet (P > 0.05; Table 2), which was below 5.8, indicating normal values.

The addition of essential oils to the diet of heifers did not affect (P > 0.05) meat moisture, ash, crude protein and total lipids percentages and mg/g collagen of protein (Table 2).

There was no interaction between diet and aging (P < 0.05) in any of the studied variables (Table 3 and Table 4). Thus, the results were presented and discussed as principal effects.

Meat colour (L*, a* and b* variables) did not differ (P > 0.05) among diets (Table 3). Also, aging time did not modify (P > 0.05) redness (a*) and yellowness (b*). Lightness (L*) was affected (P < 0.05) by aging time in E.00 and E7.0 groups .The greatest value of L* was found on 14 days of meat aging.

There was no difference (P > 0.05) on thawing loss and cooking loss among diets and aging time (Table 4).

Essential oils did not affect (P > 0.05) lipid oxidation (Table 4). However, lipid oxidation increased with aging for all the treatments (P < 0.05). The average of lipid oxidation of meat was 0.16, 0.24 and 0.34 mg malonaldehyde/kg of meat in 1, 7 and 14 days of aging respectively.

Meat texture characteristics were not affected ($P > 0.05$) by the diets (Table 4) but were affected by aging time ($P < 0.05$), wherein shear force values decreased with aging time.

Essential oils had no effect ($P > 0.05$) on the composition of fatty acids. The percentages of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), *n*-3 fatty acids, *n*-6 fatty acids, PUFA:SFA, and *n*-6:*n*-3 ratios.

4. Discussion

4.1 .pH

The $pH_{(24h)}$ observed (average 5.5) was considered normal (Page, Wulf, & Schwotzer, 2001). Similar results were found by Prado (2009) and Rivaroli et al. (2016) in meat from crossbred bulls finished in feedlot and fed with the similar diet and treatments. The lack of effect in this attribute suggests a good handling practice before slaughtering.

4.2. Chemical composition

The chemical composition in *Longissimus* muscle was similar among three groups. The absence of differences on chemical composition in *Longissimus muscle* among groups was predictable because the animals had the same energy and protein diet (basal diet was the same in all groups). The moisture, ash, crude protein and lipids total percentages varied from 72.3 to 72.7%; 1.5 to 1.7%; 22.6 to 22.8% and 4.2 to 5.0%, respectively. Still, the collagen varied from 1.4 to 1.5 mg/g of protein.

The collagen content in *Longissumus* muscle of heifers was low (1.45 mg/g of protein). It seems that variations in collagen are mainly related to changes in breeds, productive aptitude, and age as shown by Christensen, Ertbjerg, Failla, Sañudo, Richardson, Nute et al. (2011). In the current study, the animals were young heifers (14 months) and, in general, young animals present a low collagen content (Lepetit, 2008; Rivaroli et al., 2016). Also, according to Aberle et al. (1981), animals fed diets in intensive systems that provide rapid growth can have increased rates of protein turnover, including collagen, that can result in more tender meat since new collagen has higher solubility (Bailey, Jayas, Holley, Jeremiah, & Gill, 1997; Burson & Hunt, 1986).

In addition, the essential oils inclusion in the diets of animals finished in feedlot did not alter the chemical composition of *Longissimus* muscle (Prado et al., 2016; Rivaroli et al., 2016; Valero et al., 2014).

4.3. Meat colour

The addition of the essential oils blend in the diets did not influence L*, a* and b* values. Other studies related the same lack of natural oils from cashew and castor oils and propolis effects on meat color (Prado et al. 2016; Valero, et al., 2016). Monteschio et al. (2017) also observed similar results with clove, eugenol, rosemary, thymol and vanillin addition in the diets of heifers finished in feedlot. Still, Rivaroli et al. (2016) with the same essential oils blend did not observe difference in meat color from bulls finished in feedlot in the same conditions this work.

Lightness was greater in the meat from E0.0 and E7.0 groups at 14 days of aging compared with the other days of aging (1 and 7 days). During the muscle proteolysis (causing by the aging process), changes occur in the chemical structure of the protein, therefore an increase in L* value was expected (Renerre, 2004). The lack of aging effect on meat from E3.5 suggests an antioxidant protection effect on the protein during the maturation process, since there was no increase in light dispersion. Even as the study realized by Rivaroli et al. (2016) with young bulls feedlot-finished, with the same essential oil blend and dosage, the values for the variable L* was also superior in relation to others works with cattle finished in feedlot. In the case of both studies, the meat could be attractive to consumers that prefer lighter meat, since the value of L* found for all treatments was greater than 38.5, thus being considered a “light meat” (Abularach, Rocha, & Felício, 1998).

All of the studied color variables showed normal values according to other data for Angus × Nellore crossbred cattle finished in feedlots Rivaroli et al. (2016). Dark meat is classed as having an L* value below 30, and light meat from L* 38 to 40 (Abularach et al., 1998; Page et al., 2001), as in the current study, the color of the meat of the studied animals could be attractive to consumers that prefer lighter meat.

4.4. Thawing and cooking losses

Water holding capacity (WHC) was not influenced by the addition of essential oils blend in the diet and meat aging. The pH_{24}, cut fiber orientation and fat quantity are parameters that influence WHC (Huff-Lonergan & Lonergan, 2005), as in our study these parameters were similar for the three treatments.

The thawing and coking losses were considered normal (Prado et al., 2014) and had mean 9.3 and 25.5% respectively. Our results were similar as found by Rivaroli et al. (2016), which studied the influence of essential oil blend on the diet of young bulls in the same conditions. Though, they found differences on thawing loss caused by meat aging in E7.0 group. The high dosage of essential oil blend (7 g animal/day) had an effect pro-oxidant in the muscle cells which increased thawing loss. Probably this effect was not observed in this study because the feedlot time, that is, the period that heifers were fed with essential essential oil blend was below than young bulls were finished on feedlot (90 *vs* 120 days).

Monteschio et al. (2017), also studying the effect of essential oils in the diet of Nellore heifers finished in feedlot for 73 days, found lower values in comparison to this study. However, the pH_{24} in their study was above than our study (5.8 *vs* 5.5). Under normal conditions, pH 5.5-5.6 is anticipated (Roça, 2009).

4.5. Lipid oxidation (TBARS)

The lipid oxidation of beef was not affected by the inclusion of essential oils but was increased by aging time. The lipid and protein oxidation are an important factor that can affect meat quality deterioration (Falowo et al., 2014; Scollan et al., 2006).

Rivaroli et al. (2016) found effect of essential oils inclusion in lipid oxidation on E7.0 groups in relationship with the other groups. We observed in our study, a tendency (P=0.06) of lipid oxidation on 14 days of meat aging. The E7.0 group presented meat with higher values of TBARS than the other groups. Essential oils at high dosages, can permeabilize the mitochondria of cells and damage them, thus producing more free radicals (Reactive oxygen species – ROS) and causing the oxidation of lipids and proteins (Bakkali et al., 2008; Van Houten, Woshner, & Santos, 2006).

The values found for lipid oxidation are considered low (less than two) for all times of aging (Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006). After the heifers entered in feedlot, they were kept in pasture under tropical conditions which content β-

Carotene. The accumulation of β-Carotene can protect the meat against lipid oxidation, thus explaining the low values found for TBARS (Realini et al., 2004).

4.6. Warner-Blaxtler Shear Force (WBSF)

During the meat aging the values of shear force decreased. These results were expect once that the proteolysis occurred during the aging makes the muscle fibers become less rigid decreasing the shear force (Monsón, Sañudo, & Sierra, 2004).

At day one, the mean shear force was around 50 N, which is considered a tender meat (shear force > 51 is considered firm) (Shackelford, Wheeler, & Koohmaraie, 1999).

4.7. Fatty acid composition

Fatty acids composition was not influenced by the addition of the essential oils' blend in the diet, probably because the basal diet was the same for all. As well as in the study carried out by Rivaroli et al. (2016), the addition of essential oil blend reduced C18:0 by approximately 11%. The stearic acid (C18:0) is the final product of biohydrogenation (Tamminga & Doreau, 1991) and a change in the ruminal biohydrogenation can be increased the levels of C18:0 on the meat. Nanon, SuksombatYang (2014) suggested that plant extracts may change the ruminal fermentation.

On average, oleic acid comprised more than 42% of the total identified fatty acids. Similar values were observed by Ducatti et al. (2009) in crossbred feedlot cattle. Oleic acid is important in reducing the concentration of LDL - cholesterol and increasing the concentration of HDL - cholesterol in blood. Thus, the presence of this fatty acid is associated with a reduced risk of cardiovascular problems.

The recommended ratio of PUFA:SFA was higher than 0.45 (HMSO, 1994), and it has an important role in reducing cardiovascular risks (Wood et al., 2008). However, the ratio that was found in the present study was 0.07, typical of meat from animals from intensive rearing conditions (Rivaroli et al., 2016). The PUFA:SFA and *n-6:n-3* were not affected by essential oils blend. The mean ratio of *n*-6/*n*-3 observed was 31.07. The high ratio of *n*-6:*n*-3 was due to the high consumption of linoleic acid from the common use of cereals (eg maize, soybean).

5. Conclusions

High dosages of essential oils blend, as such 7.0 g/animal/day, could have some pro-oxidant effects, since it was showed a tendency to increased lipid oxidation and increased lightness meat during 14 of aging meat. Inclusion of 3.5 g/animal/day of essential oil blend influenced the lightness stability during aging meat showing an antioxidant effect on colour meat. The chemical composition, water holding capacity, shear force and fatty acid profile were not influenced by the addition of essential oils.

6. Acknowledgements

The Conselho Nacional de desenvolvimento cientifico – CNPq (research support and scholarship).

7. References

Aberle, E. D., Reeves, E. S., Judge, M. D., Hunsley, R. E., & Perry, T. W. (1981). Palatability and muscle characteristics of cattle with controlled weight gain: time on a high energy diet. *Journal of Animal Science, 52*(4), 757-763.

Abularach, M. L. S., Rocha, C. E., & Felício, P. E. (1998). Características de qualidade do contrafilé (M. L. dorsi) de touros jovens da raça Nelore. *Ciência e Tecnologia de Alimentos, 18*(2), 205-210.

AOAC. (2005). - *Association Official Analytical Chemist (2005)* (Official Methods of Analysis (18th ed.) ed.). Gaitherburg, Maryland, USA: AOAC.

Bailey, C. G., Jayas, D. S., Holley, R. A., Jeremiah, L. E., & Gill, C. O. (1997). Design, fabrication, and testing of a returnable, insulated, nitrogen-refrigerated shipping container for distribution of fresh red meat under controlled CO2 atmosphere. *Food Research International, 30*(10), 743-753. doi: http://dx.doi.org/10.1016/S0963-9969(98)00037-4

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical toxicology, 46*(2), 446-475.

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology, 145*(1-4), 209-228. doi: 10.1016/j.anifeedsci.2007.04.014

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911-917.

Burson, D. E., & Hunt, M. C. (1986). Heat-induced changes in the proportion of types I and III collagen in bovine *longissimus dorsi*. *Meat Science, 17*(2), 153-160.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311. doi: 10.1016/j.meatsci.2005.07.015

Chaves, A. V., Dugan, M. E. R., Stanford, K., Gibson, L. L., Bystrom, J. M., McAllister, T. A., . . . Benchaar, C. (2011). A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science, 141*(2-3), 213-220. doi: 10.1016/j.livsci.2011.06.006

Chaves, A. V., Stanford, K., Gibson, L. L., McAllister, T. A., & Benchaar, C. (2008). Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. *Animal Feed Science and Technology, 145*(1-4), 396-408. doi: 10.1016/j.anifeedsci.2007.04.016

Christensen, M., Ertbjerg, P., Failla, S., Sañudo, C., Richardson, R. I., Nute, G. R., . . . Williams, J. L. (2011). Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. *Meat Science, 87*(1), 61-65. doi: 10.1016/j.meatsci.2010.09.003

Cruz, O. T. B., Valero, M. V., Zawadzki, F., Rivaroli, D. C., Prado, R. M., Lima, B. S., & Prado, I. N. (2014). Effect of glycerine and essential oils (*Anacardium occidentale* and *Ricinus communis*) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. *Italian Journal of Animal Science, 13*(4), 3492.

Ducatti, T., Prado, I. N., Rotta, P. P., Prado, R. M., Perotto, D., Maggioni, D., & Visentainer, J. V. (2009). Chemical composition and fatty acid profile in crossbred (*Bos taurus vs. Bos indicus*) young bulls finished in a feedlot. *Asian-Australasian Journal of Animal Sciences, 22*(3), 433-439.

Eiras, C. E., Marques, J. A., Prado, R. M., Valero, M. V., Bonafé, E. G., Zawadzki, F., . . . Prado, I. N. (2014). Glycerin levels in the diets of crossbred bulls finished in feedlot: Carcass characteristics and meat quality. *Meat Science, 96*(2), 930-936.

Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International, 64*, 171-181.

Fasseas, M. K., Mountzouris, K. C., Tarantilis, P. A., Polissiou, M., & Zervas, G. (2008). Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry, 106*(3), 1188-1194.

Gillis, A. T., Eskin, N. A. M., & Cliplef, R. L. (1973). Fatty acid composition of bovine intramuscular and subcutaneous fat as related to breed and sex. *Journal of Food Science, 38*(3), 408-411.

HMSO. (1994). England Department of Health Nutritional. Aspects of cardiovascular disease. *Report on Health and Social Subjects, 46*, 37-46.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science, 49*(4), 447-457.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194-204.

ISO. (1978). *Animal and Vegetable Fats and Oils – Preparation of Methyl Esters of Fatty Acids. Method ISO 5509.* Geneva, Switzerland: International Organization for Standardization.

Jayasena, D. D., & Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology, 34*(2), 96-108.

Kazama, R., Zeoula, L. M., Prado, I. N., Silva, D. C., Ducatti, T., & Matsushita, M. (2008). Características quantitativas e qualitativas da carcaça de novilhas alimentadas com diferentes fontes energéticas em dietas à base de cascas de algodão e de soja. *Revista Brasileira de Zootecnia, 37*(2), 350-357.

Kearl, L. C. (1982). *Nutrient Requirements of Ruminants in Developing Countries* (1st ed.). Utah, UT, USA: International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University.

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects. *Meat Science, 80*(4), 960-967. doi: 10.1016/j.meatsci.2008.06.016

Macedo, L. M., Prado, I. M., Rotta, P. P., Prado, R. M., Souza, N. E., & Prado, I. N. (2008). Chemical composition and fatty acids profile of five carcass cuts of crossbred heifers finished in feedlot. *Semina: Ciências Agrárias, 29*(3), 597-608.

Maggioni, D., Marques, J. A., Perotto, D., Rotta, P. P., Ducatti, T., Matsushita, M., . . . Prado, I. N. (2009). Bermuda grass hay or sorghum silage with or without yeast addition on performance and carcass characteristics of crossbred young bulls finished in feedlot. *Asian-Australasian Journal of Animal Sciences, 22*(2), 206-215.

Marques, J. A., Prado, I. N., Moletta, J. L., Prado, I. M., Prado, J. M., Macedo, L. M. A., . . . Makoto, M. (2006). Características físico-químicas da carcaça e da carne de novilhas submetidas ao anestro cirúrgico ou mecânico terminadas em confinamento. *Revista Brasileira de Zootecnia, 35*(4), 1514-1522.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International, 85*(6), 1217-1240.

Monsón, F., Sañudo, C., & Sierra, I. (2004). Influence of cattle breed and ageing time on textural meat quality. *Meat Science, 68*(4), 595-602.

Monteschio, J. O., Souza, K. A., Vital, A. C. P., Guerrero, A., Valero, M. V., Kempinski, E. M. B. C., . . . Prado, I. N. (2017). Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers. *Meat Science, 130*, 50-57. doi: http://dx.doi.org/10.1016/j.meatsci.2017.04.002

Nanon, A., Suksombat, W., & Yang, W. Z. (2014). Effects of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle. *Animal Feed Science and Technology*(196), 50-59.

NRC. (2000). *Nutrient Requirements of Beef Cattle* (7th rev. ed.). Washington, DC, USA: Natl. Acad. Press.

Ornaghi, M. G., Passetti, R. A. C., Torrecilhas, J. A., Mottin, C., Vital, A. C. P., Gurerrero, A., . . . Campo, M. M. (2017). Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Animal Feed Science and Technology, 234*, 274-283.

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science, 79*(3), 678-687.

Patra, A. K., & Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *Phytochemistry, 71*(11–12), 1198-1222. doi: http://dx.doi.org/10.1016/j.phytochem.2010.05.010

Pfalzgraf, A., Frigg, M., & Steinhart, H. (1995). Alpha tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. *Journal of Agricultural and Food Chemistry, 43*(5), 1339-1342.

Prado, I. N., Campo, M. M., Muela, E., Valero, M. V., Catalan, O., Olleta, J. L., & Sañudo, C. (2014). Effect of castration age, protein level and lysine/methionine ratio in the feed on animal performance, carcass and meat quality of Friesian steers intensively reared. *Animal, 8*(10), 1-8.

Prado, I. N., Cruz, O. T. B., Valero, M. V., Zawadzki, F., Eiras, C. E., Rivaroli, D. C., . . . Visentainer, J. V. (2016). Effects of glycerin and essential oils (*Anacardium occidentale* and *Ricinus communis*) on the meat quality of crossbred bulls finished in a feedlot. *Animal Production Science, 56*(12), 2105-2114. doi: http://dx.doi.org/10.1071/AN14661

Prado, I. N., Marques, J. A., Rotta, P. P., Prado, R. M., Visentainer, J. V., & Souza, N. E. (2009). Meat quality of the *Longissimus* muscle of bulls and steers (½ Nellore *vs.* ½ Simmental) finished in feedlot. *Journal of Animal and Feed Sciences, 18*, 221-230.

Prado, I. N., Oliveira, A. N., Rotta, P. P., Perotto, D., Prado, R. M., Silva, R. R., . . . Moletta, J. L. (2009). Chemical and fatty acid composition of *Longissimus* muscle of crossbred bulls finished in feedlot. *Asian-Australasian Journal of Animal Sciences, 22*(7), 1054-1059.

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., & Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*(3), 567-577. doi: http://dx.doi.org/10.1016/S0309-1740(03)00160-8

Renerre, M. (2004). La couleur de la viande et sa mesure. *INRA, 1,* 63-68.

Rivaroli, D. C., Guerrero, A., Valero, M. M., Zawadzki, F., Eiras, C. E., Campo, M. M., . . . Prado, I. N. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Science, 121*, 278-284. doi: http://dx.doi.org/10.1016/j.meatsci.2016.06.017

Rivaroli, D. C., Ornaghi, M. G., Mottin, C., Prado, R. M., Ramos, T. R., Guerrero, A., . . . Prado, I. N. (2017). Essential oils in the diet of crossbred (½ Angus vs. ½ Nellore) bulls finished in feedlot on animal performance, feed efficiency and carcass characteristics. *Journal of Agricultural Science, in press*. doi: doi:10.5539/jas.v9n10pxx

SAS. (2004). *SAS/STAT User guide, Version 9.1.2*. Cary, NC, USA: SAS Institute Inc.

Scollan, N., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science, 74*(1), 17-33.

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1999). Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. *Journal of Animal Science, 77*(10), 2693-2699.

Tamminga, S., & Doreau, M. (1991). Lipids and rumen digestion. In J. P. Jouany (Ed.), *Rumen microbial metabolism and ruminant digestion* (pp. 151-164). Paris, FR: Institut National de la Recherche Agronomique.

Valero, M. V., Farias, M. S., Zawadzki, F., Prado, R. M., Fugita, C. A., Rivaroli, D. C., . . . Prado, I. N. (2016). Feeding propolis or functional oils (cashew and castor oils) to bulls: performance, digestibility and blood cells counts. *Revista Colombiana de Ciencias Pecuarias, 29*, 33-42. doi: doi: 10.17533/udea.rccp.v29n1a04

Valero, M. V., Torrecilhas, J. A., Zawadzki, F., Bonafé, E. G., Madrona, G. S., Prado, R. M., . . . Prado, I. N. (2014). Propolis or cashew and castor oils effects on composition of *Longissimus* muscle of crossbred bulls finished in feedlot. *Chilean Journal of Agricultural and Research*, *74*(4), 445-451.

Van Houten, B., Woshner, V., & Santos, J. H. (2006). Role of mitochondrial DNA in toxic responses to oxidative stress. *DNA Repair, 5*(2), 145-152. doi: http://dx.doi.org/10.1016/j.dnarep.2005.03.002

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., . . . Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78*(4), 343-358. doi: 10.1016/j.meatsci.2007.07.019

Young, O. A., West, J., Hart, A., & Van Otterdijk, F. F. H. (2004). A method for early determination of meat ultimate pH. *Meat Science, 66*(2), 493-498.

Zawadzki, F., Prado, I. N., Marques, J. A., Zeoula, L. M., Rotta, P. P., Sestari, B. B., . . . Rivaroli, D. C. (2011). Sodium monensin or propolis extract in the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics. *Journal of Animal and Feed Sciences,* 20(1), 16-25.

Table 1. Composition of basal diets

Parameters	Ingredients, g/kg on DM							
	SCBP[1]	Corn grains	Soybean meal	Limestone, 36%	Yeast	Mineral salt	Urea	Diets, g/kg on DM
Dry matter	947.0	889.3	886.0	993.0	980.0	993.0	980.0	881.3
Organic matter	980.0	991.0	937.0	107.1	-	107.0	5.6	973.4
Ash	19.7	9.50	62.5	832.9	-	893.0	994.4	26.60
Crude protein	18.3	89.9	490.0	-	300.0	-	2600	125,0
Ether extract	36.0	35.0	13.0	-	-	-	-	22.0
Neutral detergent fiber	787.4	177.0	137.0	-	-	-	-	303.0
Acid detergent fiber	492.0	44.0	59.7	-	-	-	-	148.0
Total Digestible nutrients	430.0	900.0	840.0	-	-	-	-	703.0
Diets	100	819.5	65.1	4.60	0.50	4.10	6.20	

[1]Sugarcane bagasse pellet
[2] g/kg on fresh basis
Diet energy was calculated according to NRC (2000)

Table 2
Effect of essential oils blend inclusion final meat pH and chemical composition on *Longissimus* muscle of crossbred heifers

Items	Essential oils			SEM[4]	P-*value*
	E0.0[1]	E3.5[2]	E7.0[3]		
pH24	5.53	5.58	5.55	0.07	0.33
Chemical composition					
Moisture, %	72.26	72.46	72.69	0.20	0.68
Ash, %	1.53	1.61	1.68	0.03	0.59
Crude protein, %	22.58	22.65	22.76	0.12	0.84
Total lipids, %	4.96	4.17	3.89	0.20	0.06
Collagen, mg/g of protein	1.54	1.45	1.45	0.02	0.10

[1]Without essential oils; [2]3.5 g essential oils/animal/day; [3]7.0 g essential oils/animal/day.
[4]SEM: Standard error of mean.

Table 3
Effect of essential oils blend inclusion on meat colour of crossbred heifers

	Essential Oils			SEM[4]	P -*value*
	E0.0[1]	E3.5[2]	E7.0[3]		
Days		L*			
1	42.22b	42.56	42.61b	0.23	0.46
7	42.41b	42.24	42.29b	0.32	0.96
14	43.08a	42.97	43.42a	0.26	0.43
SEM	0.12	0.21	0.16		
P-*value*	0.01	0.36	0.01		
		a*			
1	18.49	18.26	18.48	0.20	0.69
7	18.43	18.09	18.28	0.27	0.65
14	18.72	18.85	18.80	0.22	0.19
SEM	0.14	0.17	0.13		
P-*value*	0.22	0.23	0.22		
		b*			
1	6.01b	6.28b	6.43	0.17	0.11
7	6.47b	6.50b	6.59	0.15	0.84
14	6.77a	6.73a	6.94	0.14	0.54
SEM	0.09	0.11	0.10		
P < value	0.11	0.12	0.11		

[1]Without essential oils; [2]3.5 g essential oils/animal/day; [3]7.0 g essential oils/animal/day.
[4]SEM: Standard error of mean.
a, b: different superscripts represent significant differences during ageing ($P \leq 0.05$).
There was no interaction between diet and ageing ($P > 0.05$)

Table 4

Effect of essential oils' blend inclusion and ageing on water holding capacity, shear-force and lipid oxidation (mg malonaldehyde/kg of meat) of meat from crossbred heifers

Days	Essential oils			SEM[4]	P *-value*
	E0.0[1]	E3.5[2]	E7.0[3]		
	Thawing Loss, %				
1	8.53	8.47	8.87	0.63	0.61
7	9.52	9.23	9.88	0.58	0.45
14	9.48	9.77	10.21	0.72	0.55
SEM	0.86	0.76	0.55		
P-*value*	0.40	0.65	0.36		
	Cooking Loss, %				
1	27.30	25.73	25.55	0.69	0.24
7	25.90	25.18	25.03	0.86	0.57
14	25.28	25.07	25.01	0.70	0.31
SEM	1.00	0.71	0.79		
P-*value*	0.85	0.89	0.91		
	Lipid Oxidation, mg MDA/kg muscle				
1	0.18a	0.16a	0.15a	0.02	0.22
7	0.25b	0.26b	0.24b	0.02	0.08
14	0.31c	0.32c	0.37c	0.02	0.06
SEM	0.02	0.03	0.01		
P-*value*	0.00	<.0001	<.0001		
	WBSF, kg				
1	4.94a	5.50a	4.88a	0.033	0.32
7	3.56b	3.76b	3.65b	0.034	0.27
14	2.29c	2.57c	2.62c	0.032	0.43
SEM	0.031	0.029	0.033		
P-*value*	<.0001	<.0001	<.0001		

[1]Without essential oils; [2]3.5 g essential oils/animal/day; [3]7.0 g essential oils/animal/day.
[4]SEM: Standard error of mean.
a, b: different superscripts represent significant differences during ageing ($P \leq 0.05$).
There was no interaction between diet and ageing ($P > 0.05$)

Table 5

Effect of essential oils blend inclusion on fatty acids composition (%identified fatty acid methyl esters) in *Longissimus* muscle of crossbred heifers

	Essential Oils				
Faty acids	E0.0[1]	E3.5[2]	E7.0[3]	SEM[4]	P-*value*
SFA[5]	46.44	46.52	44.93	0.71	0.62
UFA[6]	53.56	53.48	55.07	0.71	0.62
MUFA[7]	50.22	50.32	51.54	0.72	0.73
PUFA[8]	3.35	3.17	3.52	0.12	0.53
n-3	0.10	0.10	0.10	0.01	0.97
n-6	2.97	2.83	3.18	0.12	0.49
PUFA:SFA	0.07	0.07	0.08	0.00	0.37
n-6/*n*-3	31.42	29.61	32.20	1.52	0.79
12:0	0.04	0.04	0.05	0.00	0.54
14:0	2.63	2.65	2.50	0.07	0.70
14:1 *n*-9	0.54	0.54	0.53	0.02	0.98
15:0	0.37	0.35	0.32	0.02	0.43
15:1 *n*-9	0.55	0.57	0.64	0.04	0.64
16:0	32.19	31.89	32.07	0.42	0.96
16:1 *n*-7	2.55	2.55	2.65	0.06	0.77
17:0	0.95	0.90	0.85	0.04	0.62
17:1 *n*-9	0.96	0.87	0.91	0.04	0.62
18:0	10.26	10.68	9.15	0.34	0.19
18:1 *n*-9	44.06	44.41	45.35	0.71	0.76
18:1 *n*-7	0.84	0.82	0.79	0.02	0.78
18:2 *n*-6	2.66	2.52	2.86	0.11	0.47
18:3 *n*-3	0.10	0.10	0.10	0.01	0.97
18:2 *c*9-*t*11	0.21	0.17	0.18	0.01	0.57
20:1 *n*-9	0.07	0.06	0.07	0.00	0.86
20:3 *n*-6	0.10	0.12	0.13	0.01	0.22

[1]Without essential oils; [2]3.5 g essential oils/animal/day; [3]7.0 g essential oils/animal/day; [4]SEM: Standard error of mean; [5]saturated fatty acids; [6]unsaturated fatty acids; [7] monounsaturated fatty acids; [8] polyunsaturated fatty acids.

CAPÍTULO 3.

Consumer acceptability of beef from two sexes supplemented with essential oil mix

Consumer acceptability of beef from two sexes supplemented with essential oil mix

Abstract

Essential oils are natural extracts from plants which can be included in cattle diets as an alternative to additives, such as ionophores, to improve the shelf life of meat. The aim of this study was to evaluate the sensory acceptability of meat from 57 crossbred (½ Angus - ½ Nellore) animals of different sexes [heifers *n*=30, and young bulls *n*=27], finished in feedlot with isoenergetic and isonitrogenous diets, which differed in the level of supplementation (0.0, 3.5 or 7.0 g/animal/day) with a commercial mix of essential oils (Mixoil®) from seven plants: oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange. *120 consumers who scored tenderness acceptability, flavor acceptability and overall acceptability using a hedonic nine-point scale evaluated the Longissimus thoracis muscle, aged for 24 hours*. The inclusion of essential oils affected tenderness acceptability and flavor acceptability ($P \leq 0.01$), with a tendency ($P < 0.10$) for overall acceptability. Meat from heifers was more appreciated by consumers than that from young bulls in all attributes ($P \leq 0.001$), however, different groups of consumers had different preferences. The inclusion of a mix of essential oils in beef diets was well accepted by consumers, and could improve the acceptability of meat from young bulls, when is added at a concentration of 3.5 g/animal/day during the last 4 months of fattening.

Keywords: Beef acceptability, crossbred, heifers, natural additives, young bulls.

Implications

The use of essential oils in animal diets and its effect on performance and products is starting to be studied as a natural alternative to improve meat quality. Works about this subject are still, however, scarce. Consumer opinion matters in the food sector. Our results provide new information about the acceptability of beef from animals supplemented with essential oils. The results were partially favorable, which encourages deeper research about the wider possibilities of these products.

1. Introduction

Essential oils are natural, volatile, and complex compounds characterized by a strong odour and synthesized by different plants. Due to the huge natural diversity of plants, more than 3,000 different essentials oils are known, with approximately only 10% used in diverse industries (Bakkali *et al.* 2008).

It has been reported that combinations of different essential oils modify their individual effects, due to the possible synergism between compounds, with different functions and mechanisms of action, enabling their interaction and increasing their effect when mixed with other active ingredients (Langhout 2000; Kamel 2000). Several studies have reported the varied actions and diverse uses of essential oils on animal performance and meat quality (Benchaar *et al.* 2008; Cruz *et al.* 2014; Valero *et al.* 2014a; Valero *et al.* 2014b). Their rapid development in the last decade, and the interest in the use of those natural products as feed additives in animal production has been favored by the prohibition in some areas, such as the European Union, of growth promoters due to possible risk to human health (Franz *et al.* 2010).

Essential oils have many possibilities as feed additives in livestock because they can improve feed efficiency and animal productivity by improving the palatability of feed, having an antimicrobial effect, acting as a digestive modulator in the ruminal metabolism, and having anti-inflammatory and antioxidant effects, as has been shown in studies on various species (Bakkali *et al.* 2008; Patra 2011; Cruz *et al.* 2014).

However, consumers are becoming more health conscious, and this is leading to a growing preference for quality, safety, healthier and more natural food products, and the use of plant compounds as natural alternatives to replace other chemical additives (Franz *et al.* 2010; Jian and Xiong 2016). According to Haugaard *et al.* (2014), the use of plants in meat and meat product preservation is well accepted by consumers, if visual and sensory characteristics are retained in the meat, because it is perceived as a natural, understandable and familiar technique to replace those chemical additives which are perceived by many consumers as undesirable. According to Barcellos *et al.* (2010), in reference to beef acceptance, the purchase decision of modern consumers involves several criteria, such as price and sensory attributes (related to appearance, texture, flavor and odour). Consumers also consider other aspects related to health, convenience or the means of production, which add value to beef products. Some studies have assessed the effect that essential oils have on meat quality, however those works added the product directly to the meat and used frequently processed meat as minced beef or beef patties (Fernández-Ginés *et al.* 2005; Hussein and Hayan 2012; Hulankova *et al.* 2013). It is important to study the effect that the addition of those natural oils has on the final product (meat quality), and the effect through the animal diets, and how it could affect consumer acceptance.

Beef quality is modified by many factors, such as breed, age, gender, and production system. These factors determine particular commercial types, and consequently they could influence consumer acceptability. In Brazil, more intensive systems and the application of technological knowledge are allowing production of a higher quality of meat from younger animals (slaughtered between 13-15 months of age), of both genders, and using commercial crossbred *Bos taurus* and *Bos indicus* (Ferraz and Felício 2010; Lobato *et al.* 2014).

No studies evaluating the sensory acceptability by consumers of beef from animals supplemented with essential oils in the diet have been previously performed. Consequently, due to the possibility that essential oils affect the final meat characteristics and the current and future interest in producing these type of meats, the aim of this study was to evaluate consumer acceptability of meat from heifers and young bulls, finished with different levels of a mix of essential oils.

2. Materials and methods

2.1.Animals and diets

Fifty-seven 12 month-old crossbred (½ Angus - ½ Nellore) young bulls (*n* = 27) and heifers (*n* = 30), average weight of 243.2 ± 11.7 kg and 219.8 ± 8.8 kg, respectively, were randomly assigned to one of three finishing diets (n = 9 for young bulls and n=10 for heifers, per treatment).

The basal diet was the same for all animals, formulated according to NRC (2000) recommendations for a 1.5 kg/day average daily gain (Table 1). The three experimental treatments were: (E0.0) diet without addition of essential oils, (E3.5) diet with 3.5 g/animal/day of essential oils, and (E7.0) diet with 7.0 g/animal/day of the essential oils. The mixed oils used consisted of seven plants extracts: oregano (*Origanum vulgare*), garlic (*Allium sativum*), lemon (*Citrus limonium*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus saligna*) and sweet orange (*Citrus aurantium*), registered as Mixoil® (Animal Wellness Products – Oakland – Nebraska – USA). The mixture of essential oils had a powdery texture and it was mixed with the feed from concentrate in a commercial mixer every two week, where diets were prepared during the experiment. All diets were isoenergetic and isonitrogenous.

Young bulls and heifers were finished under intensive conditions (90:10; concentrate: forage) on individual feedlots. The chemical composition of diets and percentage of different ingredients in the feed concentrate is compiled in Table 1. Sugar cane bagasse pellets were used as forage.

Animals were finished for four (young bulls, YB) or three (heifers, H) months, until reaching their respective commercial weighs (440.3 ± 17.0 kg and 345.0 ± 9.8 kg, respectively) and, consequently, a comparable fat thickness, according to Brazilian meat industry requirements for beef commercialization where the recommended minimum is between 3-5 mm (Müller 1980). The experimental animals were slaughtered at a commercial abattoir 20 km from the feedlot (Maringá, Paraná) according to commercial practices (Brasil 1997). The carcass weights of all animals were recorded before and after chilling in order to calculate hot and cold carcass weights and their respective dressing percentages (Table 2).

2.2.Sampling and sampling preparation

Twenty-four hours after slaughtering and chilling, the left sixth rib of each animal was removed, and fatness thickness was measured on the subcutaneous fat using a caliper and averaged over three different points. The ribs were later dissected into muscle, fat (subcutaneous plus intermuscular), bone and other tissues (tendons, fascia, blood vessels), according to Robelin and Geay (1975), in order to define tissue composition.

The left *Longissimus thoracis* (LT) muscle was removed from each carcass and used in the consumer study. Cuts (two 2 cm-thick steaks) were obtained at the 10th *thoracic vertebrae* level. Each sample was immediately vacuum packaged and frozen at -18 ºC and retained for less than 3 months.

Samples were thawed at 4ºC for 24 hours prior to the analysis. Each piece was covered with aluminum foil and cooked in a pre-heated grill (Philco Grill Jumbo Inox, PHILCO S.A., Brazil) at 200ºC until they had reached an internal temperature of 70ºC monitored with a penetration thermocouple (Incoterm, 145mm, Incoterm LTDA, Brazil). Each steak was cut into eight 2 x 2 cm cubes and kept warm (50ºC) until consumer evaluation (less than 10 minutes after cooking).

2.3.Experimental design

Consumer testing was performed during a National Livestock Exhibition in Maringá (Brazil) in a private room adequately adapted to perform a sensory test. One hundred and twenty consumers were selected randomly within quotas of gender (58 men and 62 women) and age (from 18 to 70 years) according to the Brazilian national profile (IBGE 2013) among the exhibition visitors. Table 3 shows the characteristics of the participating consumers.

Fifteen sessions were carried out, each with eight different consumers. Per session, each consumer evaluated six samples codified with a random three digit code, corresponding to all possible combinations between the type of diet and sex. One steak from each animal on each experimental diet was evaluated. In order to reach 120 consumers and equilibrate the consumer design a second steak from 5 or 6 animals per treatment (both heifers and young bulls respectively) was also used. Those animals were randomly selected. Meat was served following a randomized design to avoid order and carry-over effects (Macfie *et al.* 1989). Consumers were only informed that they would be evaluating beef. They were requested to taste the meat samples and evaluate the acceptability of three attributes. They were asked to eat unsalted toasted bread and rinse their mouth with water before evaluating each sample, including the first one.

2.4.Questionnaire

For each sample, consumers evaluated: tenderness acceptability, flavor acceptability and overall acceptability; using a 9-point scale ranging from 1 = dislike extremely to 9 = like extremely, where a medium level was not included according to methodologies described by Font i Furnols *et al.* (2008).

A supplementary survey was also distributed in order to increase the information about consumption habits and preferences for different types of meat (Table 3). One hundred and twenty consumers participated on the sensory test; however, consumers with missing data and outliers on the questionnaire were not considered for statistical analyses, leaving one hundred and sixteen consumers overall.

2.5.Statistical analyses

Carcass quality and meat sensory attributes were assessed via analysis of variance using general lineal model (GLM) procedures with SPSS (v.15.0) (IBM SPSS Statistics, SPSS Inc., Chicago. USA.) for Windows.

In all statistics analyses, diet and sex and its (their) interaction were considered fixed effects, and the consumer was included as a random effect on the consumer test. Mean and standard error of mean (SEM) were calculated for each variable. Differences between means were evaluated using Duncan's test ($P \leq 0.05$). To identify similarities among consumers, hierarchical cluster analysis with the Ward's method was used to determine the different segments of consumers depending on overall acceptability using XLSTAT (v.7.5.3). The number of clusters was selected from the dendrogram, trying to find a compromise between homogeneity within clusters and heterogeneity between clusters. Principal Component Analysis (PCA) was used to identify the relationships between treatments and meat attributes (fat thickness and total fat percentage). The correlations between attributes were evaluated using the Pearson correlation coefficient. Both analyses were performed using XLSTAT (v.7.5.3).

3. Results and discussion

3.1.Carcass characteristics

There were no statistically significant interactions between essential oil addition and sex. Also, no significant effects were found as a result of the addition of essential oils (0.0, 3.5 and 7.0 g/animal/day) in the diet for carcass characteristics and tissue composition (Table 2), but the sex demonstrated a strong effect ($P \leq 0.001$) on animal and carcass characteristics.

Young Bull live weight and carcass weights were greater than for heifers ($p < 0.001$) as expected and seen elsewhere, but the proportions of muscle, fat and bone percentages presented were comparable results to those observed in other studies realized under similar production conditions (Francozo et al. 2013; Valero et al. 2014b).

As fat thickness did not differ between sexes, the characteristic that defined the moment of slaughter for these commercial animals and the heifers were finished in 30 days fewer, they had fattened more quickly (Lancaster *et al.* 2008).

3.2.Characterization of consumer sample

Socio-demographics and the complementary survey were developed to obtain more detailed characteristics and the meat preferences of the consumers. The results are compiled in Table 3. Thus, participants in the test were habitual consumers of beef, with a high frequency of beef consumption. Thirty five percent of consumers ate beef more than 5 times per week, 79.2% of consumers consumed beef more than 3 times per week. Nobody reported that their beef consumption was less than once a week. These frequencies are higher than those reported in Europe (Realini *et al.* 2009), where in spite of existing variations between European countries, the average beef consumption frequency was once a week for 36.0% of consumers and once every 15 days for 14.4% of consumers. It has been reported in other Brazilian beef studies that the already high beef demand in Brazil, resulting from traditional eating habits, is increasing as a consequence of socioeconomic and demographics changes in this country (Delgado et al. 2006; Kirinus et al. 2014). Annual beef consumption was an average of 42.5 kg/person/year (FAPRI, 2014), which is three times higher than in most European countries. Hence, beef was the most consumed type of meat for 75% of participants in the test, followed by poultry (20%) (Table 3). Other kinds of meat, such as pork or lamb, were the main meat consumed by only 4.2% or 0.8% of interviewed.

In agreement with the acceptability results, 66.4% of consumers said they preferred meat from heifers, compared to 12.1% who preferred meat from young bulls and 21.6% who preferred meat from steers.

3.3.Consumer acceptability

There was a significant interaction between essential oil addition and sex for tenderness ($P \leq 0.01$) and overall acceptability ($P \leq 0.05$). Sex had significant effect on all studied attributes. Essential oil addition (0.0, 3.5 and 7.0 g/animal/day) had a significant effect ($P \leq 0.01$) on tenderness and flavor and showed a tendency ($P < 0.1$) for overall acceptability, as shown in Table 4.

Tenderness acceptability was significantly ($P<0.05$) higher in all heifer meat and in meat from YB supplemented with 3.5 g/animal/d of essential oil mix. Similarly, overall acceptability was higher for heifer meat (independently on the essential oil supplementation) and YB supplemented with 3.5 g/animal/d. Moreover overall acceptability of these YB (with E3.5) was not significantly different from that of YB not supplemented (E0.0) and significantly higher from that of YB supplemented 7.0 g/animal/d. The flavor of heifer meat was scored significantly ($P<0.001$) better (+0.43 points) than meat from YB, and meat from animals supplemented with 3.5 g/animal/d had significantly higher scores for flavor acceptability than meat from animals supplemented with 7 g/animal/d; scores for the control group were intermediate and not statistically significantly different from these two.

Tenderness has been identified as one of the most important attributes in determining eating quality and the major criteria that contributes to eating satisfaction (Font-i-Furnols and Guerrero 2014). Consumers are willing to pay more if meat tenderness is guaranteed (Realini *et al.* 2009). The present study was developed within the Brazilian population, whose traditional eating habits involve the consumption of beef from *Bos indicus*, which produces meat less tender than *Bos taurus* or crossbred animals (as in the current study) due to differences in protein breakdown *postmortem* and calpain- calpastatin activity (O'Connor *et al.* 1997), and where meat is not aged for very long. Hence, they are possibly less sensitive to toughness than consumers from other parts of the world. Although beef consumers are able to perceive- distinguish tender from tough beef when eating samples with different Warner-Braztler shear force, they are probably more interested in flavor attributes, as described by Delgado *et al.* (2006), as a consequence of traditional habits in the consumption of beef from zebuin breeds and short aging periods. Presumably, Brazilian consumers would be less sensitive to tenderness modifications, although as expected this variable showed high correlation with overall acceptability ($r = 0.710$). The effect of essential oils on beef texture characteristics has not yet been reported. In the current animals, according to Rivaroli *et al.* (2016), there was no effect in Warner-Blaztler Shear Force between the three diets or different aging periods. According to results from Monsón *et al.* (2004), at the first stage of aging (1 day) there is much variability between animals of the same breed, which could have influenced the results, and these differences decrease along aging period. It is also possible that a pro-oxidant effect of essential oils, when added in high concentrations (Bakkali *et al.* 2008), could have had an effect, because the young bulls supplemented with 7 grams per day presented higher water losses (Rivaroli *et al*. 2016) during ageing, which produces a less juicy meat and could produce differences in tenderness perception.

As has been described in other beef consumer tests flavor was a key sensory parameter in determining overall acceptability, and influencing willingness to pay for steaks (Font-i-Furnols and Guerrero 2014). Flavor is influenced by several parameters related to fat characteristics, such as marbling or fatty acid composition (Realini *et al.* 2009). According to the preliminary results of the meat quality from the animals involved in our study, there were no significant differences between sexes in both fatness score and fatty acid profile (Rivaroli 2014).

With respect to flavor quality, however, the antioxidant effect of those products has been reported when they are added directly to the final product, for instance ground beef for hamburger preparation (Hussein and Hayan 2012), where the addition of essential oils of marjoram and rosemary prevents the formation of secondary oxidation compounds and, consequently, decreases the rate of flavor deterioration, increasing acceptability scores. In the present study, the addition of a higher concentration of essential oils in the animal diet was not synonymous with better flavor, according to preliminary results from lipid oxidation tests (results not shown), where the addition of 3.5 g/animal/day resulted in meat with the lowest lipid oxidation; however greater quantities (7.0 g) did not show this antioxidant effect, especially when beef ageing time increase (Rivaroli *et al*. 2016). In the current study, the aging period was very short (24 hours), however, and consequently the flavor processes were not very well established. The lack of studies about the profile of volatile compounds in meat from animals fed with essential oils, which is not yet well defined, makes it difficult to explain the flavor acceptability variations found.

Overall acceptability is strongly related to the other two analyzed parameters (flavor and tenderness), as previously reported in other studies (Guerrero *et al.* 2014; Lepper-Blilie *et al.* 2014; Pérez-Juan *et al.* 2014). As it happens in the other two variables previously commented the E3.5 group in young bulls presented the highest scores indicating a possible optimal quantity of essential oil inclusion. The differences between diets in heifers were not significant.

Although there were no difference between young bulls and heifers in subcutaneous fat thickness or tissue composition (Table 2), some inherent differences between sexes or the time necessary to reach slaughter weight, could explain differences between groups. Our results agree with reviews from Reddy *et al.* (2015) and Rotta *et al.* (2009), who reported that meat from heifers is usually preferred to that from bulls, as it is associated with better global sensory qualities, such as being more tender due to a higher marbling content, differences in enzyme levels that breakdown proteins, as well as variations in fatty acid composition. Due to their precocity, females reach commercial characteristics one month before young bulls. Although Brazilian consumers are more familiar with the low tenderness of *Bos indicus* breeds, greater tenderness obtained higher scores as reported by Delgado *et al.* (2006) independent of social demographic variables.

Due to the interaction between diet and sex with tenderness and overall acceptability (Table 4), the results were presented segmented on the six treatments. There were differences (P=0.003 and P= 0.016) between groups for the cited attributes respectively. However due to the lack of interaction between both factors (diet and sex), flavor scores have been presented separately.

The highest values for tenderness and overall acceptability were obtained in meat from heifers, and were especially different from those of young bulls fed E7.0. In spite of presenting a lower value, there was no difference between the treatment E7.0 and the control group of young bulls for tenderness or overall acceptability. Flavor scores from heifers were significant higher (P<0.001) than those from young bulls. Also independently of sex, beef from diets supplemented with 7 g/animal/day were not statistically different from control group (E0.0) on flavor, however flavor acceptability was significant lower (P= 0.003) for E7.0 than E 3.5 group. Consumers gave higher tenderness scores to the heifer meat in all three treatments studied and similar to overall acceptability in the E3.5 group for young bulls. On the other hand, the control and E7.0 young bulls presented significantly lower values.

As previously described, meat from heifers was preferred compared to that which originated from young bulls; however according to the results in Table 4, it seems that the addition of 3.5 g/animal/day for young bulls over 4 months could improve sensory attributes, making their meat comparable to heifer's meat, as shown in the Principal Component Analysis (Figure 1). Maybe this fact could be related with the lower amount of intramuscular fat in young bulls in comparison with heifers, being consequently more unsaturated and oxidative. According to the antioxidant effect of essential oils, 3.5g/animal/day has been proved a dose more antioxidant and effective than 7.0g/animal/day.

3.4.Principal component analyses

The first two principal component (PC) axes explained 91.76% of the total variance. The first axis explained 70.5% of the variation and it is mainly explained by the sensory attributes, being the positive part related with high acceptability scores. Attributes of tenderness, flavor and overall acceptability are on the right side of F1, closely located on the graph to the three treatments for heifers and the meat from young bulls supplemented with 3.5 g/animal/day, as well as near to variables related to fat characteristics. The control group and the addition of 7.0 g/animal/day for young bulls were placed on the negative side of the F1 axis, negatively related to acceptability scores, especially the E7.0 group. According to Pearson coefficients ($P< 0.001$), the overall acceptability of this study was slightly more correlated with flavor ($r=0.770$) than texture acceptability ($r=0.710$). Being lower the correlation between both attributes (texture-flavor) ($r=0.561$). However, as demonstrated in other beef consumer studies (Oliver *et al*., 2006, Realini *et al*., 2013) there are different groups of consumers with differentiated perceptions and preferences in relation to beef acceptability and those groups may constitute significant market segments that demand beef with different characteristics (Oliver *et al.*, 2006, Font-i-Furnols and Guerrero, 2014).

3.5.Cluster analyses

As shown in Table 5, there three clusters (groups) of consumers with different preferences were identified, related to the overall acceptability of meat in the current work.

The largest cluster (1) of consumers (58.6%) evaluated all treatments with high scores, being their smallest value of 7.8 on a 9.0 point scale. That cluster included a similar number of men (44%) and women (55%), distributed in a similar percentage of people in each of the four age ranges analyzed (almost 25%). Those consumers preferred meat from heifers ($P \leq$ 0.001) without supplementation or with 7.0 g/animal/day of essential oil mix to that from (E3.5 heifers) and young bulls independently on the essential oil supplementation, a significant interaction (P≤ 0. 014) between sex and diet was observed in this cluster Frequency of beef consumption was similar to the average calculated for all consumers (Table 2), with 82.3% of sample consuming beef more than 3 times per week. In relation to the species more consumed the data also agrees with the general percentages for poultry and cattle, as well as in the type of animal preferred for beef. However it is remarkable that Cluster 1 involves all pig consumers in the global sample, representing 5.9% of participants in this cluster.

Cluster 2 comprised 10.4% of consumers (83% women, and all people less than 40 years old). For this group of consumers, diet and sex were significant factors (P= 0.019 and P= 0.002, respectively) without a significant interaction between them (P> 0.05). This segment of consumers preferred meat from heifers (6.36 points) than those from young bulls (4.44). Also this cluster disliked meat from the control group and the

E7.0 young bulls (with scores of 4.71 and 4.96, respectively), awarding meat from E3.5 group which presented higher scores (6.54). Only 25% of this cluster eats beef less than 3 times per week, being for the 83.3% meat for cattle the type of meat more consumed, following by 16.7% that answered poultry, data according to global answers (Table 3). The type of animal preferred for Cluster 2 was heifers for 75%, and steers for 25% of group.

Cluster 3 included 31.0% of participants, with a predominance of men (70%) and was mainly between 26-40 years old. There was significant interaction between diet and sex (P=0.012), being each factor (diet or sex) considered independent not significant (P> 0.05). The treatment which differed from the others, with lower scores, was the E7.0 young bulls, being scored with a value of 5.9. In reference to questionnaire answers, as in the other clusters, the frequency of beef consumption was similar to that globally (77.8% people eat beef more than 3 times per week), and the most eaten species were cattle and poultry at 75.0% and 22.2% respectively. Cluster 3 involves all habitual sheep consumers in the global sample, representing 2.8% of participants in this cluster. In relation to the type of animal preferred, 61.1% of sample answered heifers versus 25.0% and 11.1% that answered steers and young bulls respectively.

According to these results, meat form heifers are globally preferred by the main groups of consumers and the use of this mix of essential oils would improve the overall acceptability of meat, affecting views on the meat of young bulls more than opinions of meat from heifers at at 3.5 g/animal/day level of addition of essential oils.

4. Conclusions

Essential oil additives in the diet of crossbred beef cattle did not negatively affect consumer meat acceptability scores or product perception. In this study, the sex used (young bulls *vs.* heifers) had a greater effect than diet, however the addition of 3.5 grams/animal/day of a mix of essential oils could improve sensory meat characteristics, especially for young bulls, which were globally less appreciated than meat from heifers. The addition of higher concentrations of essential oils is not synonymous with higher acceptance. According to the high scores obtained in sensory evaluation of the final product, and the lack of negative effects on carcass and instrumental meat quality, the use of essential oils and the ongoing study of these natural products should be encouraged. More studies of the concentration, different compound mixtures of essential oils, time of administration in animal diets, aging times, or other breeds and consumers from different countries, would be desirable in order to better understand the effect that the inclusion of essential oils in the diet has on meat characteristics and animal performance.

5. References

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils–a review. *Food and Chemical toxicology* **46**, 446-475. doi:10.1016/j.fct.2007.09.106

Barcellos M D, Kügler JO, Grunert KG, Van Wezemael L, Pérez-Cueto FJA, Ueland Ø, Verbeke W (2010) European consumers' acceptance of beef processing technologies: A focus group study. *Innovative Food Science & Emerging Technologies* **11**, 721-732. doi:10.1016/j.ifset.2010.05.003

Benchaar C, Calsamiglia S, Chaves AV, Fraser GR, Colombatto D, McAllister TA, Beauchemin KA (2008) A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology* **145**, 209-228. doi:10.1016/j.anifeedsci.2007.04.014

Brasil (1997) Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (pp. 241). Brasília: Ministério da Agricultura, Pecuária e Abastecimento.

Cruz OTB, Valero MV, Zawadzki F, Rivaroli DC, Prado RM, Lima BS, Prado IN (2014) Effect of glycerine and essential oils (*Anacardium occidentale* and *Ricinus communis*) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. *Italian Journal of Animal Science* **13**, 790-797. doi:10.4081/ijas.2014.3492

Delgado EF, Aguiar AP, Ortega EMM, Spoto MHF, Castillo CJC (2006) Brazilian consumers' perception of tenderness of beef steaks classified by shear force and taste. *Scientia Agrícola* **63**, 232-239.

FAPRI (2014) Food and Agricultural Policy Research Institute. In: Database, W. A. O. (ed.) *Food and Agricultural Policy Research Institute.* Iowa State University and University of Missouri-Columbia Ames, IA, USA.

Fernández-Ginés JM, Fernández-López J, Sayas-Barberá E, Pérez-Alvarez J (2005). Meat products as functional foods: A review. *Journal of Food Science* **70**, R37-R43. doi: 10.1111/j.1365-2621.2005.tb07110.x

Ferraz JBS, Felício PE (2010) Production systems – An example from Brazil. *Meat Science* **84**, 238-243. doi:10.1016/j.meatsci.2009.06.006

Font-i-Furnols M, Guerrero L (2014) Consumer preference, behaviour and perception about meat and meat products: an overview. *Meat Science* **98**, 361-371.doi: 10.1016/j.meatsci.2014.06.025

Font i Furnols M, Gispert M, Guerrero L, Velarde A, Tibau J, Soler, J, Hortós M, García-Regueiro JA, Pérez J, Suárez P, Oliver MA (2008) Consumers' sensory acceptability of pork from immunocastrated male pigs. *Meat Science* **80**, 1013-1018. doi:10.1016/j.meatsci.2008.04.018

Françozo MC, Prado IN, Cecato U, Valero MV, Zawadzki F, Ribeiro OL, Prado RM, Visentainer JV (2013) Growth performance, carcass characteristics and meat quality of finishing bulls fed crude glycerine-supplemented diets. *Brazilian Archives of Biology and Technology* **56**, 327-336.

Franz C, Baser KHC, Windisch W (2010) Essential oils and aromatic plants in animal feeding - a European perspective. A review. *Flavour and Fragrance Journal* **25**, 327-340. doi:10.1002/ffj .1967

Guerrero A, Campo MM, Cilla I, Olleta JL, Alcalde MJ, Horcada A, Sañudo C (2014) A comparison of laboratory-based and home-based test of consumer preferences using kid and lamb meat. *Journal of Sensory Studies* **29**, 201-210. doi:10.1111/joss.12095

Haugaard P, Hansen F, Jensen M, Grunert KG (2014) Consumer attitudes toward new technique for preserving organic meat using herbs and berries. *Meat Science* **96**, 126-135. doi:10.1016/j.meatsci.2013.06.010

Hulankova R, Borilova G, Steinhauserova I (2013) Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. *Meat Science* **95**, 190-194. doi:10.1016/j.meatsci.2013.05.003

Hussein MHM, Hayan MA (2012) Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. *LWT-Food Science and Technology* **45**, 79-87. doi:10.1016/j.lwt.2011.07.031

IBGE (2013) Contas nacionais trimestrais. Jul.-set. 2013. 20th ed. Available in: www.ibge.gov.br, Rio de Janeiro, BR.

Kamel C (2000) A novel look at a classic approach of plant extracts. *Feed Mix* **8**, 19-23.

Kirinus JKC, Sañudo C, Fruet APB, Dörr A C, Nörnberg JL (2014) Caracterización socieconómica de consumidores con elevada ingesta de carne bovina. *Enciclopédia Biosfera* **10**, 2830-2848.

Jian J, Xiong YL (2016) Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science* **120**, 107-117.

Lancaster PA, Carstens GE, Ribeiro FRB, Davis ME, Lyons JG, Welsh TH (2008) Effects of divergent selection for serum insulin-like growth factor-I concentration on performance, feed efficiency, and ultrasound measures of carcass composition traits in Angus bulls and heifers. *Journal of Animal Science* **86,** 2862-2871. doi:10.2527/jas.2008-1083

Langhout P (2000). New additives for broiler chickens. *World Poultry* **16**, 22-27.

Lepper-Blilie AN, Berg EP, Germolus AJ, Buchanan DS, Berg PT (2014) Consumer evaluation of palatability characteristics of a beef value-added cut compared to common retail cuts. *Meat Science* **96**, 419-422. doi:10.1016/j.meatsci.2013.08.002

Lobato JFP, Freitas AK, Devincenzi T, Cardoso LL, Tarouco JU, Vieira RM, Dillenburg DR, Castro I (2014) Brazilian beef produced on pastures: sustainable and healthy. *Meat Science* **98,** 336-345. doi:10.1016/j.meatsci.2014.06.022

Macfie HJ, Bratchell N, Greehoff K, Vallis LV (1989) Designs to balance the effect of order of presentation and first order carry over effect in hall tests. *Journal of Sensory Studies* **4**, 129-148. doi: 10.1111/j.1745-459X.1989.tb00463.x.

Monsón F, Sañudo C, Sierra I (2004) Influence of cattle breed and ageing time on textural meat quality. *Meat Science* **68**, 595–602. doi:10.1016/j.meatsci.2004.05.011

Müller L (1980) Normas para avaliaçao de carcaça e concurso da carcaça de novilhos. Universidade Federal de Santa María,Santa María, RS,Brasil.

NRC (2000) *Nutrient Requirements of Beef Cattle,* 7th rev. edn. Natl. Acad. Press, Washington, DC, USA.

O'Connor SF, Tatum JD, Wulf DM, Green RD, Smith GC (1997) Genetic effects on beef tenderness in *Bos indicus* composite and Bos taurus cattle. *Journal of Animal Science* **75,** 1822-30. doi:/1997.7571822x

Oliver MA, Nute GR, Font i Furnols M, San Julián R, Campo MM, Sañudo C, Cañeque V, Guerrero L, Alvarez I, Díaz MT, Branscheid W, Wicke M, Montossi F (2006) Eating quality of beef, from different production systems, assessed by German, Spanish and British consumers. *Meat Science* **74,** 435-442. doi: 10.1016/j.meatsci.2006.03.010

Patra AK (2011) Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. *Asian Journal of Animal Veterinary Advances* **6,** 416-428. doi:10.3923/ajava.2011

Pérez-Juan M, Realini CE, Barahona M, Sarriés MV, Campo MM, Beriain MJ, Vitale M, Gil M, Albertí P (2014) Effects of Enrichment with Polyunsaturated Fatty Acids (Omega-3 and Conjugated Linoleic Acid) on Consumer Liking of Beef Aged for 7 or 21 d Evaluated at Different Locations. *Journal of Food Science* **79**, S2377-S2382. doi: 10.1111/1750-3841.12667

Realini CE, Font i Furnols M, Guerrero L, Montossi F, Campo MM, Sañudo C, Nute GR, Alvarez I, Cañeque V, Brito G, Oliver MA (2009) Effect of finishing diet on consumer acceptability of Uruguayan beef in the European market. *Meat Science* **81,** 499-506. doi:10.1016/j.meatsci.2008.10.005

Realini CE, Font i Furnols M, Sañudo C, Montossi F, Oliver MA, Guerrero L (2013) Spanish, French and British consumers' acceptability of Uruguayan beef, and consumers' beef choice associated with country of origin, finishing diet and meat price. *Meat Science* **95**, 14-21.doi:10.1016/j.meatsci.2013.04.004

Reddy BV, Sivakumar A, Jeong DW, Woo YB, Park SJ, Lee SY, Byun JY, Kim CH, Cho SY, Hwng G.I (2015) Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Animal Science Journal* **86**, 1-16. doi: 10.1111/asj.12266

Rivaroli DC (2014) Níveis de óleos essenciais em dietas alto grão para bovinos de corte terminados em confinamento: desempenho, temperamento animal, características da carcaça e qualidade da carne. Master Thesis. Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Brasil.

Rivaroli DC, Guerrero A, Valero MV, Zawadzki F, Eiras CE, Campo MM, Sañudo C, Jorge AM, Prado IN (2016) Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Science* **121,** 278-284. doi :10.1016/j.meatsci.2016.06.017

Robelin J, Geay Y (1975) Estimation de la composition de la carcasse des taurillons a partir de la 6ème côte. Bulletin Technique. *Centre de Recherches Zootechniques et Veterinaires de Theix* **22**, 41-44.

Rotta PP, Prado RM, Prado IN, Valero MV, Visentainer JV, Silva RR (2009) The effects of genetic groups, nutrition, finishing systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. *Asian-Australasian Journal of Animal Sciences* **22**, 1718-1734.

Valero MV, Prado RM, Zawadski F, Eiras CE, Madrona GS, Prado IN (2014a) Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. *Acta Scientiarum. Animal Sciences* **36**, 419-426. doi:10.4025/actascianimsci.v36i4.23856

Valero MV, Torrecilhas JA, Zawadzki F, Bonafé EG, Madrona GS, Prado RM, Passetti RAC, Rivaroli DC, Visentainer JV, Prado IN (2014b) Propolis or cashew and castor oils effects on composition of *Longissimus* muscle of crossbred bulls finished in feedlot. *Chilean Journal of Agricultural and Research* **74**, 445-451. doi:10.4067/S0718-58392014000400011

Table 1. Composition of basal diets

Parameters	Ingredients, g/kg on DM							
	SCBP[1]	Corn grains	Soybean meal	Limestone, 36%	Urea	Mineral salt	Yeast	Diets, g/kg on DM
Dry matter	947.0	889.3	886.0	993.0	980.0	993.0	980.0	881.3[2]
Organic matter	980.0	991.0	937.0	107.1	5.6	107.0	-	973.4
Ash	19.7	9.50	62.5	832.9	994.4	893.0	-	26.60
Crude protein	18.3	89.9	490.0	-	2600	-	300.0	125.0
Ether extract	36.0	35.0	13.0	-	-	-	-	22.0
Neutral detergent fiber	787.4	177.0	137.0	-	-	-	-	303.0
Acid detergent fiber	492.0	44.0	59.7	-	-	-	-	148.0
Total digestible nutrients	430.0	900.0	840.0	-	-	-	-	703.0
Diets	100	819.5	65.1	4.60	6.20	4.10	0.50	

[1]Sugarcane bagasse pellet
[2] g/kg on fresh basis
Diet energy was calculated according to NRC (2000)

Table 2. Effect of Diet (D) and Sex (S) on carcass characteristics of beef finished in feedlot

	Diets			Sex			P value		
	E 0.0	E 3.5	E 7.0	YB	H	SEM	D	S	DxS
n	19	19	19	27	30				
Initial weight, kg	228.12	230.52	228.04	243.41	219.77	3.94	0.954	0.004	0.943
Final weight, kg	378.33	382.96	384.78	440.30	345.35	7.38	0.937	<0.001	0.541
Hot carcass weight, kg	203.25	206.07	206.88	242.88	181.82	4.65	0.944	<0.001	0.821
Cold carcass weight, kg	198.86	201.35	202.40	238.11	177.44	4.59	0.942	<0.001	0.837
Hot dressing carcass, %	53.57	53.60	53.56	55.08	52.63	0.26	0.959	<0.001	0.422
Cold carcass dressing, %	52.40	52.37	52.39	53.99	51.37	0.26	0.983	<0.001	0.383
6º rib									
Fat thickness, mm	6.38	6.83	6.88	6.68	6.71	0.29	0.779	0.965	0.568
Muscle, %	59.68	60.05	61.17	60.33	60.28	0.54	0.481	0.965	0.301
Bone, %	14.66	15.89	14.95	15.61	14.77	0.40	0.449	0.303	0.860
Total fat, %	19.72	18.02	18.20	17.49	19.68	0.60	0.457	0.068	0.443
Others, %	5.95	6.04	5.68	6.58	5.27	0.24	0.743	0.005	0.187

E00: Control diet; 0.0 g/animal/day essential oil; E3.5: 3.5 g/animal/day essential oil; E7.0: 7 g/animal/day essential oils. YB: Young bulls. H: heifers.

Table 3. Characteristics of the valid consumers involved in the trial (age and gender) and habits of consumption.

	Total population	Men	Women
Age (%)			
< 25 years	28.4	26.3	30.5
26-40 years	31.0	35.1	27.1
41-55 years	19.8	17.5	22.0
> 56 years	20.7	21.1	20.3
Total (%)	100	49.1	50.9
Total (*n*)	116	57	59

Frequency of beef consumption (%)	> 5 times/week	35.0
	3-5 times/week	44.2
	1-2 times/week	20.8
Type of meat more consumed (%)	Cattle	75.0
	Poultry	20.0
	Sheep	0.8
	Pig	4.2
Type of animal preferred (%)	Heifer	66.4
	Steer	21.5
	Young bull	12.1

Table 4. Acceptability§ of sensory attributes of beef from young bulls and heifers and three level of essential oil mix supplementation assessed by consumers (n=116)

Acceptability	Young bulls			Heifers			Diet			Sex			P value		
	E0.0	E3.5	E7.0	E0.0	E3.5	E7.0	E0.0	E3.5	E7.0	YB	H	SEM	D	S	D x S
Tenderness	6.98b	7.59a	6.87b	7.76a	7.68a	7.62a						0.059	0.009	<0.001	0.003
Flavor							7.33ab	7.49a	7.09b	7.07	7.53	0.062	0.003	<0.001	0.630
Overall	7.09bc	7.47ab	6.85c	7.66a	7.63a	7.62a						0.063	0.061	<0.001	0.016

E00: Control diet, 0 g/animal/day essential oil; E3.5: 3.5 g/animal/day essential oil; E7.0: 7 g/animal/day essential oil. SEM: Standard error of mean. D: effect of type of diet (P-value); S: effect of sex (P-value); DxS: interaction between diet and sex (P-value).

a,b,c: indicate statistical differences in the same row ($P \leq 0.05$).

§Based on a 9-point scale (1: dislike extremely; 9: like extremely).

Table 5. Overall acceptability scores[§] of beef from young bulls and heifers and three level of essential oil mix supplementation in three clusters of consumers (n=116).

	n	% consumers	Young bulls			Heifers			Diets			Sex		P value			
			E0.0	E3.5	E7.0	E0.0	E3.5	E7.0	E0.0	E3.5	E7.0	YB	H	SEM	D	S	D x S
Cluster 1	68	58.62	7.84c	7.99bc	7.84c	8.40a	8.03bc	8.21ab						0.045	0.446	0.001	0.014
Cluster 2	12	10.35							4.71b	6.54a	4.96b	4.44	6.36	0.279	0.019	0.002	0.399
Cluster 3	36	31.03	6.94a	7.00a	5.94b	6.78a	7.06a	7.08a						0.096	0.081	0.164	0.012

E00: Control diet, 0 g/animal/day essential oil; E3.5: 3.5 g/animal/day essential oil; E7.0: 7 g/animal/day essential oil. SEM: Standard error of mean. D: effect of type of diet (P-value); S: effect of sex (P-value); DxS: interaction between diet and sex (P-value).

a,b,c: indicate statistical differences in the same row between treatments (P≤0.05).

[§]Based on a 9-point scale (1: dislike extremely; 9: like extremely).

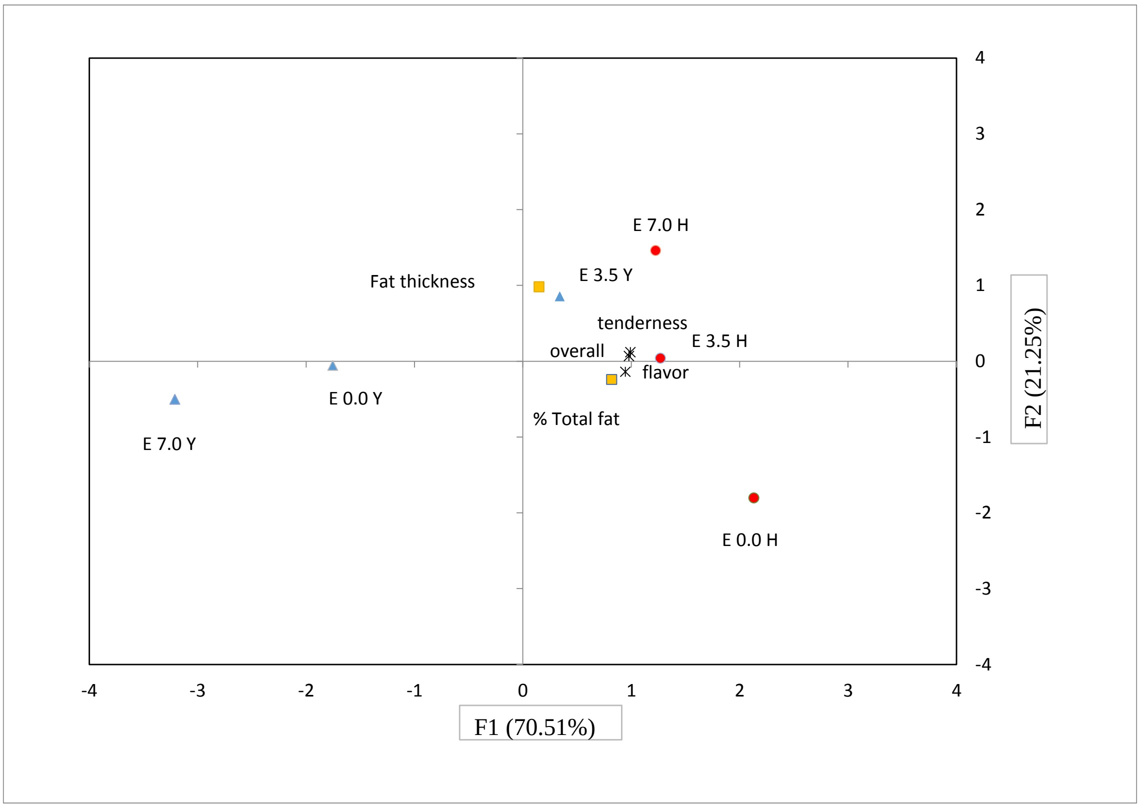

Figure 1. Principal component analysis of the scores for tenderness acceptability, flavor acceptability and overall acceptability of beef from young bulls (YB) and heifers (H) supplemented with different level of essential oil mix (E0.0: 0 g/animal/day; E3.5:3.5 g/animal/day; E7.0: 7 g/animal/day). Fat thickness and % total fat from the 6º rib.

CAPÍTULO 4.

Effect of sainfoin supplementation on fat skatole and indole concentration, meat quality, odour and flavor in lambs grazing alfafa pasture

Effect of sainfoin supplementation on fat skatole and indole concentration, meat quality, odour and flavor in lambs grazing alfafa pasture

Abstract

It was investigated the influence of sainfoin pellets supplementation on fat skatole and indole concentration, fat and meat color and chop sensory attributes in grazing lambs. Fifty-four lambs (Romane) were separated in three treatments for at least 87 days before slaughter: feedlot (SI), pasture-fed alfalfa (*M. sativa*) (AF) and pasture-fed alfalfa plus sainfoin (*O. viciifolia*) pellets (AS). Perirenal and dorsal fat skatole concentrations were below the quantification limit for all SI lambs. Sainfoin pellet supplementation in grazing lambs reduced perirenal and dorsal fat skatole concentration. The treatments did not affect subcutaneous caudal fat and *Longissimus thoracics et lumborum* muscle color ($P > 0.05$), except for lightness (L*) on fat ($P < 0.05$). The sainfoin pellets supplementation for grazing lambs can be a strategy to reduce perirenal and dorsal fat skatole concentration.

Key words: bioactive compounds, lamb meat, pastoral flavor, sainfoin,

1. Introduction

Animal production in grazing systems can be a good alternative for marketing of meat, once for consumers, grazing is associated with organic farming livestock, animal welfare and healthiness. However, the high protein levels in grazing can negatively affect the meat organoleptic quality (Prache et al., 2011; Devincenzi, et al., 2014; Girard et al., 2015).

The ruminal protein digestion occurs in two main steps: solubilization (this can be different in each protein derived of diet) and degradation. The rapid and extensive aminoacids ruminal degradation, like tyrosine and tryptophan, may result in the formation of compounds called "off-flavors", such as indole and skatole (3-methylindole) (Young, et al., 2003). Skatole and indole have been related to contribute to meat off-flavor of animals finished on pasture compared with those fed grain (Young et al., 2003). In this context, management strategies, as feed manipulation, have been studied to reduce the unpleasant pasture flavour ("grassy", "milky", "animal" and even "fecal") (Schreurs et al.; 2007; Devincenzi et al.; 2014; Girard et al.; 2015).

Plants bioactive compounds appear to reduce protein degradation velocity in rumen. Thus, feeding animals with forage legumes rich in condensed tannins (CT), such Sainfoin (*Onobrychis viciifolia*), have been considered as a promising strategy to reduce the occurrence of off-flavor and off-odours in lamb meat (Girard et al.; 2015; Schreurs et al.; 2007).

Flavor and color play a major role, being sensory properties by which meat quality is readily assessed (Liu, Lunari, Schaefer 1995). Therefore, in addition to the effect of CT on meat quality, CT could interfere with rumen metabolism as biohydrogenation modulator, as well as to decrease the meat oxidative process and increase color stability (Vasta and Luciano, 2011). The dehydration of CT rich forages, could be a possibility to improve the stability and conservation of them (Terrill et al., 2007; Gujja et al., 2013; Kommuru et al., 2015). The aim of this study was to investigate the effect of sainfoin pellets addition in the diets of graze finished lambs on fat indole and skatole concentrations, fat and meat color and chop odour and flavor attributes.

2. **Materials and methods**

The study was conducted at the Herbivore Research Unit at the INRA, Clermont-Ferrand city France. The experiment was approved by the committee for animal care and use (approval number 5287-2016050318599795_v2).

2.1. Experimental design, animals and diets

The experiment was carried out starting on June 25, 2016 with fifty-five non-castrated male Romane lambs. Lambs were born within 8 days (10 April-18 April 2016) from 9 rams and 39 dams and they were weaning until 60 days of age. After weaning the animals were separated in blocks and randomly assigned to three diets according to birth weight, average daily gain (ADG) and parenthood. The diets consisted of: feedlot (SI, n=18), pasture-fed alfalfa (*M. sativa*) (AF, n=18 + 1 replacement) and pasture-fed alfalfa plus sainfoin (*O. viciifolia*) pellets supplementation (AS, n=17). During the study, one AS lamb died from causes unrelated to the experimental treatment.

The sainfoin pellets contained 42 g of condensed tannins (CT) / kg dry matter (DM). Lamb blocks were housed in a sheepfold by 10 days for concentrate intake adaptation. To the SI and AF lambs were offered a commercial concentrate, while AS received commercial concentrate plus sainfoin pellets. When it was observed that all AS lambs fed sainfoin pellets, the pasture period adaptation was started. The adaptation period to the pasture was carried out in one week, in which it was progressively increased the grazing time of AF and AS lambs until full permanence at alfalfa pasture.

The plots of alfalfa were divided in two, and the AF and AS groups were switched between them regularly, three times a week. This procedure was done to reduce a plot effect and to ensure that the pasture conditions were the same for both groups. Sainfoin pellets (15 g DM / kg animal day) were distributed for AS lambs while SI lambs were offered only hay and concentrate. Feed was distributed every day in the morning, and the daily refusals were weighed, recorded and discarded.

Feed intake of SI lambs was restricted and adjusted according to AF weights in order to obtain similar average growth profiles between the SI and AF groups.

2.2.Slaughter procedures

The slaughtering took place at the experimental slaughterhouse of INRA Clermont-Ferrand Centre and was done according to European Union welfare guidelines. The lambs had access to food and water until thirty minutes before slaughter. As soon as the animals arrived at the facility, they were electrically stunned and had their throat cut. Lambs mean age was 159 days including the experimental period of 87 days (range: 72-100 days) with an average weight of 48.69 ± 4 kg.

2.3.. Measurements

2.3.1. Experimental period

2.3.1.1. Animal body weight

The lambs were weighed once a week before sainfoin pellets, hay and concentrate distribution.

2.3.1.2. Dry matter (DM) and DM intake of sainfoin, hay and concentrate

During approximately 8 hours (08:45 a.m – 16:30 p.m) for two days (d10 and d15 after the beginning of experimental period), the animals were visually observed with the aid of a binoculars every 5 minutes. The number of times that each lamb went to the trough and time that animal spent feeding, was observed. Then, the mean time that each lamb remained at the feeder consuming sainfoin pellets was estimated

The estimation of the sainfoin pellets DM content was made daily using the standard oven method, while DM of hay and concentrate was made once a week. The daily feed intake was estimated by the difference between the supplied feed and the refused feed left in the trough.

The voluntary DM intake level of alfalfa was assumed to be 78.1g DM/LW$^{0.75}$, where LW was the average live weight of the group (Dulphy, Faverdin, & Jarrige, 1989). Therefore, mean percentage of sainfoin pellets was calculated in relation to the total amount of voluntary DM intake.

2.3.3. Carcass characteristics and meat and fat sampling

At slaughtering lambs testes were weighed. The carcasses were chilled at 4°C for 24h. At 24h post-mortem, the pH was measured on *Longissimus thoracis et lumborum* (LTL) muscle using a pH-meter. Carcass and perirenal fat were weighed. A sample of approximately 15 g of perirenal and dorsal fat was taken from the carcasses, wrapped in aluminium foil, vacuum packed in sealable polyamide bags, labelled and frozen at -20°C for posterior analyses of indole and skatole concentrations. The fat thickness was estimated over the last thoracic rib. Firmness of thoracic fat was manually assessed by a trained person, and a grade from three to nine (from less firm to more firm) was assigned on a subjective scale.

2.3.4. Perirenal and dorsal fat skatole and indole concentrations

The concentrations of skatole and indole in perirenal and dorsal fat were measured using the HPLC according to the methodology described by Batorek et al. (2012). Concentrations were expressed in µg/gram of the lipid fraction from the fat. The minimum for the quantification was 0.03 µg/g of liquid fat.

2.3.5. Color of subcutaneous caudal fat and Longissimus thoracis et lumborum muscle

Subcutaneous caudal fat (SCF) and LTL muscle color were assessed 24 h after slaughtering at wavelengths between 400 and 700 nm using a MINOLTA CM-700d spectrophotometer with a 10° view angle and a D65 illuminant. The following color coordinates were expressed in the CIELAB uniform color space (CIE, 1986): lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue angle (h). For the measurements of SCF, a plane surface was cut with a knife to allow the fat to adhere perfectly to the eye of the apparatus. Five measurements were done at 5 randomly-selected locations. Color measures on LTL muscle were made after2 h of blooming. Afterwards, LTL muscle was stored on a tray, wrapped with stretchable food film and chilled at 4 ° C for 9 days of storage. On days 2, 3, 6, 7, 8 and 9, after 2h of blooming time, color measurements on LTL were made again following the same procedures describe above.

3.3.6. Lamb chop sensory evaluation

Lamb chop sensory evaluation was performed by 12 trained panelists at INRA Magneraud Experimental Unit (UE1206 EASM), according to the rules set out in AFNOR NF ISO 8586-1 and ISO 8586-2.

The panelists were all women, of an average age of 55 years. Before evaluation of the experimental chops, a preliminary session was held, using additional chops from one lamb with the highest skatole concentration in perirenal fat (0.41 µg/g of liquid fat), and one lamb with the lowest skatole concentration in perirenal fat (<0.03 µg/g of liquid fat), for the panelists to experience perceptions linked to skatole and to agree on common criteria describing these perceptions. The criteria chosen by the panelists were ‘animal’ odour and ‘animal’ flavor. The panelists therefore had a common understanding of the term ‘animal’ odor/flavor which here was referred to as the odor/flavor associated with a lamb chop from an animal with skatole > 0.41 µg/g liquid fat.

Two additional training sessions were held on additional randomly chosen lamb chops before evaluation of the experimental chops. Lamb chop sensory evaluation was performed on twelve chops per experimental lamb in eighteen panel sessions (one panel session per block). At each panel session, each panelist evaluated the four lambs from one block, with lambs from each group presented in randomized order. Before each session, chops to be evaluated were thawed for 24 h at +4 °C. Each chop was individually put into a covered aluminum foil container . Then, 2 chops per group were contact-grilled ‘bone in’ to an internal temperature of 75 °C, and served warm to the 12 panelists (2 chops per group being served to 3 panelists). The bone part was removed, then pieces (about 2 cm x 2 cm each) were cut from the lean part (LTL muscle) and pieces (about 1 cm x 2 cm each) were cut from the fat part (the rest of the chop) to provide a piece of both for all the panelists. The panelists were asked to taste each part separately and evaluate successively: the lean part odor, the lean part flavor, the fat part flavor then the fat part flavor. There were asked to use 10 cm unstructured line scales (from 0 to 10) to evaluate the intensity of ‘animal’ odor and of ‘animal’ flavor in the fat and the lean parts of the chops. Assessments were subsequently scanned scored as the distance in cm from the left end of the line using the FIZZ ® software. Panelists were asked to drink water and eat toast and a piece of apple between assessments to ensure that each sample was assessed with a cleansed palate.

2.4. Statistical analyses

The data for ADG, testes weight, cold carcass weight (CCW) perirenal fat weight (PFW), fat thickness, firmness and SCF color underwent a two-way analyses of variance (ANOVA). The slaughter session was set as a random effect and the treatment as fixed effect. Pairwise differences were assessed by using the Tukey test.

Skatole and indole concentrations in perirenal and dorsal fat among treatments presented different variances. For this reason, we utilized the non-parametric Kruskal-wallis test to assess whether or not the treatment effect was present and the Wilcoxon-Moann-Whitney Test for comparison between groups (two by two).

The variances of AF and AS groups had a similar magnitude, so it was compared AF and AS groups via ANOVA. The slaughter section effect was considered as random, while the treatment and adipose tissue location as fixed effects. Differences between means among groups were evaluated with the Tukey test.

The distribution in number of lambs presenting skatole concentration in dorsal fat above or below the threshold value of 0.22µg / g (Devincenzi et al., 2014) was found out with the chi-square test.

The data for LTM muscle color underwent an ANOVA using a mixed model approach, with treatment and days of display (1, 2, 3, 6, 7, 8 and 9) as fixed factors and slaughter session as random factor, and using the Tukey test for pairwise comparisons.

The data for chop sensory evaluation muscle color underwent an ANOVA using a mixed model, with treatment and panel session as fixed factors and panelist as random factor and using the Tukey test for pairwise comparisons.

In this study all ANOVAs were performed with SAS® (2005) package and using the MIXED procedure.

The SCF and LTL muscle color differences between two treatments was calculated as $\Delta Eab^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{0.5}$, where the Δ of the formula represent the differences between the corresponding coordinates of the stimuli (Brainard, 2003).

3. Results

3.1. Intake, performance, testes weight and carcass characteristics

During 8 hours, we observed that all the animals of AS group consumed sainfoin pellets. The range of time observed in which the animals remained at feeder was approximately 40 minutes.

The average sainfoin pellets DM intake by AS lambs was 440 g /animal day (38.28 kg /animal / 87 days of experiment). The mean dietary proportion of sainfoin pellets was 7.96%. Average concentrate and hay DM intake were 1.16 and 0.31 kg / animal day, respectively.

There was no significant effect ($P > 0.05$) of the treatment on testes and perirenal fat weight, fat thickness and firmness, except for ADG ($P < 0.01$) and CCW ($P < 0.001$) (Table 1). Lambs of SI and AS groups presented greater ADG than AF group ($P = 0.00$). Cold carcass weight was greater for AS compared with the others treatments (SI and AF).

3.2. Perirenal and dorsal fat skatole and indole concentrations

Perirenal and dorsal fat skatole concentrations remained below quantification limit for all SI lambs. Perirenal and dorsal fat indole concentrations were below quantification limit for 13 and 17 out of 18 SI lambs, respectively; when indole was detected in SI lambs, its concentration was just above the detection limit, i.e., it reached 0.04 to 0.05 µg/g.

Skatole was detected in the dorsal fat of all AF and AS lambs and in the perirenal fat of 16 out of 19 AF lambs, and 14 out of 17 AS lambs. Indole was detected in the dorsal fat of all AF and AS lambs and in the perirenal fat of all AF lambs and 15 out of 17 AS lambs.

There was a treatment effect on perirenal and dorsal fat skatole and indole concentration ($P < 0.001$ for all analyses). Perirenal and dorsal fat skatole and indole concentrations were significantly lower ($P < 0.001$) in SI lambs compared to AF and AS lambs ($P < 0.001$ for all analyses).

Skatole and indole concentrations were lower in AS lambs than in AF lambs ($P < 0.05$ and $P < 0.001$) (Table 2). Skatole concentration was lower in perirenal fat than in dorsal fat ($P < 0.001$), but indole concentration was higher in perirenal fat than in dorsal fat ($P < 0.001$) (Table 2). There was no interaction between treatment and tissue analyzed ($P > 0.05$). Mean perirenal fat skatole concentration was 0.14 µg/g (range : 0.00 to 0.41 µg/g) and 0.09 µg/g (0.00 to 0.25 µg/g) in AF and AS lambs respectively. Mean dorsal fat skatole concentration was 0.23 µg/g (range : 0.07 to 0.52 µg/g) and 0.17 µg/g (range : 0.09 to 0.35 µg/g) in AF and AS lambs respectively. Mean perirenal fat indole concentration was 0.12 µg/g (range : 0.07 to 0.20 µg/g) and 0.07µg/g (range : 0.00 to 0.16 µg/g) in AF and AS lambs respectively. Mean dorsal fat indole concentration was 0.08 µg/g (range : 0.05 to 0.11 µg/g) and 0.07 µg/g (range : 0.04 to 0.07 µg/g) in AF and AS lambs respectively.

The proportion of lambs whose skatole concentration in dorsal fat was above the threshold value was 53 % (10 out of 19) and 18 % (3 out of 17), for AF and AS lambs, respectively ($P < 0.05$).

3.3.Color of subcutaneous caudal fat

There was an effect ($P < 0.01$) of the treatment on lightness of SC fat (Table 3). Lightness of SC fat was higher ($P < 0.05$) in AF lambs than SI and AS lambs. The others color coordinates were not affected by the treatment. The SC fat ΔEab* values were 3.72, 1.27 and 2.54 for the comparisons between SI and AF, SI and AS and AF and AS lambs, respectively.

3.4.Color of Longissimus thoracis et lumborum muscle

There was an effect ($P < 0.0001$) of the treatment and the measure time ($P < 0.0001$) on lightness LTL muscle (Table 4) but there was no interaction between treatment and measure time. The treatment did not affected ($P < 0.05$) the others color coordinates (Redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (h)) while measure time had effect for all color coordinates ($P < 0.0001$). The SI lambs showed great lightness values than AF and AS groups ($P < 0.0001$) (Table 4)**.** The ΔEab* value was 1.70, 1.35 and 0.55 for the comparison between SI and AF, SI and AS and AF and AS lambs, respectively.

3.5.Lamb chop sensory evaluation

The intensity of the “animal” odour in the lean part of the chops ranged between 0.01 and 6.50 units. It was affected ($P<0.001$) by treatments (Table 5). The panelist found a higher intensity of “animal” odour for AF and AS lambs.

The intensity of the “animal” odour in the fat part of the chops ranged between 0.01 and 6.62 units. It was affected by treatments (P<0.0001) (Table 5). The panelist found a higher intensity of “animal” odour for AF and AS lambs. However, fat odour liking was not affected (P>0.05) by treatments but tended to be affected by the panel sessions (P=0.006) (Table 5).

The intensity of the “animal” flavor in the lean part of the chops ranged between 0.01 and 7.09 units. It was affected by treatments (P<0.05) (Table 5) and varied between panel sessions (P<0.05). The panelist found a higher intensity of “animal” flavor for AS lambs.

The intensity of the “sheepy” flavor in the lean part of the chops ranged between 0.12 and 5.59 units. It was affected by the treatments (P<0.05) (Table 5) and varied between panel sessions (P<0.001). The panelist found a higher intensity of “animal” flavor for AF and AS lambs. However, flavor liking was not affected by the treatments (P>0.05) (Table 5).

The intensity of the “rancid”, “livery” and “grassy” flavor in the lean part of the chops did not affected by the treatments (P<0.05) (Table 5).

4. Discussion

During visual observations, it was verified that all the animals of AS group ingested sainfoin pellets, so all the statistical results obtained among the groups can be attributed to different treatments

Cold carcass weight was higher for AS lambs in comparison with the other treatments. This is probably due to the fact that feed intake of SI lambs was restricted and adjusted according AF lamb weights. In addition, AS lambs were feed not only with alfalfa but also with sainfoin pellets.

Because the aim of this study was to investigate the influence of sainfoin pellets supplementation on fat indole and skatole concentration, measuring PFW was considered as well. It was expected that increasing of perirenal fat would influence the dilution fat-stored of these compounds, thus altering their concentration. However, the values observed in our study for PFW did not show treatment effect. Hence, in this study the effect of sainfoin supplementation on skatole and indole concentration could not be attributed to the PFW.

It was observed that the dorsal fat skatole was higher than perirenal fat, however with indole the opposite was true. There are not many studies comparing the concentration the skatole and indole concentrations in lamb meat. However, previous studies (Priolo et al., 2001; Devincenzi et al., 2014) rep**o**rted that skatole was higher in perirenal fat than dorsal fat. Due to the lack of studies, the reasons for this difference remain unclear.

In the present study skatole concentration in perirenal and dorsal fat was always below minimum quantification for the lambs from SI group. The same results were found by Young et al., (2001) and Priolo et al., (2005) in an experiment with Romney lambs slaughtered at 132 and 232 days of age, after grazing on ryegrass/clover or receiving concentrate on a feedlot.

In the present study, the sainfoin supplementation was effective in reducing perirenal and dorsal concentration of indole and skatole. The obtained skatole concentration result is in line with previous results of Girard et al., (2015), who observed a decrease in perirenal skatole concentration of lambs fed sainfoin silage in contrast to those fed on alfalfa silage, but the same did not occur for indole concentration, which was not influenced by treatment. Priolo et al. (2009), who studied the effect of quebracho tannins supplementation (40 g of tannic acid equivalents kg -1 DM) on indole and skatole concentration in the lambs caudal fat, obtained a similar result.

The literature suggested that the odour and flavor detection is approximately 0.22 μg/g of dorsal liquid fat for lamb meat (Devincenzi et al., 2014). The values that were observed for AF lambs was mostly above the target (10 out 19 lambs) while for AS lambs was mostly below (3 out 17 lambs).

The lightness in subcutaneous caudal fat was lower ($P < 0.05$) (Table 3) in SI and AS lambs at 24h post mortem. Although, this difference was statistically significant, it has a small biological amplitude, as ΔEab*values were lower than the threshold value of 5.9 suggested by Schwarz, Cowan, and Beatty (1987). Similar results were found by Ripoll et al., (2012), where two lambs groups finished in grazing systems resulted in greater lightness than the two lamb indoor system. Priolo et al., (2002a); Priolo et al., (2002b) and Dian et al., (2007) did not find any differences between forage and concentrate diets for lambs. Thus, the effect of grazing on lightness remains unclear.

The pH was the same for all treatments (Table 1), indicating that the effect on muscle color properties was inexistent. The lightness of LTL muscle was influenced by treatments ($P < 0.0001$) (Table 4), but as SC fat, did not have high biological amplitude, as ΔEab*values were lower to the threshold value of 2.0 suggested by Schwarz, Cowan, and Beatty (1987). Girard et al., (2015) did not find differences on muscle color of lambs when they compared lambs fed sainfoin silage with lambs fed alfalfa silage.

Animal odour and animal flavor on the fat part was stronger in AF and AS group compared with SI group but the values of flavor liking were higher for AF and AS group. The same occurred on lean part. Girard et al., 2015 also found no significant difference comparing lambs finished with addition of alfalfa and another group with addition of sainfoin.

5. Conclusions

In the present study it was demonstrated the potential of sainfoin pellets in the diet of lambs finished on graze to decreased the fat skatole and indole concentration. The fat and meat color and acceptability of lamb meat were not negatively influenced by the sainfoin pellets supplementation.

6. **References**

Batorek, N., Škrlep, M., Prunier, A., Louveau, I., Noblet, J., Bonneau, M., & Čandek-Potokar, M. (2012). Effect of feed restriction on hormones, performance, carcass traits, andmeat quality in immunocastrated pigs. Journal of Animal Science, 90, 4593–4603.

Brainard, D. H. (2003). Color Appearance and Color Difference Specification. In K. S. Steven (Ed.), The Science of Color (2 ed., pp. 191-216). Amsterdam: Elsevier Science Ltd.

Devincenzi, T., Prunier, A., Meteau, K., & Prache, S. (2014). Influence of Fresh alfafa supplementation on fat skatole and indole concentration and chop odour and flavour in lambs grazing a cocksfoot pasture. Meat science, 98, 607-614)

Dian, P., Chauveau-Duriot, B., Prado, I. & Prache, S. (2007). A dose-response study relating the concentration of carotenoid pigments in blood and reflectance spectrum characteristics of fat to carotenoid intake level in sheep. Journal of Animal Science, 85, 3054-3061.

Dulphy, J. P., Faverdin, P., & Jarrige, R. (1989). Feed intake: The fill unit systems. I.N.R.A., ruminant nutrition. recommended allowances & feed tables (pp. 61–70). London-Paris: Editions John Libbey Eurotext. Goering, H. D., & Van Soest, P. J. (1970).

Girard, M., Dohme-Meier, F., Silacci, O., Kragten, S. A., Kreuzer, M., & Bee, G. (2015). Forage legumes rich in condensed tannins may increase n-3 fatty acid levels and sensory quality of lamb meat. Journal of Science Agriculture 96, 1923-1933.

Gujja, S., Terrill, T.H., Mosjidis, J.A., Miller, J.E., Mechineni, A., Kommuru, D.S., Shaik,S.A., Lambert, B.D., Cherry, N.M., Burke, J.M., (2013). Effect of supplemental sericea lespedeza leaf meal pellets on gastro intestinal nematode infection ingrazing goats. Veterinary Parasitology, 191, 51–58.

Kommuru, D.S., Barker, T., Desai, S., Burke, J.M., Ramsay, A., Mueller-Harvey, I., Miller, J.E., Mosjidis, J.A., Kamisetti, N., Terrill, T.H., (2014). Use of pelleted sericea lespedeza (Lespedeza cuneata) for natural control of coccidian and gastrointestinal nematodes in weaned goats. Veterinary Parasitology, 204, 191–198.

Liu, Q., Lanari M.C. & Schaefer D.M., (1995). A review of dietary vitamin E supplementation for improvement of beef quality. Journal of animal science, 73, 3131-3140.

Prache, S., Gatellier, P., Thomas, A., Picard, B., & Bauchart, D. (2011). Comparison of meat and carcass quality in organically reared and conventionally reared pasture-fed lambs. Animal, 5(12), 2001-2009.

Priolo, A., Bella, M., Lanza, M., Galofaro, V., Biondi, L., Barbagallo., D., Ben Salem, H., & Pennisi, P. (2005). Carcass and meat quality of lambs fed fresh sulla (Hedysarum coronarium L.) with or without polyethylene glycol or concentrate. Small Ruminant Research. 59, 281-288.

Priolo, A., Micol, D., & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. Animal Research, 50, 185-200.

Priolo, A., Prache, S., Micol, D., & Agabriel, J. (2002). Reflectance spectrum of adipose tissue to trace grass feeding in sheep: influence of measurement site and shrinkage time after slaughter. Journal of Animal Science, 80(4), 886-891.

Priolo, A., Vasta, V., Fasone, V., Lanza, C. M., Scerra, M., Biondi, L., Bella, M., & Whittington F. M. (2009). Meat odour and flavour and indoles concentration in ruminal fluid and adipose tissue of lambs fed green herbage or concentrates with or without tannins. Animal. 3:3, 454-460.

Ripoll, G., Albertí, P., & Joy, M. (2012). Influence of alfalfa grazing-based feeding systems on carcass fat colour and meat quality of light lambs. Meat Science, 90(2), 457-464.

Schreurs, N. M., Marotti, D.M., Tavendale, M. H., Lane, G. A., Barry, T.N., Lopez-Villalobos N., & McNabb, W. C. (2007). Concentration of indoles and other metabolites in sheep after a meal of fresh write clover, perennial ryegrass or

Lotus corniculatus and the appearence of indoles in the boold. Journal of the Science of Food and Agriculture, 87, 1042-1051.

Schwarz, M. W., Cowan, W. B., & Beatty, J. C. (1987). An experimental comparison of RGB, YIQ, LAB, HSV, and opponent color models. Association for Computing Machinery Transsactions Graphics, 6(2), 123-158.

Statistical Analysis Systems Institute (1999). SAS/STAT user's guide, version 8. Cary, NC: SAS Institute Inc.

Terrill, T.H., Mosjidis, J.A., Moore, D.A., Shaik, S.A., Miller, J.E., Burke, J.M., Muir, J.P., Wolfe, R., (2007). Effect of pelleting on efficacy of sericea lespedeza hay as a naturaldewormer in goats. Veterinary Parasitology, 146, 117–122.

Vasta, V., & Luciano, G., (2011). The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Ruminant Research, 101, 150-159.

Young, O.A., Lane, G.A., Priolo A. & Fraser K., (2001), Pastoral and species flavour in lambs raised on pasture, lucerne or maize. Journal of the Science of Food and Agriculture, 83, 93-104.

Table 1. Means and statistical effects of treatments on animal performance and pH

	Treatment			SEM[4]	P-value
	SI[1]	AF[2]	AS[3]		
Number of lambs	18	19	17		
Cold carcass weight (kg)	22.80B	22.34B	24.60A	0.251	<0.0001
Fat thickness (mm)	3.70	3.41	3.71	0.113	0.451
Perirenal fat weight (g)	346	357	387	15.3	0.544
Testes weight (g/kg body weight)	9.51	10.55	11.26	0.310	0.063
Subcutaneous fat firmness	6.9	7.2	7.8	0.23	0.297
Longissimus thoracis et lumborum pHu	5.6	5.6	5.6	0.01	0.471

[1]Stall-fed concentrate and straw indoors; [2]pasture-fed alfalfa; [3]pasture-fed alfalfa with inclusion of sainfoin pellet. [4]SEM: Standard error of mean.

A, B: different superscript letters represent significant differences between treatments ($P \leq 0.001$).

Table 2. Effect of the treatment on skatole and indole concentration (µg/g of liquid fat) in dorsal and perirenal adipose tissues

	AF[1]		AS[2]		SEM [3]	P-value		
	Dor[4]	Per[5]	Dor[4]	Per[5]		Treatment	Tissue	Interaction
Skatole concentration	0.23	0.14	0.17	0.09	0.012	0.013	<0.0001	0.4679
Indole concentration	0.08	0.12	0.05	0.07	0.004	<0.0001	<0.0001	0.4038

[1]Pasture-fed alfalfa; [2]pasture-fed alfalfa with sainfoin pellet supplementation. [3]SEM: Standard error of the mean. [4]Dorsal adipose. [5]Perirenal adipose.

Table 3. Means and statistical effects of treatments on colour measured at 24h *post-mortem* on subcutaneous caudal fat

	Treatments				P-*value*
	SI[1]	AF[2]	AS[3]	SEM[4]	
Lightness (*L**)	64.76b	68.44a	65.91b	0.441	0.001
Redness (*a**)	3.33	3.18	3.35	0.152	0.889
Yellowness (*b**)	13.47	14.01	14.01	0.312	0.723
Chroma (*c*)	13.91	14.44	14.43	0.312	0.748
Hue angle (*h*)	76.27	77.15	76.81	0.590	0.837

[1]Stall-fed concentrate and straw indoors; [2]pasture-fed alfalfa; [3]pasture-fed alfalfa with inclusion of sainfoin pellet. [4]SEM: Standard error of mean.
a, b: different superscript letters represent significant differences between treatments ($P \leq 0.05$).

Table 4. Statistical effects treatment and measurement time and their interaction on *Longissimus thoracis et lumborum* muscle colour

	P-value		
	Treatment	Measurement time	Interaction
Lightness (*L**)	<0.0001	<0.0001	0.4679
Redness (*a**)	0.3274	<0.0001	0.4038
Yellowness (*b**)	0.2057	<0.0001	0.6940
Chroma (*C**)	0.3532	<0.0001	0.6971
Hue angle (*h*)	0.0791	<0.0001	0.7604

Table 5. Means and statistical effects of treatments on chop odour, flavour and acceptability in lambs

Item	Treatments			SEM[4]	P-*value*
	SI[1]	AF[2]	AS[3]		
Fat part					
Animal odour	1.24B	1.65A	1.71A	0.054	<0.0001
Normal odour	3.44b	3.53ab	3.69a	0.064	0.012
Abnormal odour	0.93	0.90	0.80	0.039	0.167
Odour liking	3.40	3.35	3.55	0.068	0.214
Animal flavour	1.49b	1.74a	1.84a	0.054	0.002
Sheepy flavour	3.59B	3.64B	3.94A	0.056	<0.0001
Rancid flavour	1.04	1.01	1.01	0.039	0.869
Livery flavour	0.60	0.56	0.57	0.020	0.617
Grassy flavour	2.21	2.26	2.32	0.057	0.675
Flavour liking	3.18β	3.21 αβ	3.44α	0.069	0.096
Lean part					
Animal odour	1.27B	1.55A	1.60A	0.051	0.000
Normal odour	3.62B	3.83A	3.94A	0.061	0.000
Abnormal odour	1.00a	0.87ab	0.82b	0.039	0.021
Tenderness	3.93b	4.30a	4.17ab	0.064	0.005
Juiciness	2.65b	2.94a	2.86ab	0.053	0.006
Chop overall liking	3.22β	3.46αβ	3.49α	0.068	0.060

[1]Stall-fed concentrate and straw indoors; [2]pasture-fed alfalfa; [3]pasture-fed alfalfa with inclusion of sainfoin pellet. [4]SEM: Standard error of mean.
a, b: different superscript letters represent significant differences between treatments ($P \leq 0.05$).
A, B: different superscript letters represent significant differences between treatments ($P \leq 0.001$).
α, β: different superscript letters represent significant differences between treatments ($P \leq 0.10$).