UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS AGRÁRIAS

PROGRAMA DE PÓS GRADUAÇÃO EM ZOOTECNIA

DIETAS SUPLEMENTADAS COM ÓLEOS ESSENCIAIS DE CRAVO E ALECRIM E ÓLEOS PROTEGIDOS (EUGENOL, TIMOL E VANILINA) SOBRE A QUALIDADE DA CARNE DE NOVILHAS TERMINADA EM CONFINAMENTO

Autor: Jéssica de Oliveira Monteschio

Orientador: Prof º Dr. Ivanor Nunes de Prado

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Estado do Paraná

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BIOGRAFIA

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Em Março de 2007 iniciou o curso de graduação em Zootecnia pela Universidade Federal da Grande Dourados- UFGD (Dourados, Mato Grosso do Sul, Brasil). Em dezembro de 2011 cumpriu as exigências para obtenção do título de Zootecnista.

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DIETAS SUPLEMENTADAS COM ÓLEOS ESSENCIAIS DE CRAVO E ALECRIM E ÓLEOS PROTEGIDOS (EUGENOL, TIMOL E VANILINA) SOBRE A QUALIDADE DA CARNE DE NOVILHAS TERMINADA EM CONFINAMENTO

RESUMO

Quarenta animais (novilhas Nelore) foram alimentados por setenta e três dias com um dos seguintes tratamentos: CON (sem adição de óleos essenciais); BLE - Mistura protegida de eugenol, timol e vanilina (4 g / animal / dia); ROS - Óleo essencial de alecrim (4 g / animal / dia); BCL - Mistura protegida - eugenol, timol e vanilina (2 g / animal / dia) + óleo essencial de cravo (2 g / animal / dia) e BRC - mistura protegida - eugenol, timol e vanilina (1:33 g / animal / dia)) + Óleo essencial de alecrim (1:33 g / animal / dia) + óleo essencial de cravo (1:33 g / animal / dia). O pH (24 horas), a temperatura, a perda de gotas, as perdas descongelantes, a espessura da gordura, a área do bife e o marmoreio foram avaliados no Longissimus dorsi. Além disso, avaliamos os efeitos do envelhecimento (um, sete e 14 dias) sobre a perda de carne, textura, cor da carne, oxidação de lipídios e antioxidante. Os óleos essenciais na dieta das novilhas não tiveram efeito (P> 0,05) sobre o pH da carne, temperatura, perda de gotas e descongelação, marmoreio, área do bife e espessura da gordura, mas tratamento com óleos essenciais juntamente com a mistura protegida tiveram a menor perda de cozimento, suavidade e intensidade da carne vermelha. A adição de óleos essenciais diet reduziu a oxidação lipídica na carne durante a maturação e resultou em maiores valores de oxidação lipídica após 14 dias de envelhecimento. O óleo essencial apresentou resultados satisfatórios independentemente de ser administrado sozinho ou com a mistura protegida. As dietas com óleo essencial e uma mistura de princípio ativo mostraram uma melhor aceitação pelo consumidor e o 7º dia de maturação recebeu pontuações mais altas (P <0,05). Além disso, o óleo essencial e os princípios ativos ou sua mistura apresentaram as maiores pontuações para a avaliação visual da carne, e as análises em exibição mostraram resultados semelhantes aos de fotos aleatórias.

Palavras- chave: Qualidade da carne, Óleo essencial, Aceitabilidade, Fêmeas.

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Abstract

Forty animals (Heifers Nellore) were fed for seventy-three days with one of the following treatments: CON (without addition of essential oils); BLE - Protected blend of eugenol, thymol and vanillin (4 g / animal / day); ROS - Rosemary essential oil (4 g / animal / day); BCL - Protected blend - eugenol, thymol and vanillin (2 g / animal / day) + clove essential oil (2 g / animal / day) and BRC - Protected blend - eugenol, thymol and vanillin (1:33 g / animal / day) + rosemary essential oil (1:33 g / animal / day) + clove essential oil (1:33 g / animal / day). The pH (24 hours), temperature, drip loss, losses thawing, fat thickness, ribeye area and marbling were assessed in the *Longissimus dorsi*. In addition, we evaluated the effects of aging (one, seven, and 14 days) on the cooking loss of meat, texture, meat color, oxidation of lipids and antioxidant. The essential oils in the diet of Heifers had no effect (P> 0.05) on the pH of the meat, temperature, drip loss and thawing, marbling, ribeye area and fat thickness, but treatment with essential oils along with the protected mixture (BRC) had the lowest cooking loss, softness and intensity of red meat. The addition of essential oils diet decreased lipid oxidation in meat during maturation and resulted in higher values for lipid oxidation after 14 days of aging. The essential oil showed satisfactory results regardless of whether administered alone or with the protected mixture. The diets with essential oil and an active principle blend showed a better acceptance by consumer and the 7^{th} aging day received higher scores (P <0.05). Also, the essential oil and the active principles or their mixture showed the highest scores for the meat visual evaluation, and the analyses in display (tray) showed similar results to random photos.

Keywords: Meat quality, Essential Oil, Aceptability. Heifers.

1. Introdução

O número de animais provenientes de confinamento vem aumentando consistentemente, mostrando sua presença ativa na pecuária brasileira. A demanda mundial por alimentos e mercados globalidades e de alta competitividades, fazem da pratica de confinamento uma necessidade para a intensificação do sistema de produção, permitindo abate de animais mais jovens, melhor conversão alimentar, podendo esperar melhorias na qualidade da carcaça e da carne. Fatos como estes fortalecem a utilização do confinamento como ferramenta estratégica no sistema na produção de bovinos e mostra a necessidade de conhecer e explorar cada vez mais este recurso de maneira tecnificada e economicamente eficiente (Prado et al., 2008; Silva et al., 2014).

O uso de aditivos alimentares já é uma pratica comum na pecuária brasileira e na bovinocultura ganha um novo apelo com o surgimento de mercados que visam o bem estar animal e a segurança alimentar. Com base no princípio da precaução, a União Européia proibiu o uso de aditivos alimentares antibióticos como promotores de crescimento para bovinos, sob a hipótese de surgimento de microrganismos patogênicos, para o ser humano, resistente a tratamento convencional com antibióticos incentivando as pesquisas em busca de alternativas para a alimentação animal (Barton, 2000; Verbeke et al., 2010).

Importantes estudos avaliam o uso de compostos secundários de plantas como aditivos na alimentação animal. Comumente referidos como óleos essenciais, estas substancias, ou conjunto delas vem adquirindo relevância dentro do mercado de produtos comerciais disponíveis para uso. Os óleos essenciais são componentes secundários dos organismos vegetais obtidos a partir de partes de plantas (flores, brotos, sementes, folhas, galhos, cascas, ervas, madeira, frutas e raízes) e podem ser extraídos mediante destilação a vapor ou extração com solventes (Greathead, 2003; Patra & Saxena, 2010; Valero et al., 2014). Extrato de plantas vem sendo explorados por suas propriedades aromáticas, antisséptica e conservante. A ação antioxidante, anti-inflamatória e antimicrobiana observada em grande numero de substancias extraídas de plantas confere a estes compostos um potencial importante como aditivo alternativo para uso na nutrição animal de bovinos (Benchaar et al., 2008; Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007) além de melhorar a eficiência alimentar, produtividade animal e efeitos moduladores digestivas sobre o metabolismo ruminal (Benchaar et al., 2008).

Foi demonstrado que existe efeito usando óleos essenciais de plantas diferentes, sendo que cada planta tem componentes ativos específicos que determinam as características do seu extrato. Ao usar uma mistura, os óleos essenciais podem ter um efeito sinérgico, influenciando seu modo de ação no metabolismo dos animais e afetar a qualidade da carne (Rivaroli et al., 2016).

O alecrim (*Rosmarinus officinalis*) possui propriedades e características organolépticas desejadas pelo consumidor e foi usado durante muitos anos como um aditivo aromatizante de alimentos e uma erva medicinal. Várias pesquisas têm sido realizadas e tem-se observado que o alecrim possui uma alta atividade antioxidante. Vários compostos fenólicos têm sido isolados do alecrim, como por exemplo o carnosol, rosmano, rosmaridifenol e rosmariquinona (Assis et al., 2009). Várias pesquisas têm sido realizadas e tem-se observado que o alecrim possui uma alta atividade antioxidante na carne. (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2003). O óleo essencial de cravo (Syzygium aromaticum) é de muita atenção devido ao seu alto conteúdo e amplo espectro de compostos fenólicos, propriedades antimicrobianas e antioxidantes, e potencial para uso em carnes e produtos derivados (Barbosa et al., 2009; Bensid, Ucar, Bendeddouche, & Özogul, 2014; Gutierrez, Barry-Ryan, & Bourke, 2008, 2009; Jayasena & Jo, 2013). O uso de OE é relatado na literatura para melhorar a vida de prateleira de carne (Jayasena & Jo, 2013; Lucera, Costa, Conte, & Del Nobile, 2012).

1.1. Óleo essencial na alimentação de ruminantes

Por causa da globalização, as exigências dos mercados consumidores ampliaram-se, visando à saúde humana em razão do risco de intoxicação e da possível resistência das bactérias, o uso de ionóforos é considerado um risco crescente para a saúde humana, e os mercados consumidores têm se mostrado intolerantes aos produtos originados de animais alimentados com esse tipo de aditivo. Os órgãos oficiais e associações de consumidores da União Européia são a favor da proibição do uso desses ionóforos, por questão de prevenção (Martinele, Eifert, Lana, Arcuri, & D'Agosto, 2008). Por este motivo, os compostos naturais alternativos vêem de encontro às necessidades do mercado nacional e especialmente internacional como uma alternativa para substituir os aditivos antibióticos na produção animal.

Os óleos essenciais podem modificar os mecanismos de digestão no rúmen, alterando a fermentação ruminal, melhorando a eficiência energética, reduzindo a produção de CH_4, que representa uma das principais perdas de energia ao animal (Benchaar et al., 2008). Ainda, os óleos essenciais podem contribuir para a redução do efeito estufa, melhoram a relação da proporção de acetato:proprionato e diminuem a incidência de desordens ruminais, pois podem diminuir a produção de acido lático (Hart, Yáñez-Ruiz, Duval, McEwan, & Newbold, 2008).

Devido ao grande numero de compostos existentes nos óleos, o modo de ação não é atribuído a um mecanismo específico levando a uma importante característica dos óleos que seria o seu poder hidrofílico, que lhes permite atravessar a membrana da célula bacteriana e da mitocôndria, mudando a estrutura e favorecendo a troca de íons de dentro da célula (Hart et al., 2008; Newbold & Rode, 2006). Na tentativa de manter o equilíbrio osmótico, a célula ativa a bomba iônica, um mecanismo que regula o balanço químico entre o meio interno e externo, com grande perda de energia; assim a célula não consegue ser eficiente e ocorre um desequilibro celular. Com o aumento da pressão osmótica, a água entra em excesso e a membrana celular é rompida, ocasionando a morte da bactéria.

Os compostos antioxidantes mais utilizados na indústria são polifenóis de origem sintética, com destaque para: butil-hidroxi-anisol (BHA), butil-hidroxi-tolueno (BHT), terc-butil-hidroxiquinona (TBHQ) e propil galato (PG) (Ramalho & Jorge, 2006), mas são suspeitos por representarem risco à saúde humana, assim os óleos essenciais seriam uma possibilidade de serem utilizados como uma alternativa por apresentarem ações antioxidantes, serem substâncias naturais e principalmente comestíveis.

2. Caracterização de óleos essenciais

2.1. Definição e características

A International Standard Organization (ISO) define que os óleos essenciais são produtos obtidos a partir da matéria prima natural de origem vegetal, por meio de destilação por arraste a vapor d’água, ou por processos mecânicos, no caso do pericarpo de frutos cítricos. Os óleos essenciais podem se apresentar isoladamente ou misturados entre si, retificados ou concentrados.

Os óleos essências são formados a partir de vias metabólicas secundarias e podem ser definidos como misturas complexas de substancias voláteis, lipofílicas, geralmente odoriferas e liquidas (Sousa et al., 2007).

Segundo (Burt, 2004) os óleos essenciais são representados por complexas misturas de substâncias voláteis, de forma geral lipofílicas, cujos componentes incluem uma série de hidrocarbonetos terpênicos, ésteres, ácidos orgânicos, aldeídos, cetonas, fenóis, entre outros, em diferentes concentrações, nos quais um composto farmacologicamente ativo é majoritário. Sendo bem conhecidos os efeitos antibacterianos, antiparasitários e, mais recentemente, antioxidantes de substâncias bioativas, originárias de extratos de plantas, com excelente efeito na dieta dos animais.

Eles são normalmente obtidos mediante destilação por arraste com vapor d'água ou extração por solventes, sendo que os óleos voláteis obtidos de diferentes órgãos de uma mesma planta podem apresentar composição química, caracteres físico-químicos e odores bem distintos (Dorman & Deans, 2000).

A composição do óleo essencial de uma planta é determinada geneticamente, mas as condições ambientais como a influência do ciclo vegetativo, em que a concentração de cada um dos constituintes do óleo pode variar durante o desenvolvimento do vegetal são capazes de causar variações perceptíveis, assim como o ambiente no qual o vegetal se desenvolve e o tipo de cultivo também influem sobre a composição química dos óleos essenciais (Williams, Stockley, Yan, & Home, 1998).

Geralmente os componentes majoritários determinam as propriedades biológicas dos óleos essenciais. Os componentes abrangem dois grupos de origem biossintética distinta (Betts, 2001; Pichersky, Noel, & Dudareva, 2006). O principal grupo é composto de terpenos e terpenóides e outro de constituintes aromáticos e alifáticos, todos caracterizados por baixo peso molecular. Os derivados terpênicos, especialmente os monoterpenos e os sesquiterpenos são os principais constituintes dos óleos essenciais, sendo às vezes inclusive referidos como sinônimos destes sendo descritos como componentes de óleos essenciais mais de 150 monoterpenos e 1.000 sesquiterpenos, de um total de mais de 22.000 compostos terpênicos identificados (Rice-Evans, Miller, & Paganga, 1997).

2.1.1. Modo de ação

O mecanismo de ação pelo qual a maioria dos óleos essenciais exerce seu efeito antibacteriano envolve a parede celular bacteriana, onde os óleos essenciais desnaturam e coagulam proteínas. Mais especificamente, eles atuam alterando a permeabilidade da membrana citoplasmática aos íons hidrogênio (H+) e potássio (K+). A alteração dos gradientes de íons conduz ao comprometimento dos processos vitais da célula como transporte de elétrons, translocação de proteínas, processo de fosforilação e outras reações dependentes de enzimas, resultando em perda do controle quimiosmótico da célula afetada e, consequentemente, na morte do microrganismo (Dorman & Deans, 2000), ainda segundo os autores a maioria dos óleos essenciais exerçam suas atividades, interagindo com os processos associados à membrana celular bacteriana, incluindo o transporte de elétrons, os gradientes de íons, a translocação da proteína e outras reações enzimodependentes. Por serem lipofílicos, possuem a capacidade de interagir com lipídios da membrana celular e das mitocôndrias bacterianas, alterando sua estrutura, tornando-as mais fluidas e permeáveis, permitindo o extravasamento de íons e outros conteúdos citoplasmáticos (Carson, Mee, & Riley, 2002; Lambert, Skandamis, Coote, & Nychas, 2001).

Nas bactérias Gram-positivas, os óleos essenciais podem interagir diretamente com a membrana celular, entretanto, a parede celular externa que envolve a membrana celular das bactérias Gram-negativas é hidrofílica e impede a entrada de substâncias hidrofóbicas como os óleos essenciais (Smith, Stewart, & Fyfe, 1998).

2.2. Alecrim (*Rosmarinus officinalis*)

O óleo essencial de alecrim é retirado das folhas e flores, sendo um condimento muito utilizado como flavorizante em carnes (Zhang, Kong, Xiong, & Sun, 2009) e vem sendo bastante estudado devido a sua atividade antimicrobiana e antioxidante. (Bozin, Mimica-Dukic, Samojlik, & Jovin, 2007; Genena, Hense, Smânia Junior, & Souza, 2008).

O alecrim pode ser usado fresco, seco ou como óleo essencial e seu óleo essencial são constituídos por hidrocarbonetos monoterpênicos, ésteres terpênicos, linalol, verbinol, terpineol, 3- octanona e acetato de isobornila. Os terpenóides são representados pelo carnosol, ácidos carnosílico, oleânico, ursólico, entre outros. Os flavonóides incluem diosmetina, diosmina, gencuanina, luteolina, hispidulina e apigenina. Apresenta ainda os ácidos rosmarínico, caféico, clorogênico, neoclorogênico e labiático (Assis et al., 2009), apresentando em sua composição compostos com atividade inibitória sobre as bactérias, tanto as Gram-negativas quanto as Gram-postivas (Baratta, Dorman, Deans, Biondi, & Ruberto, 1998)

De maneira geral, bactérias Gram positivas são mais sensíveis a compostos bioativos que Gram negativas, mostrando o gênero *Pseudomonas* um dos menos sensíveis e para outros autores, o óleo essencial de alecrim apresenta menor eficiência sobre as Gram negativas, possivelmente devido ao fato dos componentes da membrana externa que envolvem a célula Gram negativa restringir a passagem de compostos de características hidrofóbicas. (Ceylan & Fung, 2004; Pintore et al., 2002; Trajano, Lima, Souza, & Travassos, 2009)

2.3. Tomilho (*Thymus vulgaris*)

O tomilho apresenta de 2 a 5% de sua massa seca como óleo essencial. Esse óleo é constituído de timol (40%), carvacrol, 1-8 cineol, borneol, linalol e tanino (Pensel et al., 2014). A atividade biológica do óleo essencial do tomilho tem sido relatada com maior ênfase em relação aos constituintes timol e carvacrol. O timol tem mostrado efeito antibiótico, antifúngico e anti-helmíntico (Chemat, Cherfouh, Meklati, & Belanteur, 2012).

O óleo essencial de tomilho possui atividades antimicrobianas (bactérias e fungos) carminativa e expectorante, atividades estas atribuídas ao timol e ao carvacrol, componentes fenólicos do óleo, sendo o timol o mais potente. As atividades antifúngicas, pesticidas e antibacterianas do óleo essencial de tomilho foram demonstradas por diversos investigadores como (Bagamboula, Uyttendaele, & Debevere, 2004; Daferera, Ziogas, & Polissiou, 2000). Atividades espasmolíticas bem como antioxidantes foram relatadas também para o extrato alcoólico da planta (Hudaib, Speroni, Di Pietra, & Cavrini, 2002). De acordo com os estudos de (Lee, Umano, Shibamoto, & Lee, 2005), os principais compostos de aroma encontrados em extratos voláteis de manjericão e tomilho exibiram diferentes potenciais antioxidante. Os resultados encontrados demostraram que o manjericão e tomilho, possuíam atividade antioxidante potente, comparável à do conhecido antioxidantes, butil—hidroxitolueno (BHT) e α-tocoferol. Em um estudo, (Teissedre & Waterhouse, 2000) relataram que o óleo essencial de tomilho mostrou moderado inibição da oxidação de LDL (20-27%).

2.4. Cravo da índia (*Eugenia caryophyllus*)

Dentre os inúmeros óleos essenciais, tem-se o cravo que é uma planta usada como tempero desde a antiguidade. Sua espécie tem sido explorada principalmente para extração industrial do óleo essencial obtido a partir dos botões florais, folhas e outras partes (Costa et al., 2011). Possui em sua planta uma grande quantidade de óleo essencial, contendo o eugenol, responsável pela sua atividade antibacteriana e antifúngica e antioxidante tornando-se importante nas pesquisas de nutrição animal (Pereira et al., 2008; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2010).

(Trajano, Lima, Souza, & Travassos, 2010) identificaram os compostos químicos do óleo essencial extraído das folhas de E. caryophyllata obtendo em sua pesquisa 18 compostos do óleo. Dentre eles o eugenol (74%) foi o composto majoritário, seguido pelos α-humuleno (9,62%), d-cadineno (4,64%), trans-β-cariofileno (4,69%) e óxido de cariofileno (1,63%). Outros compostos, como eucaliptol (0,96%), γ-cadineno (0,86%), humuleno (0,83%), e torreiol (0,62%) foram encontrados em percentual menor.

Segundo (Chaieb et al., 2007), o óleo essencial de S. aromaticum tem potente ação antioxidante, devido à eliminação de radicais, à ação quelante com íons de metais (Fe+3) e à participação em reações fotoquímicas, com aplicabilidade estratégica na indústria, por exemplo.

(Viuda-Martos et al., 2010) testaram a atividade antioxante dos óleos essenciais de orégano (Origanumvulgare), tomilho (Thymusvulgaris), alecrim (Rosmarinusofficinalis), sálvia (Salviaofficinalis) e cravo (Syzygiumaromaticum), e observaram que o óleo de cravo obteve melhores resultados de ação antioxidante, contendo maiores quantidade de fenólicos totais.

2.5. Baunilha (*Vanilla planifolia*)

A vanilina (4-hidroxi-3-metoxibenzaldeído) é um dos compostos aromáticos mais apreciados no mundo e um importante flavorizante para alimentos, bebidas e é usada também em produtos farmacêuticos. Ela possui vários efeitos como prevenção de doenças, antimutagênico, antioxidante, conservante e antimicrobiano (Cerrutti & Alzamora, 1996; Shaughnessy, Setzer, & DeMarini, 2001).

O aroma de baunilha, ou seja, a vanilina, é obtida da planta Vanilla planifolia na forma de gluco-vanilina, na proporção de 2% em peso. A fonte natural da gluco-vanilina (a vagem da baunilha) pode fornecer apenas 20 t métricas das 12000 t métricas consumidas anualmente (cerca de 0,2%) (Daugsch & Pastore, 2005).

A vanilina possui propriedades antimicrobianas e antioxidantes. O fato de o fenol e seus derivados possuírem ação antimicrobiana é conhecido devido ao modo de ação onde o fenol e seus derivados lesam as células microbianas pela alteração da permeabilidade seletiva da membrana citoplasmática, causando uma perda das substâncias intracelulares vitais. Esses compostos também desnaturam proteínas como as enzimas (Lampman et al., 1977). As propriedades antioxidantes da vanilina devem-se ao fato de esta interagir com espécies radicalares, evitando, dessa forma, os processos oxidativos (Angelo & Jorge, 2007). Tais características justificam o amplo uso da vanilina em alimentos na condição de conservantes. Ainda assim, o efeito antimicrobiano da vanilina bem como de vários outros extratos vegetais ainda não é totalmente compreendido (Antoniolli, Benedetti, Souza Filho, & Borges, 2004). Além disso, algumas pesquisas ilustram o seu potencial antimutagênico (Maurya, Adhikari, Nair, & Devasagayam, 2007).

3. Consumidores e qualidade da carne

A carne, seja ela bovina, ovina, suína, de aves ou de pescado, deve corresponder às expectativas do consumidor no que se refere aos atributos de qualidade sanitária, nutritiva e organoléptica, além, obviamente, de ter preço criteriosamente estabelecido pelo justo valor (Felicio 2006). Os consumidores estão cada vez mais preocupados com questões que envolvam riscos, tanto ao uso de aditivos na alimentação quanto à contaminação de produtos alimentícios, principalmente em relação ao aumento de renda e a exigência de um produto final de qualidade dentro da cadeia de carne para comercialização (Font-i-Furnols & Guerrero, 2014, Fernqvist & Ekelund, 2014).

A primeira percepção de qualidade do produto que o consumidor tem é pela avaliação sensorial, da qual fazem parte os fatores de aparência como cor, aroma, posteriormente textura e sabor (Behrens et al., 2010). Atualmente, com a expansão do mercado de carne, os atributos sensoriais não são suficientes, assim outros conceitos de qualidade estão surgindo, como os atributos tecnológicos (pH, capacidade retenção de água, oxidação lipídica, oxidação proteica), os atributos ligados ao nutricional (teor de lipídeos, proteína, perfil de ácidos graxos) e aqueles sanitários.

A palatabilidade da carne deriva de uma complexa interação sensorial e física durante o processo de mastigação. Entre as várias características subjetivas que determinam a palatabilidade, a maciez é a mais relevante (Caine et al., 2003). Para o consumidor, a maciez é o atributo sensorial mais importante para julgar a qualidade da carne, juntamente com a cor que seria a primeira característica que o consumidor observa para comprar a carne, relacionando o vermelho brilhante à carne fresca. A suculência e a maciez estão intimamente relacionadas, a carne mais macia libera rapidamente os sucos presentes que aumentam a salivação e conseqüentemente, a sensação de suculência (Cross, 1994) (Monin & Ouali, 1991; Cross, 1994; Jorge, 2001; Vestergaard et al., 2000; Koohmaraie, 2003; Roça, 1997) sendo características importantes e que determinam as características de qualidades desejáveis pelo consumidor.

4. Objetivo geral

O objetivo deste estudo foi avaliar o efeito da adição de óleos essenciais (alecrim, cravo e um composto a base de timol, vanilina e seus mix) sobre a qualidade da carne: cor, perda por cocção, gotejamento e descongelamento, textura, oxidação lipídica e capacidade antioxidantes com distintos tempos de maturação (1, 7 e 14 dias) da carne de Novilhas Nelore terminadas em confinamento.

Objetivou-se avaliar os atributos sensoriais (odor, sabor, maciez e aceitabilidade geral) da carne (um e sete dias de maturação) e avaliação visual da carne de Novilhas Nelore terminadas em confinamento recebendo dieta com adição de óleos essenciais (alecrim, cravo e um composto a base de eugenol, timol, vanilina).

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CAPÍTULO I - Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers

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ABSTRACT

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Forty Nellore heifers were fed (73 days) with different diets: with or without essential oils (clove and/or rosemary essential oil) and/or active principle blend (eugenol, thymol and vanillin). The pH, fat thickness, marbling, muscle area and water losses (thawing and drip) were evaluated 24h *post mortem* on the *Longissimus thoracis,* and the effects of aging (14 days) was evaluated on the meat cooking losses, color, texture and lipid oxidation. Antioxidant activity was also evaluated. Treatments had no effect (P > 0.05) on pH, fat thickness, marbling, muscle area, thawing and drip losses. However, treatments affected (P < 0.05) cooking losses, color, texture and lipid oxidation. The diets with essential oil and the active principle blend reduced the lipid oxidation and reduced the color losses in relation to control diet. Aging affected (P < 0.05) texture and lipid oxidation. The essential oil and active principles or its blend have potential use in animal feed aiming to maintain/improve meat quality during shelf-life.

Keywords:

Aging time

Antioxidant activity

Beef

Lipid oxidation

Meat quality

1. Introduction

The addition of antibiotics in livestock production systems is a common practice to prevent diseases and metabolic disorders and to improve feed efficiency, particularly when animals are intensively reared (Goodrich et al., 1984; Valero et al., 2014; Zawadzki et al., 2011). However, due to antibiotic resistance and possible risks to human health (residues in the final products) (Russell & Houlihan, 2003), their use has been prohibited in some regions of the world. Consequently, researches are increasingly focused on natural alternatives, which are well accepted by consumers (Chaves et al., 2011; Chaves, Stanford, Dugan, et al., 2008). Thus, alternative solutions to improve cattle performance and improve the food shelf life are required to replace the antibiotics (Moyo, Masika, & Muchenje, 2014; Prado et al., 2016; Valero et al., 2014a; Valero et al., 2014b). Each plant has specific active components that determine its extract characteristics. Essential oils are aromatic extracts from plant material, such as flowers, buds, seeds, leaves, twigs, barks, wood, fruit and roots (Burt, 2004). They may be obtained by fermentation, extraction or most commonly by steam distillation (Burt, 2004; Gershenzon & Croteau, 1991). Chemically, essential oils are variable mixtures of terpenoids that primarily include monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also be present. They also include a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and N- and S-containing compounds, such as coumarins, and homologs of phenylpropanoids may also be present (Dorman & Deans, 2000). These products may act as antimicrobials (oils of clove, rosemary, thyme and vanillin are some of the most effective due to the presence of phenolic compounds) and antioxidants, benefiting the immune and digestive system of animals, which is reflected in their performance indices (Benchaar et al., 2008; Jayasena & Jo, 2013). Moreover, when a blend is used, essential oils may have a synergistic effect, influencing their mode of action in animal metabolism and affecting beef quality (Prado et al., 2016; Valero et al., 2014b).

Plant species containing thymol and carvacrol show high antioxidant potential due to the presence of phenolic terpenes (Bakkali, Averbeck, Averbeck & Idaomar, 2008). Vanillin, which has a similar structure to eugenol exhibits both antimicrobial and antioxidant properties in soft drink and fruit juice, and table grapes, respectively (Fitzgerald, Stratford, Gasson & Narbad, 2004; Konuk & Korel, 2016)

Rosemary (*Rosmarinus officinalis*) has a higher antioxidant activity than the other essential oils, and several phenolic compounds have been isolated from this oil, such as carnosol, rosmanol, rosmaridiphenol and rosmarquinone (Assis et al., 2009). Moreover, rosemary is reported to have a high antioxidant activity in meat (Djenane, Sánchez-Escalante, Beltrán & Roncalés, 2003).

Clove (*Syzygium aromaticum*) essential oil has received a lot of attention due to its high and diverse content of phenolic compounds, its antimicrobial and antioxidant properties and potential use in meat and derived products (Barbosa et al., 2009; Bensid, Ucar, Bendeddouche & Özogul, 2014; Jayasena & Jo, 2013).

Although some studies have demonstrated that essential oils can influence meat quality (Rivaroli et al., 2016) and prolong the shelf-life of meat (Jayasena & Jo, 2013; Lucera, Costa, Conte & Del Nobile, 2012), as well as studies with other natural compounds (Moyo, et al., 2014; Muthukumar, Naveena, Vaithiyanathan, Sen, & Sureshkumar, 2014). Researches concerning the effects of essential oils on meat quality (used in animal feed) are still limited. Thus, this study aimed to investigate the effects of essential oils (clove and rosemary), their encapsulated active principles (eugenol, thymol and vanillin mixture) and blends thereof on the meat quality of feedlot-finished heifers fed on high-grain diets.

2. Materials and Methods

2.1. Locality, animal and diets

This experiment was approved (no. 3624120116) by the ethical committee of Maringá State University. The study was conducted at Sector Rosa and Pedro, at the Experimental Station, Iguatemi farm, Iguatemi City, Paraná, Brazil. This region (south) has a subtropical climate, with an annual average around 22ºC; and semi-humid (approximately 1590 mm of annual rain). The lowest temperatures are between the months of May to July, while the highest temperatures are from November to March.

In relation to humidity, the city is drier between July to September, while rains are mainly between January, February and March.

Forty purebred Nellore heifers with an initial average weight of 297.6 ± 31.2 kg were randomly assigned to one of five finishing diets (n = 8 per treatment). The heifers were allocated to individual pens, with an adaptation period of 7 days. All animals received the same basal diet (Table 1), which was formulated according to the NRC (2000) and provided *ad libitum*. The heifers were used since in Brazil, part of the beef production (approximately 40%) comes from the slaughter of heifers and cows cull (Annualpec 2015). Moreover, heifers not used for breeding in the herd are destined for fattening and slaughtered at a young age (<24 months) because they produce meat with good quality (Rotta et al., 2009 and Reddy et al., 2015; Rivaroli et al., 2016).

The five experimental diets (based on previous studies) were: CON – without essential oil and active principles (eugenol, thymol and vanillin); ROS – rosemary essential oil (4 g/animal/day); BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); BCL – eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day); and BRC – eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). These concentrations were chosen according to the results of Rivaroli et al. (2016) which showed that a concentration above 3.5 g/animal/day has no effect on animal performance and meat quality. Furthermore, previous studies (Busquet et al., 2006; Benchaar et al., 2008) showed that the most adequate concentrations of the essential oils in the animal diets is between 3 and 5 g/animal /day.

Rosemary and clove essential oils were obtained from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil) and its major compounds were 1,8 cineole and eugenol, respectively (Biondo et al., 2016). The encapsulated blend (eugenol, thymol and vanillin active principles) was obtained from Safeeds® (Cascavel, Paraná, Brazil).

The diets were prepared with a pre-mix containing ground corn. The essential oils were then added to the feed mixer with the other ingredients. As reported by Zulueta, Esteve & Frígola (2009), the oxygen radical absorbance capacity of the essential oils in the diet is retained for up to 30 days of exposure.

The heifers were finished on their respective diets for 73 days until they reached a medium weight of 356.6 ± 32.6 kg. They were then slaughtered at a commercial abattoir, after a solid fasting period of 12 h, in compliance with the slaughter standards of the State Inspection Service legislation in Brazil (Brazil, 2000). The carcasses were then divided medially through the sternum and vertebral column, identified and chilled below 4 ºC for 24 h. The *Longissimus thoracis* (LT) was excised from the left side of the carcass between the 7th and the 13th ribs for subsequent analysis.

2.2. Sampling and meat quality

The LT was sliced into steaks (2.5 cm thick), weighed, vacuum-packed (99% vacuum, Sulpack SVC 620) in polyamide/polyethylene pouches (120 µm; 1 $cm^3/m^2/24$ h O_2 permeability and 3 $cm^3/m^2/24$ h CO_2 permeability, at 5 °C and 75% relative humidity; 3 $g/m^2/24$ h water vapor transmission rate at 38 °C and 100% relative humidity; 97 °C Vicat softening temperature; 1.3 g dart drop strength), and aged for either 24 h, or 7 or 14 days, before being frozen and stored (-20 °C) for 1 month for subsequent analyses.

2.3. pH measurements

At 24 h *post-mortem*, the LT pH was measured using a digital pH meter (Hanna – HI99163, Romania - Europe) with a penetration electrode placed at the point of the 3rd lumbar vertebra. The phMeter was calibrated at 20ºC using standard pH 4.0 and 7.0 buffers (Valero et al., 2014b).

2.4. Fat thickness

The thickness of subcutaneous fat from the 12th rib in the LT muscle was measured by a digital caliper at 24 h *post-mortem* and averaged over three points (Eiras et al., 2014).

2.5. Marbling (MAR)

The MAR was measured in the LT muscle area (LMA) (12th rib) at 24 h *post-mortem* using the Brazilian scoring system (18–16, abundant; 15–13, moderate; 12–10, mean; 9–7, small, 6–4, light: and 3–1, traces), according to Muller (1987).

2.6. *Longissimus thoracis* muscle Area (LMA)

The LMA (cm^2) was measured at 24 h *post-mortem* in the 12th rib by a compensating planimeter. The LT muscle area/100 kg carcass (LMC) is defined as the LMA:HCW (hot carcass weight) ratio, multiplied by 100 (Eiras et al., 2014).

2.7. Thawing, drip and cooking losses

The steaks were thawed at 4 °C for 24 h. They were then weighed and the thawing losses were calculated as the percentage difference between the fresh and thawed weights (Rivaroli et al., 2016).

Drip loss was measured using the method described by A steak of each animal was taken 24 h *post mortem*, placed in a plastic bag, and kept at 4 °C. After 24 h, the sample was removed from the bag, dried on absorbent paper, and reweighed. Amount of drip at 48 h *post mortem* was expressed as a percentage (Honikel, 1998).

$$\% \text{ drip loss} = \left(\frac{initial\ weight - final\ weight}{initial\ weight}\right) * 100$$

For cooking losses, the steaks were weighed and wrapped in aluminum foil. Each sample was cooked in a pre-heated grill (Grill Philco Jumbo Inox, Philco SA, Brazil) at 200 °C until an internal temperature of 72 °C was reached, which was monitored using an internal thermocouple (Incoterm, 145 mm, Incoterm LTDA, Brazil). The sample was then removed from the heat and left at ambient temperature to cool. Once the steaks reached 25 °C, each steak was weighed and the cooking losses calculated as the percentage difference in weight before and after cooking.

2.8. Instrumental meat color

The color was evaluated after 30 min of exposure to oxygen at 1, 7 and 14 days of display, by using the CIELab system with a Minolta CR-400 Chroma meter (Japan) (with a 10° view angle, D65 illuminant, 8 mm of aperture with a close cone). Six measurements at randomly selected points were recorded per sample, obtaining lightness (L*), redness (a*) and yellowness (b*). Chroma and hue values were calculated as follows:

Chroma= ($\sqrt{a*^2 + b*^2}$) and hue angle (h^o) = ($\arctan\left(b*/a*\right)$).

2.9. Texture measurement

The texture of the previously cooked steaks was analyzed using a Stable Micro Systems TA.XTplus (Texture Technologies Corp., Serial Number 41288, Godalming, Surrey, UK) texture analyzer with a Warner-Bratzler blade, according to Honikel (1998). The meat was cut into rectangular pieces of 1 cm^2 cross-section (eight pieces per animal), which were cut perpendicular to the direction of the muscle fibers.

2.10. Antioxidant capacity

2.10.1 Meat bioactive compounds extract

Meat extracts (1:1 w/v with methanol), were obtained according to Vital et al., 2016 using an Ultra-Turrax homogenizer (IKA® - T10, USA), followed by centrifugation (4.000 rpm, 15 min) and filtration (filter paper (grammage – 80 g/m^2, thickness - 205 µm, pores – 14 µm). Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays, using meat extracts.

2.10.2. Radical scavenging assay (DPPH)

The DPPH radical scavenging activity was measured according to Li, Hydamaka, Lowry, & Beta (2009), with modifications. Meat extract (150 μL) were mixed with 2850 μL of a methanolic solution containing DPPH (60 μM) and reacted for 30 min. The absorbance at 515 nm was measured against pure methanol. Antioxidant activity was calculated as DPPH radical scavenging activity (%) = (1- ($A_{sample\ t=0}$/$A_{sample\ t}$)*100, where: $A_{sample\ t\ =\ 0}$ is the absorbance of the sample at time zero, and $A_{sample\ t}$ is the absorbance of the sample at 30 min.

2.10.3. Radical scavenging assay (ABTS)

The ABTS assay was conducted according to Re et al., 1999, with modifications. $ABTS^+$ was generated by the interaction of 7 mM ABTS (5 mL) with 140 mM potassium persulfate (88 μL). The mixture was incubated in the dark at 25 °C for 16 h. The ABTS-activated radical was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The radical scavenging activity (%) was also measured at 734 nm. Meat extract (40 μL) were mixed with $ABTS^+$ solution (1960 μL) and the absorbance was recorded at 6 min. The ABTS radical scavenging activity (%) was calculated as 1-($A_{sample\ t=0}$/$A_{sample\ t}$)*100, where: $A_{sample\ t\ =\ 0}$ is the absorbance of the sample at time zero, and $A_{sample\ t}$ is the absorbance of the sample at 6 min.

2.10.4. The FRAP assay

The FRAP method was performed according to Zhu, Hackman, Ensunsa, Holt, & Keen, 2002. Meat extract (250 μL) was then mixed with 50 mM sodium phosphate buffer pH 7 (1.25 mL) and 1% potassium ferricyanide (1.25 mL) and incubated at 50 °C for 20 min. Then, 10% trichloroacetic acid (1.25 mL) was added and the mixture was centrifuged at 4,000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 0.1% ferric chloride (500 µL) and the absorbance was measured at 700 nm. Results were expressed as mg gallic acid equivalents (GAE) g^{-1} oil, mg GAE g^{-1} coating and mg GAE 100 g^{-1} meat. Gallic acid (0−300 mg L^{-1}) was used to establish the standard curve.

2.11. Lipid oxidation

The malondialdehyde (MDA) content in meat was quantified using the thiobarbituric acid reactive substances (TBARS) assay as was adapted by Vital et al. (2016).

The sample (5 g) was mixed with trichloroacetic acid (10 mL), homogenized using an Ultra-Turrax, and then centrifuged (4,000 rpm) at 4 ºC for 15 min. The supernatant was filtered and mixed with TBARS reagent (1:1 v/v). The mixture was boiled (100 ºC) for 15 min, cooled, then the absorbance measured at 532 nm against an MDA standard. Results were expressed as mg MDA kg^{-1} of meat. The assay was performed at 1, 7 and 14 days of display.

2.12. Statistical analysis

The experimental design was completely randomized with five treatments (finishing diets) and eight replications per treatment. Meat attributes were assessed by analysis of variance using the general linear model (GLM) with SPSS (v.19.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows. Means and standard error of mean were calculated for each variable.

On the statistical design the finishing diet was considered as fixed effect, on color, shear force and lipid oxidation (TBARS), the effect of aging (1, 7 and 14 days) was also considered as fixed effect and studied the interaction between diet and ageing days. However, there was no interaction effect among diets and ageing days. Thus, data were presented and discussed as the main effect. Differences between group means were assessed by using the Tukey Test ($P < 0.05$).

3. Results and Discussion

3.1. Meat characteristics

3.1.1. The pH, fat thickness, MAR and LMA

At 24 h after slaughter, the pH of the meat samples obtained from the heifers fed the various diets was similar ($P > 0.05$) (Table 2). The mean pH was 5.8, which, although acceptable, was considered high. Under normal conditions, pH 5.5–5.6 is anticipated (Page, Wulf, & Schwotzer, 2001). The relatively high pH obtained can be explained by the animal breed used in this experiment. In general, Zebu animals present a high pH after slaughter due to its aggressive behavior during handling (Zawadzki et al., 2011). Rivaroli et al. (2016) evaluated the effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots and found that the addition of essential oils to the diets did not affect ($P > 0.05$) the pH(24h), which was below 5.8.

The fat thickness was not influenced ($P > 0.05$) by the essential oils and active principles added to the diets (Table 2). In general, the fat thickness of feedlot-finished heifers is above 5 mm (Andreotti et al., 2015; Marques et al., 2010). According to the cattle marketing practices in Brazil, the fat thickness must be between 3–6 mm. Thus, the fat thickness of 2.5 mm found in this study is considered low. Consequently, this carcass could be penalized by the Brazilian market. Valero et al. (2014b) evaluated the effect of propolis and essential oils additives in the diets of bulls finished in feedlot and found values for fat thickness of 4.35 for control diet, 5.81 for diets with propolis diet and 4.78 for diets with essential oils, and no significant differences was observed. These differences in fat thickness occur due to the termination system, breed, sex, age, among others. In our studies, some of these factors may be determinants for low fat thickness, such as the short time confined, the Nellore breed and because they are young (heifers).

According to Luchiari Filho (2000), intramuscular fat, or MAR, is the last to be deposited in the carcass and the animal may have considerable amounts of internal and subcutaneous fat without a reasonable amount of MAR. The MAR values were low and were not affected ($P > 0.05$) by the diets evaluated (Table 2). Beef with a MAR score above 5 is deemed to be well accepted by consumers (Marques et al., 2006; Rotta et al., 2009). Low MAR is directly related to fat deposition. As observed from the fat thickness results, the fat deposition was low, which was attributed to the breed used in this experiment (Nellore heifers). Zebu animals have low meat MAR as they are less genetically predisposed to fat deposition than other breeds (Campion, Crouse & Dikeman, 1975; Rotta et al., 2009). Although low MAR values were found in this experiment, similar values have been reported by other authors for Nellore heifers (Silva et al., 2014; Sousa et al., 2015). Valero et al. (2014a) found values between 4.60 and 5.90 and no differences were observed.

The LMA, which was measured between the 12th and 13th ribs, was also not affected ($P > 0.05$) by the diet (Table 2).

Although pH, fat thickness, MAR and LMA differences were not observed, the results revealed a positive use for the essential oils and their active components. When a new compound/product is added to animal feed, the effects of the addition must be carefully studied, due to the complex digestion system of cattle. The added ingredient could cause some harm to the animal rather than the intended benefit, thereby influencing the quality of the final product. This study showed that the inclusion of clove and rosemary essential oils, and the encapsulated active principles, did not cause any damage to the meat characteristics (pH, MAR and LMA).

3.1.2. Thawing, drip and cooking losses

The water losses by thawing and dripping were not affected ($P > 0.05$) by the diets (Table 3). Freezing and subsequent thawing have a strong impact on the meat water loss due to the formation of ice crystals, which damage the structural integrity of the cell membrane, allowing water to flow out from the intracellular to extracellular region, generating considerable exudates losses (Lagerstedt, Enfält, Johansson & Lundström, 2008; Leygonie, Britz & Hoffman, 2012). However, in this study, the thawing (around 3%) and drip losses (1.62%) were low, which may be associated with the high pH value. Lactic acid formation and the consequent drop in pH *post-mortem* are responsible for the decreased ability of the meat to retain water. These reactions cause denaturation and loss of solubility of the muscle proteins as the pH approximates their isoelectric point (IP = 5.2–5.3). If the pH is higher than the pI, the proteins have an overall negative charge, which causes repulsion of the filaments, leaving more space for the water molecules (Roça, 2009) and consequently, increasing the water retention capacity.

At 1 and 7 days of aging, the diets had no effect ($P > 0.05$) on the cooking loss (Table 3). However, at 14 days of aging, the beef from heifers supplemented with BRC had a lower cooking loss than the other treatments (Table 3). Although this difference was not significant, the heifers fed a BRC diet had numerically more MAR that could be associated with its relatively lower cooking loss. Intramuscular fat acts as a barrier against muscular juice losses during cooking, increasing water retention by the meat and, consequently, juiciness (Roça, 2000). Another factor linked to low water loss is meat maturation (aging), which slightly increases the water retention capacity due to a small pH increase and a replacement of divalent for monovalent ions in meat Roça (2009). The cooking losses in this study were approximately 25% but within the normal limits. Generally, beef cooking losses range from 20–28% (Prado et al., 2014; Rivaroli et al., 2016). The meat aging (1, 7 or 14 days) had no effect ($P > 0.05$) on the cooking losses (Table 3).

3.1.3. Instrumental color

The lightness (L* value) increased with aging time ($P > 0.05$), in agreement with previous studies (Prado et al., 2015). Although chemical changes in myoglobin do not influence lightness (McKenna et al., 2005), it is affected by changes in protein structure that occur during aging (proteolysis) (Renerre, 2004). The mean L* value observed was around 36.6. Thus, the meat was slightly darker than that considered to be attractive (L* $\approx$ 38) (Page et al., 2001).

Meat color can be influenced by several factors, including the breed and age of the animals. For instance, Zebu and older animals tend to have darker meat due to the changes in myoglobin composition (Mancini & Hunt, 2005). Thus, the L* values found in this experiment could be associated with the breed (Nellore) and slaughter age (30 months).

The L* presented significant difference at day 7 and CON showed the highest value, probably caused by changes in the meat structure related to the highly oxidizing conditions, such as protein conformational changes, which may increase light dispersion (MacDougall, 1982).

The addition of essential oils and active principles in the diets had no effect (P>0.05) on the a* values (redness) at day 1 and 7 of aging. However, at 14 days of aging, the CON a* value was lower (P < 0.05) than the other diets, whose values were not affected by the aging time (P > 0.05) and, thus, demonstrated greater protection and maintenance of red color. Furthermore, the mean a* value of 11 for CON at 14 days of aging was lower than the normal value (Page et al., 2001). This may be explained by the breed (Nellore), age (30 months old) and the high pH value (5.8) observed in this experiment.

Fresh meat generally lightens and becomes less red after a few days. However, the diets with ROS and BCL maintained the lightness of the meat during 7 days of aging (L* value), while BLE and BRC maintained the original lightness even at 14 days of aging. Moreover, the diets with essential oil and active principle inclusion preserved the red color during display. A diet that can influence meat quality, in particular, maintain or intensify the redness, could lead to an extension of color display life (Cardoso et al., 2016). The conversion of oxymyoglobin to metmyoglobin results in meat discoloration and interactions between lipid oxidation and discoloration has been demonstrated (Faustman, Sun, Mancini, & Suman, 2010). Meat that showed less oxidation (those coming from heifers fed with essential oil, active principle and their blends) also has higher color preservation.

The diets and aging period did not change (P > 0.05) the meat b* values (yellowness). The b* values ranged from 9.7–11.4 and were close to the values considered normal for beef (Page et al., 2001).

Meat with low chroma values are considered pale (Cardoso et al., 2016) which may adversely affect consumer choice at purchase time. In this study, CON showed a lower chroma value than BRC at day 14 of aging. In contrast, the h^o of CON displayed the highest increase during storage, while BLE and BCL indicated minimal color deterioration (*P* < 0.05).

3.1.4. Shear force

The diet had no effect (*P* > 0.05) on meat tenderness during 7 days of aging (Table 5). At day one, the mean shear force was around 76.5 N, which is not considered a tender meat (shear force > 49 N is considered firm) (Shackelford, Koohmaraie, Miller, Crouse & Reagan, 1991).

The content of connective tissue, sarcoma length and myofibrilar breakdown are sources of meat tenderness variation (Strydom, Lühl, Kahl, & Hoffman, 2016). Meat tenderness also might be influenced by genetics, slaughter age and stress before slaughter. Animals with Zebu blood (e.g. Nellore animals, as used in this experiment) have less tender meat compared to animals with European blood (Shackelford et al., 1991) due to the calpain-calpastatin complex (the calcium-dependent proteolytic system involved in the *post-mortem* aging process) (Allais et al., 2011; Shackelford et al., 1994). Meat from Zebu animals tends to have higher shear force due to the higher calpastatin concentrations in their muscles, which inhibits calpain activity that is responsible for the degradation of myofibrillar proteins during *rigor mortis,* this proteolysis is the most important process in the establishment of tenderness (Koohmaraie, 1994; Whipple et al., 1990). After 14 days of aging, beef from animals fed the BCR diet showed the lowest shear force (*P* < 0.05). Such tenderness may be associated with the higher amounts of fat present in animals fed this diet and the greater water-holding capacity of the meat during cooking (Tables 2 and 3). Moreover, aging time (Table 5) led to more tender meat ($P < 0.05$).

3.1.5. Antioxidant activity in meat

Three methods (ABTS, DPPH and FRAP) were used to verify if the diet with essential oil and the active principles would have any effect on meat antioxidant activity. All three assays revealed similar results (Table 6).

Compounds with antioxidant activity can be incorporated into the diet, and could be transferred to the muscle, not only to prevent or reduce oxidation in muscle food but also improve meat quality (Falowo, Fayemi, & Muchenje, 2014). Antioxidants are typically added to the feed at moderate levels because high levels of inclusion may lead to adverse effects, like pro-oxidative action (Martin & Appel, 2009).

Generally, among the treatments evaluated, the BLE and BCL diets showed higher antioxidant activity. In contrast, CON showed the lowest antioxidant activity. The higher antioxidant activity may help maintain the meat quality during its shelf-life.

3.1.6. Lipid oxidation (TBARS)

Lipid and protein oxidation is considered one of the main non-microbial factors that affect meat quality deterioration (Falowo et al., 2014). However, the oxidation susceptibility can be influenced by animal species and breed, the muscle type evaluated, as well as the diet provided to the animals (Falowo et al., 2014; Min, Nam, Cordray & Ahn, 2008).

The lipid oxidation of beef, measured by MDA production, was affected by the various diets ($P < 0.05$) and increased with aging time (1, 7 and 14 days) (Table 6). The initial MAL values were comparable among the treatments evaluated ($P > 0.05$), at around 0.25 mg/kg meat on day one. At day 7 of aging, BLE, BCL and BRC presented lower MAL values among the treatments studied. At 14 days of aging, BLE and BCL had the lowest MAL contents, while CON had the highest. This difference may be associated with the antioxidant potential of the meat from animals fed the BLE and BCL diets (Table 6) that delayed its lipid oxidation, in concurrence with similar studies (Botsoglou, Govaris, Ambrosiadis & Fletouris, 2012; Dal Bosco et al., 2014).

An overall, low lipid oxidation was detected in the meats and can be explained by the history of the animals. The feedlot heifers entered confinement at around 30 months of age. Before termination in confinement, the heifers were kept in pastures under tropical conditions. During this time, there is a large accumulation of β-carotene that can protect the meat against lipid oxidation after a short period of confinement (Realini, Duckett, Brito, Dalla Rizza & De Mattos, 2004), such as that implemented in this study (73 days).

4. Conclusion

The inclusion of essential oil, active principles and their blends had no effect on pH, fat thickness, MAR, LMA and in thawing and drip losses but cooking losses were affected at 14 days of aging by the diet containing clove and rosemary essential oil and the active principles (eugenol, thymol and vanillin). In general, the dietary inclusion of these compounds lessened color degradation, increased antioxidant activity and decreased lipid oxidation in the meat. Thus, these compounds have potential use in animal feed to maintain/improve meat quality during its shelf-life.

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Table 1- Composition of ingredients and the diets fed to Nellore heifers (g kg -1of DM).

Ingredients	DM[1]	OM[2]	Ashes	CP[3]	EE[4]	NDF[5]	ADF[6]	TC[7]	NFC[8]	TDN[9]
Corn silage	306	969	30.9	71.1	27.1	424	224	870	446	656
Corn grain	853	984	16.4	96.1	47.1	175	45.8	840	665	858
Soybean meal	850	933	67.0	489	19.0	159	87.8	425	266	810
Yeast	920	954	46.1	331	21.0	26.0	9.22	572	546	
Phosphorus	995	38.0	962							
Mineral salt	986	55.0	945							
CON[10]	710	971	27.3	125	39.1	237	95.4	805	569	797
ROS[11]	701	970	28.1	127	38.6	241	98.8	804	562	793
BLE[12]	725	973	26.2	122	39.9	231	90.4	810	579	804
BCL[13]	720	972	26.7	124	39.6	234	92.7	808	574	801
BRC[14]	724	973	26.2	122	39.9	231	90.6	809	578	803

[1]Dry matter; [2]Organic matter; [3]Crude protein; [4]Ether extract; [5]Neutral detergent fiber; [6]Acid detergent fiber; [7]Total carbohydrates; [8]Non-fiber carbohydrates; [9]Total digestible nutrients; [10]CON – Without essential oil active principles ; [11]ROS – Rosemary essential oil (4 g/animal/day); [12]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [13]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [14]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day).

Table 2- Effect of essential oils and active principles on meat characteristics of Nellore heifers finished in feedlot.

	Diets						
Item	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	P <
pH	5.79	5.82	5.79	5.84	5.84	0.0	0.895
Fat thickness	2.15	2.20	2.90	2.49	2.87	0.1	0.284
Marbling, pts[6]	1.75	2.50	2.50	2.25	3.25	0.2	0.205
LMA[7], cm^2	48.25	47.62	49.57	54.00	54.37	1.2	0.223

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). [6]SEM: Standard error of means. [7]LMA- *Longissimus thoracis* muscle area. number of animals (n=8 per treatment).

Table 3- Effect of essential oils and active principles on meat losses of Nellore heifers finished in feedlot.

	Diets						
Item	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	*P* <
Thawing losses, %	3.22	3.31	2.98	3.29	3.27	0.1	0.824
Drip loss, %	1.62	1.58	1.49	1.58	1.86	0.0	0.192
Cooking losses, %, days							
1	27.27	25.77	22.98	25.88	23.79	0.7	0.247
7	25.22	25.27	23.80	23.17	23.29	0.8	0.795
14	25.40[a]	25.22[a]	22.32[ab]	23.05[ab]	19.28[c]	0.7	0.024
SEM	1.0	0.9	0.7	0.9	0.9		
P <	0.665	0.970	0.921	0.342	0.059		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Means with different lowercase letters in the same line are significantly different ($p < 0.05$). [6]SEM: Standard error of means. number of animals (n=8 per treatment).

Table 4- L*a*b*, chroma and hue values of meat from Nellore heifers finished in feedlot.

Variables	Diets						
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	*P* <
L*, Days							
1	36.25^{B}	35.18^{B}	35.38	34.07^{B}	36.35	0.3	0.184
7	38.46^{Aa}	35.44^{ABb}	36.68^{ab}	35.44^{ABb}	36.90^{ab}	0.3	0.002
14	38.84^{A}	38.41^{A}	37.27	36.85^{A}	37.82	0.3	0.359
SEM	0.5	0.6	0.4	0.4	0.3		
P <	0.044	0.026	0.170	0.006	0.141		
a*, Days							
1	11.41^{A}	11.35	11.60	11.74	11.29	0.2	0.933
7	10.12^{A}B	10.61	11.57	10.96	11.00	0.3	0.480
14	9.79^{B}b	10.40^{ab}	10.47^{ab}	10.98^{a}b	11.65^{a}	0.2	0.020
SEM	0.3	0.2	0.3	0.3	0.2		
P <	0.025	0.270	0.266	0.515	0.445		
b*, Days							
1	10.34	10.18	10.26	9.71	10.34	0.1	0.661
7	10.37	9.97	10.42	10.07	10.51	0.2	0.815
14	10.27	10.41	10.33	10.59	11.36	0.2	0.236
SEM	0.1	0.2	0.2	0.3	0.2		
P <	0.969	0.663	0.946	0.441	0.089		
Chroma, Days							
1	15.55	14.75	15.50	15.25	15.34	0.2	0.843
7	14.67	14.50	15.88	14.93	15.23	0.3	0.616
14	13.90^{b}	14.78^{ab}	14.73^{ab}	15.27^{ab}	16.32^{a}	0.2	0.020
SEM	0.3	0.3	0.4	0.4	0.3		
P <	0.076	0.927	0.456	0.921	0.185		
Hue, Days							
1	42.70^{B}	42.00	41.48^{B}	39.65^{B}	42.55	0.4	0.138
7	46.36^{A}	42.94	43.32^{AB}	42.74^{AB}	43.70	0.5	0.099
14	47.14^{A}	45.23	44.64^{A}	43.83^{A}	44.45	0.4	0.182
SEM	0.7	0.7	0.5	0.6	0.5		
P <	0.007	0.194	0.037	0.013	0.286		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means. number of animals (n=8 per treatment).

Table 5- Effect of essential oils and active principles on meat shear force of Nellore heifers finished in feedlot.

Days	CON[1]	ROS[2]	BLE[2]	BCL[4]	BRC[5]	SEM[6]	*P* <
1	77.35^{A}	80.64^{A}	72.58^{A}	74.58^{A}	77.61^{A}	2.9	0.934
7	57.43^{B}	68.19^{AB}	55.94^{AB}	55.13^{AB}	63.50^{A}	2.1	0.227
14	49.89B^{ab}	56.94^{Ba}	48.85^{Bab}	45.90^{Ba}b	41.19^{Bb}	1.7	0.041
SEM	3.3	3.0	4.1	4.3	3.8		
P <	0.000	0,002	0,051	0.014	0.000		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means. number of animals (n=8 per treatment).

Table 6- Radical scavenging activity (ABTS and DPPH radical scavenging) and ferric reducing power (FRAP) of meat of Nellore heifers finished in feedlot.

	Diets						
Method	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	P <
ABTS (%)	37.81[b]	38.49[b]	44.51[a]	44.27[a]	37.77[b]	0.6	0.001
DPPH (%)	24.91[b]	26.98[ab]	28.64[a]	27.95[a]	27.79[ab]	0.3	0.013
FRAP (mg EAG/g meat)	0.10[b]	0.11B[ab]	0.12[a]	0.12[a]	0.12[a]	0.0	0.001

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. [6]SEM: Standard error of means. number of animals (n=8 per treatment).

Table 7- Effect of essential oils and active principles on lipid oxidation (TBARS) expressed as mg malonaldehyde kg-1 of meat of Nellore heifers finished in feedlot.

	Diets						
Days	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	P <
1	0.27[C]	0.26[C]	0.24[C]	0.24[C]	0.25[C]	0.0	0.146
7	0.52[Ba]	0.47[Bab]	0.39[Bd]	0.41[Bcd]	0.45[Bbc]	0.0	0.001
14	0.70[Aa]	0.60[Ab]	0.51[Ac]	0.51[Ac]	0.59[Ab]	0.0	0.001
SEM[6]	0.0	0.0	0.0	0.0	0.0		
P <	0.001	0.001	0.001	0.001	0.001		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means. number of animals (n=8 per treatment).

CAPÍTULO II - Sensorial and visual acceptability of meat from Nellore heifers finished in feedlot and fed with clove and rosemary essential oil and active principles (eugenol, thymol and vanillin blend)

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ABSTRACT

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The objective this study was evaluates the meat acceptability (visual and sensorial analyses) of Nellore heifers finished in feedlot and fed with a diet with essential oil and active principle blend. Forty Nellore heifers were fed (73 days) with different diets: with or without essential oils (clove and/or rosemary essential oil) and/or active principle blend (eugenol, thymol and vanillin). Treatments and aging time were evaluated for consumer acceptance. The use of photographs to evaluate the meat color was compared to the face-to-face view in commercial expositor (tray) during 11 days. Treatments affected (P> 0.05) consumer acceptability and visual analysis (meat color). The diets with essential oil and an active principle blend showed a better acceptance by consumer and the 7th aging day received higher scores (P <0.05). Also, the essential oil and the active principles or their mixture showed the highest scores for the meat visual evaluation, and the analyses in display (tray) showed similar results to random photos.

Keywords

Consumers

Heifers

Meat quality

Essential Oils

1. Introduction

For intensive system in beef cattle, it is necessary to increase the use of concentrate (Ducatti, Prado, Rotta, Prado, Perotto, Maggioni et al., 2009; Monteschio, Souza, Vital, Guerrero, Valero, Kempinski et al., 2017; Rivaroli, Ornaghi, Mottin, Prado, Ramos, Guerrero et al., 2017). Thus, antibiotic and ionophores additives are used in cattle diets to modulate rumen fermentation and improve animal performance and feed efficiency (Zawadzki, Prado, Marques, Zeoula, Rotta, Sestari et al., 2011). However, the use of antibiotics and ionophores can cause the proliferation of resistant bacteria via food chain (Schäberle & Hack, 2014). Thus, new natural additive have been studied as an alternative for feed strategies to improve animal production and feed efficiency including plant extracts and essential oils (Cruz, Valero, Zawadzki, Rivaroli, Prado, Lima et al., 2014; Valero, Zeoula, Moura, Costa Júnior, Sestari, & Prado, 2015).

Essential oils are natural and a complex compounds characterized by a strong odor and are formed by aromatic compounds as secondary metabolites. They are usually obtained by steam or hydro-distillation (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). These products have several advantages than the commonly used antibiotics and ionophores; since they are residue free and are generally regarded as safe (GRAS) in the food industry (Haugaard, Hansen, Jensen, & Grunert, 2014).

Scarce and punctual studies evaluating the sensory acceptability of meat by consumers from cattle supplemented with essential oils in the diet have been previously realized (Guerrero, Rivaroli, Sañudo, Campo, Valero, Jorge et al., 2017; Rivaroli, Guerrero, Valero, Zawadzki, Eiras, Campo et al., 2016; Valero, Torrecilhas, Zawadzki, Bonafé, Madrona, Prado et al., 2014). Thus, there is a need for further studies.

The visual assessments are a gold standard for estimating consumer perception of meat (Mancini & Hunt, 2005), but are complex, expensive and consume time. Difficulties of using meat in consumer surveys could be overcame through the use of photographs for the color evaluation (Brugiapaglia & Destefanis, 2009; O'Sullivan, Byrne, Martens, Gidskehaug, Andersen, & Martens, 2003; Passetti, Torrecilhas, Ornaghi, Mottin, Oliveira, Guerrero et al., 2017). According to theses authors the images reduced the number of representative samples to explain the variations of meat color surface. Lu, Tan, ShatadalGerrard (2000) used digital images and trained panelists to predict pork loin visual color. When digital images were assessed by consumers, they were able to distinguish color before and after blooming (Brugiapaglia & Destefanis, 2009). Another advantage of digital images is their repeatability (Ngapo, Martin, & Dransfield, 2004), and being consistent, efficient and cost effective (Lu et al., 2000).

Thus, due to the current and future interest in producing meat from beef cattle finished with essential oils addition in the diets, this study was realized to evaluate consumer acceptability (sensory and visual) of meat from heifers finished in feedlot and fed with clove and rosemary essential oil and active principles (eugenol, thymol and vanillin blend).

2. Material and methods

The current experiment was approved by the ethical committee of the Universidade Estadual de Maringá (protocol nº 3624120116).

The sensory analysis protocol was previously approved by the Committee on Ethics in Research, at the Universidade Estadual de Maringá, PR, Brazil, under protocol CAAE: 56154816.2.0000.0104.

2.1. Locality, animal and diets

The study was conducted at Sector Rosa & Pedro, in the Experimental Station at Iguatemi farm, Iguatemi City, Paraná, Brazil. Heifers were allocated in individual pens, with an adaptation period of 7 days.

Forty purebred Nellore heifers with an initial average weight of 297.6 ± 31.2 kg randomly assigned to one of five finishing diets (n = 8 per treatment). The heifers were used since in Brazil, part of the beef production (approximately 40%) comes from the slaughter of heifers and cull cows (ANUALPEC, 2017). Moreover, heifers not used for breeding are destined for fattening and slaughtered at a younger age (<24 months) producing meat with good quality (Guerrero et al., 2017; Rotta, Prado, Prado, Valero, Visentainer, & Silva, 2009). Heifers were finished on their respective diets for 73 days, until they reached a medium weight of 356.6 ± 32.6 kg. Then, they were slaughtered at a commercial abattoir, after a solid fasting period for 12 h, following the slaughter standards of the State Inspection Service of the Brazilian legislation.

The basal diet was the same for all heifers (Table 1) formulated according to NRC (2000) and provided *ad libitum*. The five experimental diets were: CON – without essential oil and active principles; ROS – Rosemary essential oil (4 g/animal/day); BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day), and BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). These concentrations were chosen according to the results from Rivaroli et al. (2016) which showed that a concentration above 3.5 g/animal/day has effect on animal performance and meat quality. Furthermore, previous studies (Benchaar, Calsamiglia, Chaves, Fraser, Colombatto, McAllister et al., 2008) showed that the most adequate concentrations of e essential oils in the animal diets are between 3 and 5 g/animal/day.

2.2. Sampling and meat quality

After slaughter, carcasses were divided medially thought the sternum and vertebral column, identified and chilled (bellow 4ºC for 24 h).

Longissimus thoracis (LT) was excised from the left side of the carcass between the sixth and the thirteenth ribs for subsequent analysis, sliced into steaks (2.5 cm thick), weighed, vacuum-packed (99% vacuum, with a Sulpack SVC 620 machine, in Polyamide/Polyethylene pouches of 120 μm and 1 $cm^3/m^2/24$ h 0_2 permeability, 3 $cm^3/m^2/24$ h $C0_2$ permeability measured at 5 °C and 75% relative humidity; water vapor transmission rate (WVTR) was 3 $g/m^2/24$ h at 38 °C and 100% RH; the vicat softening point of sealing was reached at 97 °C and it had a dart drop strength of 1300 g), and aged for either 24 h or 7 days before being frozen and stored (−20 °C) for subsequent analyses.

2.3. Consumer experimental design

Consumer testing was performed at Universidade Estadual de Maringá in Maringá (Brazil) in a private room adequately adapted to perform a sensory test. One-hundred twenty seven consumers were selected randomly within quotas of gender (58 men and 69 women) and age (from 18 to 70 years) according to the Brazilian national profile. Table 2 shows the characteristics of the participating consumers. In total, 130 consumers participated in the sensory test; however, consumers with missing data and outliers on the questionnaire were not considered for statistical analyses (127 consumers are considered).

Thirteen sessions were carried out, each with ten different consumers. Per session, each consumer evaluated ten samples codified with a random three digit code, corresponding to all possible combinations between the diet and aging days. One steak from each animal of each experimental diet was evaluated. Meat was served following a randomised design to avoid order and carry-over effects (Macfie, Bratchell, Greehoff, & Vallis, 1989). Each steak was covered with aluminium foil codified with a random three-digit code and cooked in a pre-heated grill (Philco Grill Jumbo Inox, Philco S.A., Joinville, Brazil) at 200°C until reaching an internal temperature of 70°C, monitored with a penetration thermocouple (Incoterm, 145 mm; Incoterm Ltda, Porto Alegre, Brazil). Each steak was cut into ten 2 × 2 cm cubes and kept warm (50° C) until consumer evaluation (<10 min after cooking). Consumers were only informed that they would be evaluating beef.

All consumers were asked to taste the meat samples and evaluate the acceptability of four attributes: odor , tenderness, flavor and overall acceptability; this was achieved using a structured hedonic nine-point scale ranging from 1 = dislike extremely to 9 = like extremely, where a medium level was not included according to methodologies described by Prado, Campo, Muela, Valero, Catalan, Olleta et al. (2015). Before consumer testing, a questionnaire was applied (Table 3), including closed questions with multiple choices, based on previous research on beef quality attributes (Eiras, Ornaghi, Valero, Rivaroli, Guerrero, & Prado, 2016).

2.4. Visual appraisal of the meat and willingness to buy

2.4.1. Session 1 (random photos)

One LT steak (2.5 cm of thickness) from the 7th vertebra of each animal was sectioned and packaged in polystyrene trays wrapped with a retractile film (Goodyear®, Americana, São Paulo, Brazil, with oxygen permeability of 8200 cm^3/m^2 per day and rates of relative humidity of 262 cm^3/m^2 per day) and refrigerated in an illuminated display at 4°C and light (fluorescent lamp, 380 lux, 12 h/day) simulating typical Brazilian market conditions. Time of display for steaks was 11 days.

In order to produce images in standardized conditions, photographs were taken according to Chan, Moss, Farmer, GordonCuskelly (2013) and Passetti et al. (2017). The steaks were photographed every day through the eleventh days of evaluation, using a NIKON COOLPIX P500 digital camera mounted on a photographic stand containing two D65 fluorescent light tubes as the standard illuminant. An additional grey-colour cardboard was used to cover the entrance of the cabinet to provide lighting evenly distributed across the sample and also to avoid exposure to external light. The camera was fixed perpendicularly 45 cm to the surface of the meat sample. Following preliminary experiments to fix meat samples for appearing entirely in photos, the camera parameters were chosen: manual mode; shutter speed, 1/20; aperture size, F5.3; ISO, 1600; focal distance 40 mm. Images were stored and transferred to computer as JPEG file. A GretagMacbeth mini Colour-Checker (Colour – confidence, Birmingham, UK) which contains 24 colored patches was photographed with each meat sample for checking the colour reproduction capability as described by Passetti et al. (2017).

Training was done with evaluators prior to the visual analyses and it was determined that 5 s was sufficient time to evaluate one photograph and after every 40 evaluations, a 1 minute break was taken to avoid fatigue.

The analyses occurred in a comfortable place, the conference room of the Animal Science Department of Universidade Estadual de Maringá, where evaluators were distributed on individual chairs in such a way they could not have direct contact with each other. At the beginning of the analyses evaluators were instructed to evaluate the acceptance of meat color, disregarding other aspects as size, purge, marbling and subcutaneous fat. Photographs were projected individually in the room by a multimedia device (Epson Powerlite S27 2700 - UHE lamps 200 watts) in a Random method, where the photographs were randomly shown to the consumers.

2.5. Statistical analyses

Subsequent visual appraisal tests were assessed via ANOVA using GLM procedures with SPSS v.19.0 (IBM SPSS Statistics; SPSS Inc., Chicago, IL, USA) for Windows. Visual attributes were evaluated considering diet and time of display as fixed effects. Likewise, on consumer acceptability, diet and ageing time were considered fixed effects and the consumer was included as a random effect. Differences between means were evaluated using Tukey's test ($P \leqslant 0.05$). Answers related to habits and preferences of consumption were analyzed by frequency of response for each question. To identify similarities between consumers on acceptability test, hierarchical cluster analysis was used to determine the different groups of consumers depending on overall acceptability using XLSTAT (v.7.5.3). The number of clusters was selected from the dendrogram, trying to find a compromise between homogeneity within clusters and heterogeneity between clusters. Principal component analysis was used to identify the relationship between treatments and meat attributes. Correlations between attributes were evaluated using the Pearson's correlation coefficient.

3. Results and discussion

3.1. Consumer questionnaire

The supplementary questionnaire distributed to the consumers provides information about habits and preferences of consumption (Table 3).

The most frequent consumption of beef was 4 times/week (47.2%) followed by >4 times/week (34.6%). These results agreed with other Brazilian beef studies (Andrighetto, Sabbag, Sartorello, & Lupatini, 2014; Behrens, Barcellos, Frewer, Nunes, Franco, Destro et al., 2010; Eiras, Guerrero, Valero, Pardo, Ornaghi, Rivaroli et al., 2017; Eiras et al., 2016), that current socio-economic changes and the increase on the salary of Brazilian population consequently is reflected as an increase on beef consumption decreasing other basic aliment as rice or bean.

Most consumers prefer to buy meat from a supermarket (56.7%), followed by a butcher (40.2%), with a lower percentage of consumers purchasing the meat from specialized boutiques (3.1%). A similar tendency had been previously reported in Brazil by ; Andrighetto et al. (2014); Eiras et al. (2017); Guerrero et al. (2017). As Behrens et al. (2010) reported the increase of purchasing meat in supermarkets is consequence of the changes on food habits and modern life style, which demands time saving efforts and reduce time available. Supermarket helped people in purchasing different kinds of products in the same establishment.

Consumers preferred buying fresh meat (83.5%) than in the packaging forms (11% on tray and 5.5% vacuum-packed). Nevertheless, buying meat directly on trays is an increasing habit due to it being easier to freeze and store (Eiras et al., 2017; Velho, Barcellos, Lengler, Elias, & Oliveira, 2009).

Consumers considered color (44.1%) to be one of the most important attributes on beef purchase intention followed by establishment hygiene (39.4%), which is in agreement with Andrighetto et al. (2014); Eiras et al. (2017); Velho et al. (2009). On the other hand, the lowest proportion of Brazilian participants (13.4%) considered that price is the most important factor in beef purchasing decisions, as observed by Eiras et al. (2017) and as for European consumers, who considered other factors such as origin or animal feed to be more important than price (Realini, Font i Furnols, Sañudo, Montossi, Oliver, & Guerrero, 2013).

Meat from heifers (58.3%) followed by steers (26.7%) was preferred by consumers when compared with that from bulls (8.7%) or cows (6.3%). The current changes and demands have encouraged the development of highly efficient production systems in order to maximize the potential growth of the cattle, reduce the age at slaughter and increase the meat quality (Rotta et al., 2009).

The traditional Brazilian beef production was the best accepted by consumers. Thus, 42.5% of consumers answered that meat from pastures was the best option for purchase; whereas 40.2% believe that the beef production system does not influence their preferences. The feedlot (17.3%) was the least preferred production system. These percentages are close to the values observed by Eiras et al. (2017).

3.2. Consumer sensory evaluation

There was no interaction ($P > 0.05$) among diets and ageing days on sensory evaluation by consumers. Thus, the diet and ageing effects were presented and discussed as principal effect.

Diet effect: Essential oil and the active principle blend in the diets of heifers finished in feedlot did not change ($P > 0.05$) the odor acceptability score (Table 4). The score mean was 6.9 (hedonic scale from 1 to 9).

On the other hand, the scores assigned by consumer to flavor, tenderness and overall acceptability to meat from heifers fed with essential oil and the active principle blend (BCL diets), in general, were the highest ones ($P < 0.05$). These results show that the addition of clove essential oils + active principle blend in the diet improved the flavor, tenderness and overall acceptability of the meat as reported by Rivaroli et al. (2016). In contrast, rosemary essential oils addition (alone) or as part of a blend of essential oils reduced the meat score.

In relation to flavor, the antioxidant effect has been reported when they are added directly to the final product, for instance ground beef for hamburger preparation (Hussein & Hayan, 2012) where the addition of essential oils prevents the formation of secondary oxidation compounds and, consequently, decreases the rate of flavor deterioration, increasing acceptability scores. Overall acceptability is strongly related to the other two analyzed parameters (flavor and tenderness), as previously reported in other studies (Guerrero, Campo, Cilla, Olleta, Alcalde, Horcada et al., 2014; Pérez-Juan, Realini, Barahona, Sarriés, Campo, Beriain et al., 2014).

Ageing effect: The ageing days (1 or 7) did not affect ($P > 0.05$) the smell of the meat (Table 4). However, the mean scores assigned by consumers to the flavor, tenderness and overall acceptability to 7 days of ageing were better ($P < 0.001$; 7.0); than the first day (6.5). Thus, ageing improved consumer acceptability of the meat as observed by Guerrero et al. (2014); Pérez-Juan et al. (2014); Rivaroli et al. (2016) and Eiras et al. (2017). Ageing time influences the development of flavor precursors, usually improving the acceptability before reach long ageing, which could develop off-flavors (Monsón, Sañudo, & Sierra, 2005). Consumers can focus on flavor when meat is tender and becomes the major factor in acceptability (Campo, Nute, Wood, Elmore, Mottram, & Enser, 2003). In this sense, Huffman, Miller, Hoover, Wu, BrittinRamsey (1996), found that most consumers (51%) identified tenderness as the attribute that contributes most to eating satisfaction, but, when meat were evaluated at home, flavor affected overall ratings more than tenderness ratings. Neely, Lorenzen, Miller, Tatum, Wise, Taylor et al. (1998) indicated that flavor may be as important as tenderness in determining overall acceptability for fresh beef steaks as both flavor and tenderness correlated similarly with overall liking of fresh beef steaks (r^2 0.86 and 0.85, respectively).

3.3. Principal component analyses

Information about diets and ageing preferences by consumers is graphically summarized in Figure 1. The first two principal component axes explained 96.9% of the total variance. Attributes of smell, tenderness, flavor and overall acceptability are on the right side of F1, closely located on the graph to the BCL diets without ageing and CON, ROS, BLE and BCL with 7 days of aging. Meats without ageing were located on the left side of F1 (BRC, CON, BLE and ROS diets), inversely related to acceptability attributes. The tenderness, flavor and overall acceptability are located in the same quadrant for the treatment CON and BLE diets aged for 7 days. The odor is located in the upper right quadrant and associated with diets with 7 days of aging, especially the ROS and BCL diets, as well as BCL without aging. Even as demonstrated in other beef consumer studies (Eiras et al., 2017; Guerrero et al., 2017; Oliver, Nute, Font i Furnols, San Julián, Campo, Sañudo 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 (Font-i-Furnols & Guerrero, 2014; Oliver et al., 2006).

3.4. Cluster analysis

Four different clusters of consumers, related to overall acceptability, were found in the current survey (Table 5). The largest group (cluster 1) of consumers (40.2%) described differences ($P \leq 0.05$) between diets and ageing days ($P <0.05$) with acceptability scores for the five diets higher than in other clusters, with an average of 7.6 points on a nine-point scale. Meat from the BCL diets and 7 days ageing were preferred. This group included a similar percentage of women (51.0%) and men (49.0%). However, the age differed between the sexes, with 70% of men with an age below 40 years and in relation to women, they were similarly divided in all age groups (around 25%). This cluster also showed a good ace acceptance for BLE and BRC diets.

The second group (cluster 2) consisted of 16.5% consumers, being composed by 66.7% women and 33.3% of men who also observed differences ($P \leq 0.05$) among diets, preferring the treatment CON and BCL with an average acceptability of 6.19 points. This cluster was characterized with 42.8% of men with age above 56 years and with 71.4% of women between the ages of 26 and 40 years.

Cluster 3 consists of a percentage of (22.8%) consumers, being 55% men and 45% women, this segment reported differences between diets ($P < 0.05$). Meat from the BLE and BRC treatments were preferred. This group was characterized by 50% of the men aged from 41 to 55 years, presenting a better acceptance for the diet with essential oil and active principle blend and only 8% of the women with age range over 56 years presenting this acceptance.

The last group of consumers (group 4) was formed by a percentage of (20.47%) consumers who described differences ($P \leq 0.05$) between diets and aging periods ($P < 0.05$), being formed by 61,5% of females and 38.5% of males being the treatment BCL the preferred and also the meat aged by 7 days. This group was characterized by men 50% with ages ranging from 18 to 25 years and 62.5% of women under 40 years of age.

3.5. Visual appraisal

Diet effect: During all evaluated period the score assessed by the consumers to the meat from heifers fed with BRC and BCL diets was higher ($P < 0.001$) than scores assessed to CON, ROS and BLE diets (Table 6), except for ROS at day 6 which was similar to BCL. Thus, the essential oil and the active principle blend complex addition in the diets of heifers finished in feedlot with high-grain improved the visual evaluation of meat. In fact, essential oils have a natural antioxidant effect (Jiang & Xiong, 2016; Kumar, Yadav, Ahmad, & Narsaiah, 2015; Passetti et al., 2017). Thus, antioxidant and its incorporation into cell membranes may delay the oxidation of myoglobin. These compounds act to capture free radicals that are formed during lipid oxidation, delaying the conversion of the cherry red pigment (oxymyoglobin) to the brown pigment (methemoglobin) (Descalzo & Sancho, 2008). The addition of essential oils as nutritional supplements in ruminants diets can be a practice to improve animal performance, health, welfare and meat quality (Guerrero et al., 2017; Jiang & Xiong, 2016; Passetti et al., 2017). For meat, natural essential oils added to feed not only can improve the oxidative stability and sensorial properties but they also can enhance the nutritional value and the health benefit of meat products (Rivaroli et al., 2016). Recently, there has been a growing interest in supplementing animal feeds with antioxidant plant extracts or essential oils to increase the nutritional value of meat for consumers' health benefits (Brenes, Viveros, Chamorro, & Arija, 2016; Kumar et al., 2015). According to Nieto, Díaz, BañónGarrido (2010) and Serrano, JordánBañón (2014) lamb fed distilled rosemary or thyme in the diet for several months showed a higher antioxidant stability of the meat, a higher concentration of polyphenolic antioxidants, and delayed color deterioration in the meat.

Display time effect: Throughout the 11-day of display period, consumer acceptance appearance decreased significantly (Table 6). The gradual decline in visual acceptability was expected due to oxidative processes, which cause meat deterioration, and is particularly relevant in meat from concentrate-fed animals (Warren, Scollan, Nute, Hughes, Wood, & Richardson, 2008) (lower composition in natural antioxidants of the diet). Prado et al. (2015) and Eiras et al. (2017) also observed the similar decline in visual acceptability of meat from Friesian animals finished in feedlot.

The maximum exposure time was 7 (score above 5). After the eighth day of display the values were below 5; which can be considered improper for consumer acceptance.

The oxidation is a natural process which reduces the shelf life of meat and is linked to deterioration of the product due to oxidation-reduction of myoglobin which is change from oxymyoglobin to metmyoglobin (Mancini & Hunt, 2005). At the eleventh day (the last day of evaluation) the score was above 2. In general, after the eighth day of evaluation the meat has scores below 3 (Eiras et al., 2017; Prado et al., 2015).

The lowest deterioration observed in the current study may be related to the origin of the meat. Animals finished in pasture have a higher carotenoids content that protects the deterioration of meat (Realini, Duckett, Brito, Dalla Rizza, & Mattos, 2004). In the present study, heifers were raised on pasture for 20 months. In this way, the presence of increased carotenoids concentration may have prevented a more rapid deterioration of the meat (Descalzo & Sancho, 2008).

In Table 7 the equations of effects of the days of display are presented. For the different diets, the visual scores showed a higher relationship with the days of display in the form of a quadratic equation ($R^2 > 0.99$). However, according to the equations the shelf-life was different between the treatments. The CON diets presented the lowest shelf-life (6.92 days), while BCL and BRC presented the highest (7.72 and 7.77, respectively).

3.5.1. Willingness to buy

Regarding to the meat willingness to buy (random photos), until the 3rd day, 100% of the participants would by the meats and until the 5 day of exhibition, above 88.6% would buy the meat of all the treatments. From the 6th day, only 33% of the participants would by the meat from control group, while the willingness to buy for the other treatments still remains above 50%, and from the day 10, 97% of the participants would not buy the meat.

In relation to treatments (Table 8), BCL and BRC had the highest willingness to buy, while ROS and BLE had intermediate and CON presented the lower willingness to buy ($P < 0.001$). In relation to the display time, the wiliness to buy the meat decreased over time, which was expected due to the deterioration of meat, being the seventh day the turning day of the willingness (score above 1.5, where 1 represents yes, I would buy and 2 represent no, I would not buy). Thus, the meat from animals that received diet with the active compounds presented better results than the control.

4. Conclusions

Addition of essential oil and active principle blend in the diet of heifers finished in feedlot improved the consumer assessment score, especially when they are all together (BRC), just the clove and the principles blend (BCL) or only the principles (BLE). In relation to overall acceptability, BCL presented the higher scores, and the seventh day of ageing was preferred. Regarding to visual appraisal, BCL and BRC showed the better results, suggesting a longer shelf life of meat, and also showed a better willingness to buy. Thus, the use of essential oils and active principle blends (natural products) in animal diets can be an alternative to the conventional antibiotics.

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Table 1. Ingredients of experimental diets (g kg^{-1} of DM).

Ingredients	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]
Corn silage	258	276	232	244	233
Corn grain	645	621	682	665	680
Soybean meal	91.9	98.1	82.4	86.7	82.8
Yeast	0.52	0.55	0.46	0.48	0.46
Phosphorus	0.91	0.96	0.81	0.85	0.81
Mineral salt[2]	1.81	1.93	1.62	1.71	1.63
Rosemary		0.004			
Eugenol + Thymol + Vanilinn			0.004		
Eugenol + Thymol + Vanilinn				0.002	
Rosemary				0.002	
Eugenol + Thymol + Vanilinn					0.0013
Rosemary					0.0013
Clove					0.0013

[1]CON – without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –Protected blend of eugenic + thymol + vanillin (4 g/animal/day); [4]BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). [2]Mineral salt composition (kg $^{-1}$): calcium, 50 g; magnesium, 57 g; sodium, 81 g; sulfur, 3.75 g; cobalt, 20 mg; copper, 500 mg; iodine, 25 mg; manganese, 1.500 mg; selenium, 10 mg; zinc, 2.000 mg; vitamin A, 400.000 UI; vitamin D3, 50.000 UI; vitamin E, 750 UI; ether extract, 168 g; urea, 200 g.

Table 2. Characteristics (age and gender) of the consumers involved in the trial (n = 127 consumers).

Age	Total population	Men	Women
Years		%	%
< 25	29.1	32.8	26.1
26-40	31.5	25.9	36.2
41-55	22.0	25.9	18.8
> 56	17.3	15.5	18.8
Total	127	58	69
Total, %	100	45.7	54.3

Table 3. Answers of questionnaire about consumer preferences and habits of consumption (n = 127 consumers)

Questions	Answers	Consumers, %
Q1. Frequency of beef consumption	1 time/week	0.8
	2 times/week	2.4
	3 times/week	15.0
	4 times/week	47.2
	> 4 times/week	34.6
Q2. Place where you buy the meat	Butcher	40.2
	Supermarket	56.7
	Meat boutique	3.1
Q3. How do you prefer to buy meat?	Fresh cut	83.5
	Vacuum-packed	5.5
	On tray	11.0
Q4. The most important factor when you buy meat	Price	13.4
	Colour	44.1
	Hygiene	39.4
	Customer service	3.1
Q5. Which beef cattle category do you prefer?	Steers	26.7
	Bulls	8.7
	Heifers	58.3
	Cow	6.3
Q6. What system of cattle production do you prefer?	Pasture	42.5
	Feedlot	17.3
	Little importance	40.2

Table 4. Effect of essential oils and ageing time on consumer acceptability of meat from Nellore heifers finished in feedlot (n = 127 consumers)

	Diets					Ageing days			P < Value		
Item	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	1	7	SEM[6]	D	A	DxA[7]
Odor	6.94	6.83	6.83	7.00	6.98	6.92	6.93	0.041	0.404	0.892	0.510
Flavor	6.85b	6.74b	7.00a	7.10a	6.89b	6.83	7.01	0.042	0.038	0.008	0.960
Tenderness	6.39b	6.37b	6.62ab	6.98a	6.41b	6.19	6.92	0.063	0.001	0.001	0.796
Overall	6.71ab	6.62b	6.84ab	7.05a	6.67ab	6.59	6.96	0.050	0.006	0.001	0.768

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – Blend of eugenol + thymol + vanillin (4 g/animal/day); [4]BCL – Blend – eugenol + thymol + vanillin (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – Blend – eugenol + thymol + vanillin (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). a-b: different letters in the same line are different. There was no interaction among diet and aging ($P \geq 0.05$). [6]SEM: Standard error of means. [7]Interactions between diets and aging time.

Table 5. Overall acceptability of meat from Nellore heifers finished in feedlot in four consumers clusters (n = 127).

			Diets					Ageing. days			P-value		
Cluster	n	Samples, %	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	1	7	SEM[6]	Day	Aging	D x A[7]
1	51	40.15	7.40b	7.43b	7.67ab	7.83a	7.59ab	7.37	7.80	0.04	0.003	0.000	0.567
2	21	16.53	6.12a	4.98b	5.31ab	6.26a	5.00b	5.51	5.55	0.13	0.002	0.877	0.354
3	29	22.83	5.67b	6.12ab	6.83a	6.03ab	6.76a	6.11	6.46	0.10	0.000	0.095	0.131
4	26	20.47	7.00ab	6.92ab	6.46bc	7.29a	6.12c	6.48	7.03	0.09	0.000	0.002	0.000

a–c, lowercase letters indicate statistical differences in the same row between treatments ($P \leq 0.05$). [1]CON – without essential oil and active principles (eugenol, thymol and vanillin); [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); [4]BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). [6]SEM: Standard error of means. [7]Interactions between diets and aging time.

Table 6. Visual appraisal of meat from Nellore heifers finished in feedlot during display time (n = 59 appraisers).

	Diets						
Days	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	P-Value	SEM[6]
1	7.65cA	7.93bA	7.78bcA	8.54aA	8.55aA	0.000	0.062
2	7.24cB	7.61bB	7.54bB	8.21aB	8.19aB	0.000	0.063
3	7.08cBC	7.16bcC	7.22bC	7.67aC	7.71aC	0.000	0.045
4	6.91bC	6.97bD	6.87bD	7.41aD	7.44aD	0.000	0.043
5	6.12bD	6.20bE	6.23bE	6.68aE	6.72aE	0.000	0.045
6	5.43cE	5.80bF	5.60cF	5.98abF	6.04aF	0.000	0.041
7	5.19bF	5.34bG	5.34bG	5.84aF	5.90aF	0.000	0.049
8	4.27bG	4.40bH	4.42bH	4.79aG	4.85aG	0.000	0.041
9	3.22bH	3.34bI	3.33bI	3.81aH	3.81aH	0.000	0.044
10	2.25cI	2.69bJ	2.55bJ	3.25aI	3.42aI	0.000	0.074
11	1.16cJ	1.30bK	1.28bK	1.76aJ	1.80aJ	0.000	0.043
P-Value	0.000	0.000	0.000	0.000	0.000	0.000	
SEM	0.222	0.221	0.221	0.224	0.222		

A–K Different letters in the same column means significant differences (Tukey 0.05). a–c Different letters in the same line means significant differences (Tukey 0.05). [1]CON – without essential oil and active principles (eugenol, thymol and vanillin); [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); [4]BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). [6]SEM: Standard error of means.

Table 7. Regression analysis of the visual acceptability of meat from Nellore heifers finished in feedlot with or without addition of essential oil and active principle blend

	Random			
Diets	Days	Equation	P-Value	R^2
CON[1]	6.92	y = -0.052x² -0.019x +7.625	0.001	0.993
ROS[2]	7.16	y = -0.047x² -0.075x +7.953	0.001	0.994
BLE[3]	7.12	y = -0.049x² -0.048x +7.829	0.001	0.994
BCL[4]	7.72	y = -0.039x² -0.175x +8.679	0.001	0.99
BRC[5]	7.77	y = -0.040x² -0.161x +8.664	0.001	0.987

[1]CON – without essential oil and active principles (eugenol, thymol and vanillin); [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); [4]BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day).

Table 8. Willingness to buy of meat from heifers fed finished in feedlot during display time (random photos).

	Diets					Aging time												P-Value		
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BCR[5]	1	2	3	4	5	6	7	8	9	10	11	SEM[5]	Diet	Aging	DxA[6]
Willing to buy	1.50[a]	1.45[b]	1.45[b]	1.39[c]	1.38[c]	1.00[e]	1.00[e]	1.00[e]	1.01[e]	1.08[d]	1.38[c]	1.53[b]	1.94[a]	1.96[a]	1.97[a]	1.97[a]	0.004	<0.001	<0.001	<0.001

a-c: Different lowercase letters in the same line means significant differences (Tukey 0.05) between treatments. A-E: Different uppercase letters in the same line means significant differences (Tukey 0.05) between days. 1Diets: CON – without essential oil and active principles (eugenol, thymol and vanillin); ROS – Rosemary essential oil (4 g/animal/day); BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). [5]SEM: Standard error of means. [6]Interaction between diets and aging time.

Figure Captions

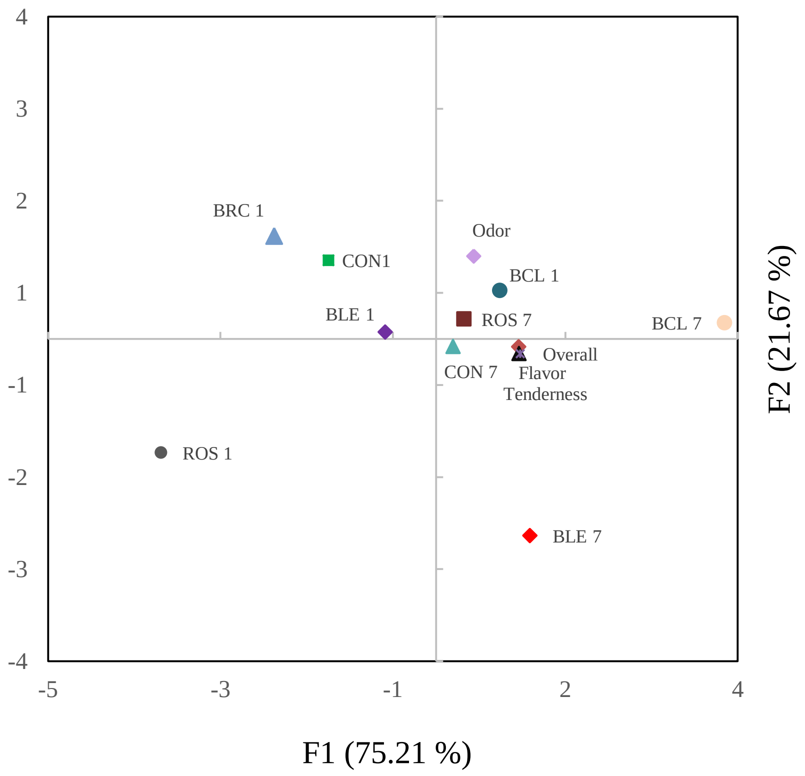

Figure 1. Principal component analysis of the scores for odor, flavor, tenderness and overall acceptability of beefs from Nellore heifers finished in feedlot and fed with or without essential oil and active principle blend and two aging times. CON – without essential oil and active principles (eugenol, thymol and vanillin); ROS – Rosemary essential oil (4 g/animal/day); BLE – eugenol, thymol and vanillin active principle blend (4 g/animal/day); BCL –eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and BRC –eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day).