UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

MIX DE ÓLEOS DE CRAVO, MAMONA E CAJU E COMPOSTO MICROENCAPSULADO DE EUGENOL, TIMOL E VANILINA NA SUPLEMENTAÇÃO DE BOVINOS TERMINADOS EM PASTAGEM DE AVEIA E AZEVÉM: DESEMPENHO E QUALIDADE DA CARNE

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Estado do Paraná

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 21 de fevereiro de 2019.

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Cada dia é uma soma de batalhas vencidas, etapas concluídas, promessas alcançadas, algumas lágrimas contidas e outras derramadas.

(Autor desconhecido)

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BIOGRAFIA DO AUTOR

CAMILA MOTTIN, filha de Nilton Mottin e Regina Celi Mottin, nasceu em 13 de setembro de 1990, em Nova Cantu, Paraná, Brasil.

Em dezembro de 2012 concluiu o curso de medicina veterinária pelo Centro Educacional Integrado. Nesse período desenvolveu trabalhos, cursos e estágios na área de grandes animais com ênfase em produção, nutrição e reprodução de bovinos de corte.

Em setembro de 2014 concluiu a especialização em bovinocultura de corte e leite: nutrição e reprodução pelo Centro Universitário Integrado de Campo Mourão. O curso abrangeu temas relacionados à cadeia produtiva, nutrição, reprodução, sanidade animal, melhoramento genético, qualidade da carne, além de aspectos gerenciais e ambientais na produção de bovinos de corte e leite.

Em março de 2016 concluiu o mestrado em Ciência Animal pela Universidade Estadual de Londrina. Desenvolveu pesquisa com o tema adição de óleos essenciais de cravo e canela na dieta de bovinos mestiços terminados em confinamento sobre o desempenho e qualidade da carcaça e da carne.

Em março de 2016 ingressou no Programa de Pós Graduação em Zootecnia da Universidade Estadual de Maringá, em nível de Doutorado, área de concentração Nutrição e Produção animal, realizando estudos na subárea de grandes ruminantes de corte com ênfase em aditivos naturais na dieta de bovinos terminados em pastagem de aveia e azevém. Também, realizou doutorado sanduíche na Universidade Federal do Recôncavo da Bahia na área de bromatologia.

Em setembro de 2018, realizou o exame de qualificação. E em fevereiro de 2019, submeteu-se a banca examinadora para defesa de doutorado.

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RESUMO

No sistema de terminação de bovinos em semi confinamento são necessárias estratégias nutricionais para que ocorra a redução do ciclo produtivo. A suplementação com aditivos pode ser uma dessas medidas para auxiliar no aproveitamento dos alimentos e produzir carne de qualidade. De modo geral, estas substâncias são ionóforos ou antibióticos. Todavia, essas substâncias estão proibidas na União Europeia e em vias de proibição nos Estados Unidos. Desta forma, é necessário o desenvolvimento de substâncias alternativas e seguras na alimentação animal. Assim sendo, os aditivos naturais tornaram-se objetivos de várias pesquisas no mundo. Entre esses aditivos, os óleos essenciais e os óleos vegetais têm merecido destaque. Entretanto, para sua adição na alimentação animal é necessário caracterizar os vários produtos de plantas, bem como conhecer o modo de ação destas substâncias, que possuem comprovado efeito flavorizante, estimulante da secreção enzimática, ação antimicrobiana, antioxidante, anti-inflamatória, antiparasitária, antiviral, entre outras. Ainda mais, esses compostos têm uma ampla variedade de efeitos sobre a qualidade da carne, podendo retardar o processo de oxidação aumentando o tempo de vida útil, além de serem incorporados nos músculos e poder contribuir na saúde do consumidor, incluindo efeitos positivos sobre as doenças cardiovasculares, alguns tumores, processos inflamatórios, e em geral, doenças nas quais ocorre uma proliferação descontrolada de radicais livres. Este trabalho foi realizado para avaliar o desempenho animal, as características de carcaça e a qualidade da carne de 40 novilhos mestiços (½ Bons Mara x ½ Nelore) com cerca de 20 meses de idade, peso corporal inicial médio de 416,9 ± 5,56, sem adição (controle) ou com níveis (1.500, 3.000, 4.500 ou 6000 mg/dia/animal) de uma mistura de aditivos naturais (AN), contendo óleo essencial de cravo, óleo de mamona, óleo de caju e uma mistura de princípios ativos microencapsulados de eugenol, timol e vanilina durante 80 dias sobre o desempenho

animal e qualidade da carne. Os resultados sugerem que, embora o uso da mistura de óleos não tenha modificado o ganho de peso dos animais, o suplemento teve efeito curvo linear na ingestão de forragem, e consequentemente na matéria seca, proteína bruta, fibra em detergente neutro e carboidratos não fibrosos. A maior ingestão de matéria seca foi observado no tratamento com 1.500 mg e a menor ingestão no tratamento com 6.000 mg. A digestibilidade da proteína foi menor e dos carboidratos não fibrosos foi maior nos tratamentos com AN em todas as dosagens. Um aumento nas concentrações de nitrogênio amoniacal ruminal, e nos ácidos graxos voláteis propiônico e isovalérico foram observados nos tratamentos com AN em todas as dosagens. Não foram observadas diferenças nos parâmetros macroscópicos do líquido ruminal (movimentos ruminais, cor, odor, consistência, sedimentação e flutuação, potencial redox e contagem e viabilidade de protozoários). As características de carcaça não foram alteradas pelos tratamentos, mas houve alteração na composição corporal, aumentando a deposição muscular nos animais suplementados com AN. Os tratamentos não tiveram efeito nas perdas por gotejamento da carcaça. As perdas de descongelamento/armazenamento, cozimento, textura, cor, atividade antioxidante e oxidação lipídica foram avaliadas ao longo do tempo de armazenamento em embalagem a vácuo nos dias 1, 7 e 14 e foram observadas diferenças. Houve um efeito quadrático nas perdas por descongelamento/armazenamento no primeiro dia de armazenamento da carne, sendo que o tratamento controle perdeu menos líquido que os demais. No entanto, nas perdas por cocção esse mesmo tratamento no sétimo dia de armazenamento perdeu mais líquidos. A força de cisalhamento foi similar entre os tratamentos no dia 1 e no dia 7 de armazenamento. No dia 14, foi observado um efeito linear; a carne do tratamento controle estava mais macia. Um efeito linear na luminosidade da carne foi observado. A carne de animais do tratamento controle estava mais clara e potencialmente mais atraente para o consumidor no dia 1 de armazenamento. Após 7 e 14 dias de armazenamento, as carnes foram semelhantes entre os tratamentos. Os parâmetros de intensidade de vermelho e amarelo não foram alterados. No entanto, ao avaliar o potencial antioxidante da carne, observou-se que no dia 1 de armazenamento houve um maior número de compostos fenólicos e uma maior atividade antioxidante (DPPH e FRAP) nos tratamentos com AN. Apesar dos valores mais altos de oxidação lipídica serem notados no primeiro dia de armazenamento nos tratamentos que receberam AN na dieta, foi observado também que tratamentos com maiores dosagens de aditivos retardaram a oxidação lipídica ao longo do tempo de armazenamento. O tempo de armazenamento afetou as perdas por descongelamento/armazenamento, perdas por

cocção, textura, cor e oxidação lipídica. No entanto esses resultados são devido ao processo de proteólise. Em conjunto, estes resultados sugerem que a mistura de aditivos naturais tem potencial no uso na alimentação animal e pode melhorar a estabilidade da carne, no entanto, ainda devem ser estudados com relação a dose a ser empregada em animais a pasto.

Palavras-chave: aditivos naturais, extratos de plantas, óleo essencial, óleo vegetal, suplementação a pasto.

ABSTRACT

In the grazing system for cattle, nutritional strategies are necessary to shorten the production cycle; supplementation with additives can be used to maximize nutrient use and meat quality. In general, these substances are ionophores or antibiotics. However, these substances are banned in the European Union and limited use in the United States. In this way, the development of safe alternative substances in animal feed is necessary. Thus, natural additives are the subject of much research around the world. Among these additives, essential oils and vegetable oils has greater prominence. However, for their addition in animal feed it is necessary to characterize the various plant products as well as to know the mode of action of these substances. These substances have proven flavoring effect, stimulating enzymatic secretion, antimicrobial action, antioxidant, anti-inflammatory, antiparasitic, antiviral, among other actions. Furthermore, these compounds have a many of effects on the quality of the meat, which can slow down the oxidation process and increase the shelf life, as well as being incorporated into the muscles and contributing to consumer health, including positive effects on cardiovascular diseases, some tumors, inflammatory processes, and in general, diseases in which there is an uncontrolled proliferation of free radicals. The objective of the study was to evaluate the animal performance, carcass characteristics and meat quality. Forty 20-month old crossbred steers (Bons Mara x Nellore) of initial body weight 416.9 ± 5,56 kg, without addition (control) or levels (1500, 3000, 43500 or 6000 mg / day / animal) of a mixture of natural additives (NA) containing clove essential oil, castor oil, cashew oil and a blend of microencapsulated active ingredients of eugenol, vanillin and thymol for 79 days. The results suggest that, although the use of the oil mixture did not modify the animals' weight gain, the supplement had a quadratic effect on forage intake, and consequently on dry matter, crude protein, neutral detergent fiber and non-fibrous carbohydrates. The greatest intake of dry matter was observed in treatment with 1500 mg and the smallest consumption in treatment with 6000 mg. Protein digestibility was smaller and non-fibrous carbohydrates were greater in AN treatments at any dosage. An increase in ruminal ammoniacal nitrogen concentrations and in propionic and isovaleric volatile fatty acids were observed in AN treatments at any dosage. No marked differences were observed in the macroscopic parameters of ruminal fluid (ruminal movements, color, odor, consistency, sedimentation and flotation, redox potential and counting and viability of protozoa). The carcass characteristics were not altered by the treatments, but there was a change in the body composition, increasing the muscular deposition in the animals supplemented with AN. The treatments had no effect on drip losses. The thawing/ ageing losses, cooking losses, texture, color, antioxidant and lipid oxidation were evaluated over the storage time in a vacuum package for 1, 7 and 14 days and differences were observed. There was a quadratic effect observed in the thawing/ageing losses on the first day of storage of the meat, and the control treatment lost less liquid than the others. However, in cooking losses that same treatment on the seventh day of storage lost more liquids. The shear force was similar between treatments on day 1 and day 7 of storage. At day 14, a linear effect was observed, and the meat from the control treatment was tender. A linear effect on meat lightness was observed. The meat from control treatment animals was clearer and potentially more attractive to the consumer on day 1 storage. After 7 and 14 days of storage, the meats were similar between the treatments. The redness and yellowness parameters were not changed. However, when evaluating the antioxidant potential of the meat, it was observed that on day 1 of storage there was a greater number of phenolic compounds and a greater antioxidant activity (DPPH and FRAP) in AN treatments. Although greater values of lipid oxidation were observed on the first day of storage in treatments receiving AN in the diet, it was also observed that treatments with greater dosages of additives delayed lipid oxidation throughout the storage time. The storage time affected the losses by thawing/ageing losses, cooking losses, texture, color and lipid oxidation, however these results are expected due to the proteolysis process. Taken together, these results suggest that the mixture of natural additives has potential use in animal feed and may improve meat stability; however, they should still be studied with respect to dose-response.

Keywords: cashew oil, castor oil, clove oil, natural plant extracts, supplementation of grazing

CAPÍTULO I

INTRODUÇÃO

A preocupação dos consumidores com alimentação saudável e balanceada, para o funcionamento e bem estar do organismo, tem levado ao desenvolvimento de pesquisas em busca de produtos mais saudáveis (Hocquette et al., 2007a; Jayasena & Jo, 2013). Nesse sentido, é necessário salientar os benefícios no consumo de carne bovina e a importância de seus nutrientes na composição da dieta, desmistificando os conceitos passados pela mídia de que, o consumo de carne aumenta o colesterol sanguíneo e o risco de desenvolver doenças cardiovasculares (Guerrero et al., 2013; HMSO, 1994; Wood et al., 2008).

A carne é fonte de proteínas, vitaminas, ácidos graxos essenciais, minerais e outros compostos (Pereira & Vicente, 2013). Todos esses componentes são sensíveis aos danos causados pelas reações de oxidação durante o armazenamento, que no decorrer da vida útil vão diminuindo o valor nutricional do alimento e com passar do tempo tornam a carne imprópria para o consumo (Ramalho & Jorge, 2006; Realini & Marcos, 2014; Wood et al., 2008).

Com objetivo de evitar os danos celulares causados pela oxidação prévia, a indústria alimentícia utiliza produtos antioxidantes, geralmente sintéticos, que atuam na remoção ou sequestro dos produtos gerados, que são os derivados de espécies reativas ao oxigênio (Biesalski, 2002; Hocquette et al., 2007b). No entanto, devido ao apelo nutricional tem-se buscado antioxidantes naturais, como os óleos essenciais biossintetizados por plantas (Guerrero et al., 2018; Kempinski et al., 2017; Monteschio et al., 2017; Rivaroli et al., 2017; Souza et al., 2019; Vital et al., 2018).

Os óleos essenciais, inicialmente foram utilizados na indústria farmacêutica e alimentícia. Contudo, devido às características odoríferas marcantes do óleo, quando aplicado diretamente no produto pode haver alteração na aparência, aroma e sabor, sendo menos notado pelo consumidor em produtos processados do que na carne *in natura* (Kim et al., 2013).

Os óleos essenciais, recentemente, começaram a ser utilizados como aditivos na alimentação animal (Ornaghi et al., 2017; Valero et al., 2014a; Valero et al., 2014b). Quando suplementados na ração, alguns autores relatam melhora na digestibilidade e desempenho produtivo dos animais, como também efeitos antimicrobianos e antioxidantes na carne (Kempinski et al., 2017; Monteschio et al., 2017; Vital et al., 2018). Os extratos vegetais podem ser uma alternativa aos antioxidantes químicos, uma vez que substâncias sintéticas têm limites restritos de inserção nos produtos alimentares (Laguerre et al., 2007).

REVISÃO DE LITERATURA: MIX DE ÓLEOS DE CRAVO, MAMONA E CAJU E COMPOSTO MICROENCAPSULADO DE EUGENOL, TIMOL E VANILINA NA SUPLEMENTAÇÃO DE BOVINOS TERMINADOS EM PASTAGEM DE AVEIA E AZEVÉM: DESEMPENHO E QUALIDADE DA CARNE

Suplementação de bovinos em semi confinamento

O sistema semi-intensivo de criação apresenta grande importância para pecuária brasileira, sendo que cerca de 30% das áreas cultivadas do território nacional são constituídas por pastagens e que cerca de 90% dos bovinos abatidos são criados exclusivamente em pastos, sendo a pastagem, muitas vezes, a única fonte de alimento para os animais (ANUALPEC, 2017; Ferraz & Felício, 2010). Nesse cenário, as pastagens se constituem como principal fonte alimentar para os animais, caracterizando-se uma forma econômica de produção de carne, embora muitas vezes deficiente em termos de produtividade e valor nutritivo. A busca pela intensificação da cadeia produtiva determina a adoção de novas tecnologias que visam aumentar a eficiência no setor. Os avanços tecnológicos disponíveis permitem a redução na idade ao abate dos animais, sendo este um dos fatores de maior impacto positivo na empresa pecuária (Ito et al., 2010; Ito et al., 2012).

As forrageiras sofrem grande influência das variações climáticas, que causam oscilações na qualidade e na quantidade (acúmulo de massa seca) (Figueiras et al., 2015; Moreira et al., 2004). O período denominado época seca (inverno) é uma fase crítica, e normalmente, os animais se alimentam de forrageira com baixo valor nutricional, com níveis de fibra indigestível elevados e baixos níveis de proteína bruta (menores que 7%). Esse conjunto de fatores indesejáveis limita o consumo pelos animais, e consequentemente a produtividade (Berchielli et al., 2011; Mertens, 1994, 2007).

No entanto, em algumas regiões do país, é possível, nessa época de escassez, modificar esse contexto com a introdução de cultivares adaptadas ao clima (Prado & Prado, 2010; Silva et al., 2009; Silva et al., 2010). Na região Noroeste do Paraná, é comum a utilização das pastagens cultivadas de inverno, sistema conhecido como integração lavoura-pecuária, desenvolvendo uma pecuária mais rentável, com a engorda de bovinos no período da entre safra proporcionando a comercialização destes animais em um período em que o preço histórico da arroba está mais elevado, permitindo ao produtor um incremento na renda da propriedade (Moreira et al., 2001; Moreira et al., 2005; Moreira et al., 2006).

As culturas forrageiras de inverno são semeadas pelo sistema de plantio direto, geralmente, entre os meses de março e abril, após colheita do milho ou soja. A utilização destas pastagens pode se prolongar até novembro, para que então dê início ao plantio da cultura subsequente, quase sempre soja ou milho. Entre as espécies mais conhecidas e adaptadas ao sistema de plantio direto destacam-se a aveia preta (*Avena sativa*) e o azevém perene (*Lolium perene*). O uso desta consorciação tem sido adotado por aliar a precocidade de produção da aveia preta com a qualidade e ciclo mais tardio do azevém, estendendo assim o período de pastejo (Lupatini et al., 1998; Macari et al., 2006; Roso et al., 2000)

A composição bromatológica observada no consórcio das pastagens cultivadas de inverno ao longo do ciclo deve-se, em grande parte, ao estágio vegetativo das mesmas. Durante o período de pastejo, pastagens de aveia e azevém podem apresentar teores médios de proteína bruta (PB), nutrientes digestíveis totais (NDT), fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA) próximos a 14, 63, 55, e 32%, respectivamente (Roso et al., 2000; Skonieski et al., 2011). Sua produção anual pode se aproximar a 10.000 kg de MS/ha, com taxa de acúmulo diário que varia de 32 a 48 kg MS/ha (Frizzo et al., 2003; Pilau et al., 2005; Rocha et al., 2003).

Mesmo com a elevada qualidade das pastagens de inverno quando comparada às pastagens tropicais, os rendimentos por animal são limitados pela ingestão de energia. Ademais, as elevadas concentrações de amônia ruminal registradas em animais alimentados com pastagens temperadas caracterizam um gasto de energia extra ao indivíduo, pois o excedente é absorvido pelo rúmen, detoxificado em ureia e finalmente excretado (Monteiro et al., 2018; Ulyatt et al., 2002).

Assim, o desempenho e a eficiência no aproveitamento dos nutrientes digeridos são dependentes do adequado balanço entre energia e proteína. Com este sincronismo, que pode ser obtido pela suplementação, o N amoniacal será incorporado à proteína microbiana. Com isso, ocorre a redução dos níveis de amônia aumentando a eficiência de síntese e elevação do fluxo de proteína microbiana para o intestino delgado elevando os ganhos (Monteiro et al., 2018; Ulyatt et al., 2002).

A utilização da suplementação, além de corrigir as deficiências nutricionais e melhorar a utilização da forragem, flexibiliza a taxa de lotação, reduz a permanência dos animais na propriedade, maximiza novas oportunidades de negócios, aumenta o retorno econômico e melhora a qualidade da carne. Outra vantagem ao fornecimento de suplementos é a vinculação de aditivos alimentares à dieta, sendo uma boa alternativa para o aumento de ganho de peso dos animais e na melhoria da eficiência alimentar, em detrimento as modificações no ambiente ruminal (Figueiras et al., 2015; Moletta et al., 2014).

Os antibióticos ionóforos são os aditivos alimentares mais utilizados no Brasil. No entanto, nos últimos anos muito se tem discutido a respeito da utilização de antibióticos e outros promotores de crescimentos sintéticos na produção animal, assim como na busca por alternativas naturais para substituição desses produtos, que no mínimo mantenham os níveis produtivos (Bergen & Bates, 1984; Raun et al., 1976; Russell & Houlihan, 2003; Schelling, 1984). Nesse sentido, o interesse em avaliar os efeitos de diversos aditivos naturais e sinergismos entre eles tem aumentado e refletido na constante realização de estudos científicos (Guil-Guerrero et al., 2016; Karre et al., 2013; Patra & Saxena, 2010).

A combinação entre aditivos pode causar sinergismo, porém essa estratégia deve ser amplamente estudada. A otimização do ambiente ruminal poderia ser melhorada pela combinação de aditivos que possuem efeitos sinérgicos, alguns têm sido estudados como, as leveduras, os óleos essenciais e os óleos vegetais (Fugita et al., 2018). Apesar de esses compostos serem sinérgicos, não se tem uma resposta clara sobre seus efeitos. A literatura é escassa de resultados de pesquisa com a associação de compostos em animais suplementados a pasto, no entanto, espera-se que em virtude do potencial dos aditivos em melhorar o desempenho (Laguerre et al., 2007).

Óleos essenciais

As plantas em seu metabolismo cotidiano produzem compostos primários e secundários para manutenção de suas funções vitais (Demirtaş et al., 2018; Wink, 2015). Os óleos vegetais e os óleos essenciais são umas dessas substâncias (Wang et al., 2017), atuam de forma secundária na proteção contra situações adversas e predadores. Podem ser extraídos de várias partes da planta na forma líquida ou oleosa, geralmente de coloração amarelada e aroma intenso (Benchaar et al., 2008; Burt, 2004). Esses compostos são instáveis na presença da luz, oxigênio, altas temperaturas e umidade e são solúveis em solventes apolares e pouco solúveis em água, formados por compostos de baixa massa molecular e por isso, voláteis (Vitti & Brito, 2003).

Os óleos essenciais, que são aditivos naturais e constituem, de forma geral, uma mistura de compostos terpenóides e aromáticos, extraídos geralmente por destilação a vapor (Calsamiglia et al., 2007). A composição química pode ser bastante variável em qualidade e em quantidade de acordo com a cultura, região anatômica da planta, ambiente de colheita, tipo de cultivo, entre outros (Amorati et al., 2013). Essa variabilidade é um dos principais questionamentos no uso dessas substâncias na dieta dos animais, a falta de uniformidade do produto, ausência de padronização da atividade antioxidante e o desafio de produção em larga escala, leva alguns pesquisadores a preferirem compostos sintéticos (Bakkali et al., 2008).

Geralmente os óleos essenciais são caracterizados em sua composição química por muitos compostos, porém observa-se dois ou três componentes principais, ou seja, em concentrações elevadas (20-70%) e outros presentes em quantidades vestigiais. Estes compostos principais determinam as propriedades biológicas do produto (Bakkali et al., 2008). Acredita-se também que exista um efeito sinérgico, onde os elementos secundários atuariam como potencializadores dos princípios ativos primários (Kamel, 2000).

Em contrapartida, os óleos vegetais, que também fazem parte dos aditivos naturais são adicionados à dieta dos animais com outro objetivo, pois desempenham funções, além do simples aporte de energia normal. Supõe-se que essas substâncias possuem capacidade antimicrobiana, atuando de forma semelhante aos antibióticos promotores de crescimento, inibindo enzimas que conferem resistência às bactérias, e possuem, ainda, atividade antioxidante e anti-inflamatória (Diao et al., 2014; Guil-Guerrero et al., 2016; Radha et al., 2014; Szczepanski & Lipski, 2014).

O óleo essencial da folha de cravo (*Eugenia caryophyllus*) contém como principal composto o eugenol, sendo encontrado em média de 83% a 90% (Biondo et al., 2017; Silvestri et al., 2010) em sua composição. Este óleo é amplamente utilizado como antisséptico por possuir um alto potencial bactericida, fungicida e nematicida (Deans & Ritchie, 1987; Mulla et al., 2017; Tomaino et al., 2005).

O óleo de mamona (*Ricinus communis* L.), também vegetal, contém predominantemente o ácido ricinoléico, que junto com outros ácidos graxos insaturados correspondem a 97% da massa da composição do óleo. Relatos que esses ácidos graxos reduzem a digestibilidade e a relação acetato:propionato, inibem a produção de metano e alteram a resistência bacteriana, aumentam a síntese microbiana e reduzem a concentração de amônia ruminal, contribuindo assim para o desempenho animal (Van Nevel, 1991).

O óleo de caju (*Anacardium occidentale*), considerado óleo vegetal, possui atividades antimicrobianas que são atribuídas aos princípios ativos ácidos anacárdico e cardol, que atuam como ionóforo monovalente. As atividades anti-inflamatória e antioxidante são atribuídas ao composto ativo cardanol (Amorati et al., 2013; Amorati et al., 2001; Trevisan et al., 2006).

Os óleos essenciais ainda podem ser microencapsulados na forma *in natura* (pouco usual) e/ou compostos sintéticos semelhantes aos componentes presentes nos óleos essenciais naturais. Nesse caso, surge uma opção para produção em larga escala e padronização da uniformidade. Esses compostos microencapsulados são utilizados no sentido de preservar a molécula do óleo, que são de natureza volátil. Geralmente são utilizados na forma de misturas, explorando diversas características de vários óleos. Não foram encontrados na literatura trabalhos que elucidem o modo de ação desses compostos, porém sugere-se que sua ação seja em nível intestinal no metabolismo dos animais (Spanghero et al., 2009b).

O sinergismo dos compostos utilizados na dieta é amplamente relatado na literatura, principalmente quando se trata de óleos. Portanto, a mistura dos compostos citados acima (óleo essencial de cravo, óleo vegetal de mamona e caju e compostos microencapsulados de eugenol, timol e vanilina) apresentam grande potencial para serem utilizados como aditivo na manipulação da fermentação ruminal em substituição aos ionóforos convencionais utilizados na terminação de bovinos. A adição do óleo essencial de cravo e de óleos vegetais na dieta de bovinos auxiliam o processo de fermentação, manutenção do pH ruminal e melhora a eficiência microbiana. Os compostos microencapsulados podem fazer seleção de bactérias no intestino e são antioxidantes (Spanghero et al., 2009a).

Óleo essencial de cravo

O cravo-da-Índia (*Eugenia caryophyllus*) pertence à família das mirtáceas (*Myrtaceae*) e é uma planta de porte arbóreo que pode atingir em média 10 metros de altura. Suas folhas possuem características aromáticas. Embora ainda desconhecidas muitas de suas propriedades terapêuticas têm sido usadas popularmente no tratamento de muitas doenças na medicina humana (Bakkali et al., 2008).

Os principais produtos derivados do cravo comercializado no mercado são o óleo essencial puro ou produtos derivados dele, cuja principal aplicação é como anestésico local em odontologia e indústria cosmética (Lalko & Api, 2006; Sritabutra & Soonwera, 2013) e mais recentemente na produção animal (Burt, 2004; Calsamiglia et al., 2007).

O óleo essencial de cravo-da-Índia pode ser extraído do caule, das flores e folhas das espécies *Eugenia* spp, e tem como princípio ativo o eugenol (4-alil-2- metoxifenol), que representa de 70 a 90% do óleo (Biondo et al., 2017). O eugenol é um produto natural, considerado seguro para consumo e tem sido utilizado como flavorizante na indústria alimentícia, e recomendado em concentrações até 1.500 µg/mL pela *Food and Drug Administration* (FDA). As propriedades conhecidas de interesse na produção animal são as funções antioxidante, antimicrobiana, antisséptica e anestésica (Karre et al., 2013; Moleyar & Narasimham, 1992).

A atividade antioxidante é atribuída aos compostos fenilpropanóides que podem atuar como antioxidantes primários pelo sequestro de radicais livres formados durante a iniciação ou propagação da reação de oxidação (Biesalski, 2000a, 2000b). Também é relatado ação bactericida por vários autores em alimentos, inibindo e/ou retardando o desenvolvimento de *Staphylococcus* sp, *Micrococcus* sp, *Bacillus* sp e *Enterobacter* sp na carne (Geraci et al., 2012) e no rúmen (Calsamiglia et al., 2007).

Além do cravo, o eugenol é constituinte de vários outros óleos essenciais, como canela, sassafrás e a mirra (Kim et al., 1997). O cariofileno ($C_{15}H_{24}$) presente nesse óleo em menor quantidade pode ser empregado na produção animal como anti-inflamatório, anti-neoplásico, antialérgico, bactericida e repelente. Ainda, possui segundo alguns estudos, ação terapêutica nas infecções produzidas por estafilococos, especialmente quando aplicado em feridas contaminadas (Legault & Pichette, 2007; Shimizu, 1990).

A produção do cravo no Brasil é em torno de 6 toneladas/ano, sendo o 3º produtor mundial. A Bahia é a maior produtora dessa especiaria, a área plantada estimada é de 8 mil hectares e produção de 4 toneladas, quase em sua totalidade exportada (CEPLAC, 2013).

Óleo de mamona

A mamona ou rícino (*Ricinus communis* L.) é o fruto da mamoneira, de origem afro-asiática e nativa de regiões tropicais, da família Euphorbiaceae. O óleo de mamona é considerado como óleo vegetal. Ele é extraído por prensagem, e é um produto da produção de biodiesel. No entanto, destaca-se economicamente pela versatilidade química no ramo industrial (Kadri et al., 2011). É um composto basicamente de ácido ricinoléico (89,5%), seguido de outros ácidos graxos em menor proporção como o ácido linoleico (4,2%), ácido oleico (3,0%), esteárico (1,0%), palmítico (1,0%), ácido hidroxi esteárico (0,7%), ácido linolênico (0,3%) e ácido eicosanoico (0,3%) (Ogunniyi, 2006; Vaisman et al., 2008). Devido sua estabilidade em temperaturas superiores àquelas usadas na extrusão (200º C) (Costa et al., 2009), permite ser classificado como um óleo estável, pois não sofre perdas por volatilização.

O ácido ricinoléico apresenta destacáveis efeitos analgésicos e anti-inflamatórios, e possui ação bactericida e citolítica, dissolvendo a quitina, constituinte da membrana celular de microrganismos. Ainda, estudos preliminares apontam efeitos anticancerígenos atribuídos ao óleo de mamona (Ogunniyi, 2006).

Óleo de caju

O cajueiro (*Anacardium occidentale*) é uma planta tropical, originária do Brasil. No processo industrial para obtenção da amêndoa origina-se o líquido da castanha de caju (LCC). Utilizado para diversas aplicações na indústria (Calo et al., 2007; Calo et al., 2015; Trevisan et al., 2006). O LLC possui altas concentrações de lipídeos fenólicos, que o torna a maior fonte de origem natural dos ácidos anacárdico, cardol e cardonol. As concentrações dos ácidos variam em função do processo de obtenção da amêndoa. A concentração dos ácidos graxos no LLC natural varia de 71,70 a 82,00 % para o ácido anacárdico, de 13,80 a 20,10 % para o ácido cardol e 1,60 a 9,20 % para o ácido cardonol (Mazzetto et al., 2009).

Mistura de compostos microencapsulados

Os compostos voláteis presentes nos óleos essenciais são quimicamente instáveis na presença de ar, luz ou quando expostos a temperaturas elevadas. Portanto, torna-se necessário preservar os compostos aromáticos de forma a impedir ou minimizar as referidas alterações, principalmente no armazenamento das rações. O encapsulamento é uma das tecnologias possíveis conducentes a esta estabilização. Esta técnica permite que compostos do aroma sejam preservados numa base inerte, retardando a perda de compostos voláteis e possibilitando a liberação na altura mais conveniente, no caso no trato digestivo do animal. Outro motivo para microencapsular essas partículas seria a estabilidade no rúmen (Spanghero et al., 2009a).

Óleos essenciais sobre a qualidade da carne bovina

O conhecimento atual do poder antioxidante dos óleos essenciais vem despertando interesse no uso desses compostos no mundo inteiro, na tentativa de reduzirem os efeitos oxidativos da carne ao longo da vida útil (Jayasena & Jo, 2013; Kempinski et al., 2017; Monteschio et al., 2017). A oxidação causa efeitos indesejáveis no produto alterando características sensoriais, como maciez, suculência, sabor e cor. A polêmica na utilização desses compostos seriam substituir o uso de antioxidantes sintéticos, como BHA, BHT e etoxiquina, pois podem apresentar efeitos nocivos à saúde, sendo que são proibidos em diversos países (Ramalho & Jorge, 2006).

Após o abate do animal e consequentemente, perda da circulação sanguínea, ocorrem diversas alterações bioquímicas em nível celular, como a queda de pH e aumento da solubilidade de íons no meio celular. Com isso, o funcionamento de todo mecanismo de ação dos componentes antioxidantes de defesa fica debilitado e a suscetibilidade à oxidação da carne é aumentada (Harris & Shorthose, 1988; Harris et al., 2001).

Nesse sentido, com o aumento do tempo de armazenamento da carne vão se formar compostos reativos ao oxigênio e reações de redox catalisadas por metais de transição, principalmente o ferro, presente em grande quantidade na carne. Esses fatores vão contribuir para ocorrer o processo de oxidação proteica e lipídica (Biesalski, 2000a, 2000b). O grau de insaturação dos ácidos graxos presentes, os pigmentos heme e metais de transição são os principais precursores das reações de degradação lipídica e proteica nas carnes (Xiong, 2000).

Algumas alternativas têm sido utilizadas buscando a redução da oxidação e seus efeitos negativos, buscando o aumento do tempo de conservação da carne nas prateleiras dos supermercados, como incorporação de agentes antioxidantes na dieta dos animais (Falowo et al., 2014; Juárez et al., 2012) e uso de embalagens inteligentes (Kim et al., 2010; Realini & Marcos, 2014).

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CAPÍTULO II

(Journal of Animal Physiology and Animal Nutrition)

Mix of clove, castor, cashew oils and a microencapsulated compound of eugenol, thymol and vanillin in the supplementation of crossbred young bulls finished in a pasture system on animal performance, feed intake, rumen fermentation and rumen microbial populations

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Abstract

Forty 20-month old crossbred steers of 416.9 ± 5.56 kg initial body weight were reared on oat and ryegrass pasture and supplemented with a natural additive blend containing clove essential oil and cashew oil and castor vegetables oils and a microencapsulated blend of eugenol, thymol and vanillin for 80 days until reaching 494.1 ± 9.11 kg slaughter weight. Treatments included a control group (no natural additive inclusion), and natural additive inclusion in dosages of 1500, 3000, 4500 or 6000 mg/animal/d. Animal performance, feed intake, rumen fermentation and rumen microbial populations were evaluated. The results suggest that although the use of the natural additive blend as supplementation in grazing steers did not modify ($P > 0.05$) the animals' body weight gain. The supplement had a quadratic effect ($P < 0.05$) on forage intake and consequently on nutrients including crude protein, neutral detergent fiber, ether extract, and non-fibrous carbohydrates. A quadratic effect ($P < 0.05$) was also observed on the digestibility of crude protein, neutral detergent fiber, and non-fibrous carbohydrates. An increase ($P < 0.05$) in the concentrations of rumen ammoniacal nitrogen, and propionic and isovaleric volatile fatty acids was recorded when comparing treatments with or without the addition of natural additives. No effects ($P > 0.05$) were observed on the microbiological population of the rumen. In conclusion, the use of a mixture of natural additives for dietary supplementation in grazing cattle did not modify the performance, but did alter food intake, digestibility rumen, ammoniacal nitrogen, volatile fatty acids and microbiological population of the rumen.

KEY WORDS: cashew oil, castor oil, cattle, clove oil, natural plant extract

1 INTRODUCTION

Recent years have seen a general increase in consumer concern regarding the profile of additives in animal feed and food sources, prompting the industry to study natural additives (NAs) have been promoted to replace synthetic products (Jiang & Xiong, 2016; Patra & Saxena, 2010; Prado et al., 2015; Valero et al., 2016).

Among the wide variety of NAs currently available, vegetable and essential oils are the most commonly used as modulators of microbial flora. The essential oil of clove (*Eugenia caryophyllus*) has shown to have a positive effect on rumen modulation *in vitro*

(Castillejos, Calsamiglia, Martín-Tereso, & Ter Wijlen, 2008; Remmal, Achahbar, Bouddine, Chami, & Chami, 2011), as well as on animal performance and carcass dressing (Fugita et al., 2018; Monteschio et al., 2017; Ornaghi et al., 2017; Rivaroli et al., 2017). Alternative vegetable oils also have a proven antimicrobial capacity, in addition to their use as energy supply, including castor oil (*Ricinus communis* L.) and cashew oil (*Anacardium occidentale*) (Cruz et al., 2014; Prado et al., 2015; Valero et al., 2016; Valero et al., 2014). Essential oils may be microencapsulated in either their natural form or as similar synthetic molecules. Such microencapsulated additives are used to preserve the oil molecules, which are volatiles (Monteschio et al., 2017; Rivaroli et al., 2017; Spanghero, Robinson, Zanfi, & Fabbro, 2009).

Previous studies on crossbred beef cattle finished in feedlots have shown that various natural compounds may improve animal performance and favorably alter rumen metabolism (Ornaghi et al., 2017; Rivaroli et al., 2017; Valero et al., 2014). However, similar studies focusing on semi-intensive or oat and ryegrass pasture systems remain scarce.

As the synergism and dose volume of NAs are considered to have a considerable impact on animal response (Ait-Ouazzou et al., 2012; Chaves, Baah, Wang, McAllister, & Benchaar, 2012), mixing of the above-mentioned additives (clove essential oil, castor and cashew vegetables oils) and the use of a microencapsulated principle blend (eugenol, thymol and vanillin) offer great potential for use as NAs in animal feed.

The present work was thus undertaken in order to evaluate the effect of NAs blend supplementation on animal performance, feed intake, rumen fermentation and rumen microbial populations in crossbred steers finished in a pasture system.

2 MATERIALS AND METHODS

2.1 Study site, animals and diets

The experimental procedures were reviewed and approved by the respective institutional animal care and use committees was registered under case nº 9827130218.

Experiments were carried out from July to October at a rural property located in Campina da Lagoa, Paraná, Brazil (24°35'34.4"S52°36'38.3"W). This study period was selected as it encompassed the regional dry-to-rainy transition season; thus making it possible to employ temperate pastures due to the lower temperatures, as well as adopt the local cultural practice used for the deposition of organic matter in the soil in the soybean off-season. The rainfall was 33 mm in July, 201 mm in August, 49 mm in September, and 58 mm in October. The average availability of forage dry matter (DM) during the experiment was 4489.6 kg ha^{-1}.

Forty 20-month old crossbred steers (Bons Mara x Nellore) of initial body weight 416.9 kg, all immunologically castrated (Bopriva®, Zoetis), were kept in a pasture of white oat (*Avena sativa*) consortium with ryegrass (*Lolium perene*), covering an area of 70 ha with continuous intensive stocking. The animals were sent daily to the paddocks where they were supplied with the concentrate containing NAs, according to table 1.

Animals were distributed in a completely randomized design comprising five treatments in which different doses of the NAs blend were tested.

The concentrate from each treatment was provided once daily (0900 h) in individual pens (with latches) in the amount of 1.77 kg DM/animal (composition g/kg, as fed: 1672.7 g cracked corn, 13.3 g soybean meal, 46 g mineral salt, 34.3 g limestone, 11.7 g dicalcium phosphate, and 4 g yeast), with only the amount of additives changed according to the dosages displayed in Table 2. Supplement intake took place as planned.

The clove essential oil contained 845 g/kg, 133 g/kg and 13 g/kg of eugenol, carofilene, and eugenyl acetate, respectively (Biondo et al., 2017); the cashew oil contained 750 g/kg anarcardic acid, 153 g/kg cardol, and 41 g/kg cardanol; and the castor oil contained 895 g/kg ricinoleic acid, 42 g/kg linoleic acid, and 30 g/kg oleic acid. Clove essential oil were obtained from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil). The cashew and castor vegetables oils and microencapsulated blend (eugenol, thymol and vanillin active principles) were obtained from Safeeds® (Cascavel, Paraná, Brazil). The liquid textured oils were first added one at a time until completely homogenized, with the microencapsulated oils added later with the concentrate in a commercial mixer every two weeks, when the diets were prepared.

2.2 Experimental procedure and sampling

Animals were adapted for 14 days and then spent 80 days in the experimental trials, which were divided into four 20-day periods. For performance evaluation, the animals were weighed on a trunk balance (Toledo® MGR 3000 JUNIOR) at the beginning and end of the experiment after 14 h fasting.

Samples used for the chemical composition analysis of the pasture consumed by the animals were obtained by hand plucking every 20 days to quantify the forage mass, making a cut approximately 1 cm above the ground in ten randomly chosen areas delimited by a metal square (0.5 m^2).

To evaluate voluntary intake and digestibility, a 12-day digestibility trial was carried out from the 40^{th} day of the experimental period. Estimation of fecal excretion was undertaken by feeding the animals titanium dioxide as an external marker (Detmann et al., 2012), supplied as a supplement at 10 g/animal/d (Titgemeyer, Armendariz, Bindel, Greenwood, & A., 2001). Forage dry matter intake (DMI) was estimated by using indigestible neutral detergent fiber as an internal marker (Zeoula et al., 2002).

The first 7 days of the experiment were used to stabilize marker flow in the gastrointestinal tract, while the last 5 days were used for feces collection at different times (at 0600, 0900, 1200, 1500 and 1800 hours, respectively). Fecal samples of approximately 200 g were collected directly from the rectum and stored in a cold chamber at -26º C. Samples were then oven-dried (60° C/72 h) and proportionally pooled per animal. On the 7^{th} day of the digestibility assay, a forage sample was obtained via the hand-plucking method to estimate voluntary intake and digestibility.

Samples of ruminal fluid were collected via oral stomach tube (11 mm diameter) and manual vacuum aspirator (TE-058, Tecnal in Piracicaba, São Paulo, Brazil), filtered through a double cotton cloth, and conditioned according to the analysis to be used. A total of 400 mL ruminal fluid was sampled from several different anatomical regions of the rumen.

The animals were slaughtered at approximately 23 months of age, at which time their average body weight was 494.1 kg, in a commercial slaughterhouse (Campo Mourão, Paraná, Brazil) following the slaughtering standards of the State Inspection Service Brazilian Legislation.

2.3 Sample processing

The samples used for quantifying chemical composition of the ingredients diets, forage and faeces were ground in a knife mill with a 2-mm sieve. The DM content was determined by oven-drying at 65° C for 24 h and then drying at 135° C for 3 h (Method 930.15) (AOAC, 2005). The organic matter (OM) content was calculated as the difference between the DM and ash contents, with ash determined by combustion at 550° C for 5 h (method 930.05) (AOAC, 2005). The N content in the samples was determined by the Kjeldahl for crude protein (CP) (method 976.05). The ether extract (EE) by Soxhlet method (method 920.39) (AOAC, 2005). For analysis of neutral detergent fiber (NDF) and acid detergent fiber (ADF), samples were treated with α-thermostable amylase without sodium sulfite and corrected for ash residue (Mertens, 2002) and residual nitrogen compounds (Licitra, Hernandez, & Van Soest, 1996).

Indigestible neutral detergent fiber (iNDF) was analyzed as described by Valente et al. (2011). Sample amounts of 1.5 g were added to pre-weighed polyester cloth Saatifil PES 12/6 (Saatitech S.p.A., 22070 in Veniano, Como, Italy) with a pore size of 12 μm and open surface area of 6%. The bags were incubated for 288 h in the rumen of 2 steers fed a diet consisting of 50% corn silage and 50% concentrate (DM basis) at maintenance level (Huhtanen, Kaustell, & Jaakkola, 1994). After removal from the rumen, the bags were rinsed, dried at 45° C for 48 h, and weighed. Residues were then analysed for NDF in an Ankom 200/220 Fiber Analyzer (Ankom Technology Corp in USA). Heat-stable α-amylase (Mertens, 2002) was used in the determination of NDF.

Non-fiber carbohydrates (NFC) were calculated according to Detmann et al. (2012). For converting metabolisable energy (ME) requirement into digestible energy requirements, the factor of 0.82 was used (NRC, 2000).

Fecal samples were evaluated for titanium dioxide content via both atomic absorption spectrophotometry (Thermo Scientific, Genesys Scanning 10 mV in USA) (Detmann et al., 2012) and colorimetric methods (Titgemeyer et al., 2001). Fecal excretion and forage DMI were estimated by rationing the quantity of TiO_2 offered and calculating the concentration in feces.

Ruminal pH was estimated using a digital potentiometer (Hanna HI 2211 in Limena, Italy). The method described by Detmann et al. (2012) was used for analysis of ammoniacal nitrogen concentrations. Short-chain fatty acid and gas quantification were conducted via gas chromatography using a SP-2560 capillary column (100 m × 0.25 mm in diameter 0.02 mm thick) (Palmquist & Conrad, 1971).

Macroscopic analyzes of color (1 – olive green, 2 – brownish green, 3 – yellowish brown color, 4 – grey and 5 – darker greenish), odor (1 – aromatic, 2 – acid and 3 – putrid) and viscosity (1 – viscous, 2 - viscous or frothy bloat and 3 – lightly viscous) were performed according to Nagaraja & Titgemeyer, 2007 and the physical-chemical analyzes of potential redox (1 – active (0 to 3 min); 2 – normal (3 to 5 min) and 3 – reduced (greater than 5 min), sedimentation and flotation time (1 – active (0 to 4 min), 2 – normal (4 to 8 min) and 3 – reduced (greater than 8 min) and density and quantification of protozoa (1 – absent, 2 – little, 3 – normal and 4 – abundant) according to Dehority (1984).

2.4 Statistical analyses

All studied variables were tested for normality, with those exhibiting a normal distribution submitted to variance analysis (ANOVA) via an adjusted regression model (animal performance, feed intake, digestibility, ruminal pH, concentration of ruminal ammoniacal nitrogen, concentration of volatile fatty acids, and microbiological protozoa viability), and those that did not subjected to the Kruskal-Wallis non-parametric method (all ruminal fluid parameters with the exception of microbiological protozoa viability). Orthogonal contrast was used to evaluate the effects of the control treatment versus natural additives. In all statistical analyses, diet was considered a fixed effect and the animals a random effect. Differences between means were compared using the Tukey test ($P < 0.05$). The statistical program used was the SPSS v.21 (IBM Corporate Headquarters in Armonk, NY).

3 RESULTS AND DISCUSSION

The chemical compositions of the forage and concentrate are shown in Table 2. Animals had restricted access to the concentrate containing the NAs (1.77 kg DM/d), and *ad libitum* access to forage.

An average CP value of 11.2% was recorded for the oat and ryegrass consortium. This value is somewhat lower than those of above 15% found by Roso, Restle, Soares, and Andreatta (2000) and Rocha et al. (2007), whose mean value above 15%, but similar to the 10.1% reported by Prohmann et al. (2004). It should be noted that in the present study, grazing began near the end of the ryegrass vegetative cycle. This consortium is widely used in southern Brazil, since oats make it possible to anticipate the use of pasture, and ryegrass prolongs this cycle.

The average NDF value was 66.0% for the pasture, with average ADF 39.6%, which may limit consumption. Mean values of DM, OM, EE and ME were 22.8%, 67.1%, 1.8% and 250.9 Mcal/kg, respectively, all of which are somewhat below levels normally found. However, in addition to the later plant stage, frosts were also recorded throughout the duration of the experiment (Prohmann et al., 2004; Rocha et al., 2007).

Although the addition of NAs did not influence the final live weight (FBW) of the animals, it did result in a linear decrease ($P < 0.07$) in the average daily gain (ADG) and consequently also the total average gain (Table 3). Nevertheless, such effects were not evident in steer performance, and can thus be explained by the decrease in forage intake (NA30, NA45 and NA60), CP intake, and fiber digestibility (NA15, NA30 and NA45).

A non-significant linear decrease in ADG was recorded as the level of natural compounds in the diet increased ($P = 0.07$). In addition, feed intake exhibited a quadratic reduction ($P < 0.05$) in all variables (DM, CP, NDF, EE and NFC). These findings are important, as the literature is very scarce regarding the effect of NAs or their components on the feed intake and performance of ruminants, especially those in pasture.

As the rumen is the anaerobic chamber in which DM and food fiber are digested, changes in the digestibility of these components are important indices used in the evaluation of NA impact on ruminant digestion. In the present study, whereas no differences were observed in the digestibility of DM ($P > 0.05$), a quadratic effect was recorded for NDF digestibility ($P < 0.05$). These results agree with those of Metwally, Deml, Carmen, and Wihelm (2016) for Friesian dairy cows fistulated with the addition of a 1g/d blend of various essential oils, including thymol, m-cresol, guaiacol, eugenol, and resorcinol.

Animal performance was found to be directly dependent on daily feed intake (Maggioni et al., 2009), with a quadratic effect recorded on the digestibility of nutrient CP and NDF ($P < 0.05$). Orthogonal contrast analysis also revealed variation in CP digestibility between treatments with and without natural additives ($P < 0.05$).

The effect of the selected additives on forage consumption and fiber digestibility varied with dose, with the highest intake of DM observed in treatment NA15, and the lowest intake in treatment NA60. This increase in DMI also influenced the intake of other nutrients (CP, NDF, EE and NFC). In fact, a number of feedlot studies have shown that high doses of NAs may inhibit the growth of certain cellulolytic ruminal bacteria, which may compromise fiber digestion and limit consumption due to an increased rumen filling effect (Maggioni et al., 2009). The results found here are similar to those reported by McIntosh et al. (2003), who fed fistulated Holstein-Friesian cows with a 1g/d mix of thymol, eugenol, vanillin and limonene essential oils, and Lin et al. (2013), who fitted Hu sheep with ruminal and duodenal fistula to investigate the effects of a 1g/d mixture of essential oils of clove, oregano, cinnamon and lemon (using 0.5 or 1.0 g/d combinations of the active components eugenol, carvacrol, citral, and cinnamaldehyde).

A lower population of cellulolytic bacteria may lead to a reduction in fiber degradation, reducing the access of proteolytic bacteria to the nitrogen bound to the fibrous fraction, and indirectly reducing protein degradation (Rízpoli et al., 2009).

The current results suggest that doses above 1500 mg/animal/d are too high for cattle grazing in temperate grassland, and thus studies involving doses below this value are required. Nevertheless, higher NFC digestibility was observed in treatments that received natural additives in the diet.

The mean ruminal pH of 7.74 was unaffected by the addition of NAs at the levels used in the present study (Table 4). Although this value is higher than that reported elsewhere for cattle, ruminal pH can be influenced by the fluid collection method employed, which frequently varies between studies (Salles, Zanetti, Del Claro, Netto, & Franzolin, 2003).

RAN concentrations exhibited both quadratic behavior ($P < 0.05$) and an orthogonal contrast effect ($P < 0.05$). The higher values observed here are potentially linked to lower NFC fermentation (Table 3), since the synthesis of microbial protein in the rumen is dependent on carbohydrate availability.

Metwally et al. (2016) found a strong increasing tendency in the degradability of crude protein in protein-rich foods such as soybean and canola meal, possibly reflecting the activation of proteolytic bacteria due to the addition of NAs. In contrast, McIntosh et al. (2003) and Newbold, McIntosh, Williams, Losa, and Wallace (2004) observed a reduction in the ammoniacal nitrogen production rate in cows and sheep fed respectively with a 1 g and 100 mg/d mix of thymol, eugenol, vanillin and limonene essential oils, suggesting that these additives inhibited the activity of ammonia-producing bacteria.

The total concentration of VFA was also similar between treatments, as found by other authors (Benchaar, Petit, Berthiaume, Whyte, & Chouinard, 2006; Metwally et al., 2016). However, when comparing the control treatment with NA addition, higher production of propionic and isovaleric acids was observed in the latter (P = 0.05). Ruminal concentrations of propionic acid indicate fermentation of soluble sugars and starch, while higher concentrations of isovaleric acid are indicative of the fermentation of amino acids, suggesting modification of the microbial population in the rumen. However, Busquet, Calsamiglia, Ferret, and Kamel (2006), who examined different doses of 12 plant extracts and 6 secondary plant metabolites, found that some oils affected rumen fermentation, with total VFAs reduced with a linear increase in the molar concentration of propionate.

Movement of the rumen-reticulum promotes rumination (Elischer, Arceo, Karcher, & Siegford, 2013). In the present study, animals in treatment NA30 exhibited a greater number of ruminal movements (P > 0.05) (Table 5), as well as lower NDF digestibility. In contrast, treatment NA60 was associated with a lower number of ruminal movements and higher NDF digestibility.

Ruminal fluid color and odor were not influenced by NA in the diet (P > 0.05), with all animals presenting olive green fluid and an aromatic odor indicative of ruminal health. Regarding consistency, treatment NA60 presented greater viscosity (P < 0.05) of content compared to the other groups, which presented a more aqueous content (P < 0.05).

The ruminal fluid of NA15 and NA30 animals had a longer sedimentation time (P < 0.05) (4 to 8 min) than that of the other groups (0 to 4 min).

According to the redox potential tests, the ruminal fluid of animals in treatment NA30 presented a more active metabolism than those in CON and NA15, whose activities were closer to those of normal metabolization (P < 0.05). Values for all other treatments were similar, at around 1.2. However, although all the parameters evaluated in this study indicated healthy rumen function, and thus the addition of NA to the diet did not affect the ruminal environment, it did not induce pathological changes such as defaunation of microflora. This finding correlates with those observed by Sallam et al. (2011) for the addition of citrus essential oil (0.5 and 0.75 mg/d) and its secondary metabolite limonene (0.45 and 0.60 mg/d). The *in vitro* study carried out by Cieslak, Zmora, Nowakowska, and Szumacher-Strabel (2009) also confirmed the potential of limonene to inhibit the power of protozoa (at 40 or 400 mg/L), while Wanapat, Cherdthong, Pakdee, and Wanapat (2008) observed similar results for the addition of lemon grass essential oil (at 100, 200 or 300 g/d).

Microbiological protozoa populations were not influenced by the inclusion of the selected NAs in the steer diet ($P > 0.05$), with an average total count of $242.1 x 10^3$/mL and mean percentages of viable protozoa of 66, 72, 76, 80 and 84% in treatments CON, NA15, NA30, NA45 and NA60, respectively. However, an increasing tendency in the percentage of viable protozoa was recorded at higher NA levels ($P = 0.10$). The average density of protozoa was 1.5 points, a value classified as abundant to moderate. On the basis of these data, no defaunation was observed, a phenomenon closely related to an increase in ruminal transit rate and an increase in the metabolism of bacterial protein.

Populations were dominated by large protozoa (1.6 points – abundant to moderate), followed by medium (2.92 points – moderate) and small protozoa at lower frequencies (3.0 points – low). No significant were recorded between the counts of any groups, indicating that the presence of the NAs did not impair ruminal fauna, and was not toxic to any specific group of protozoa. Thus, the inclusion of the selected NAs in the steer diet did not alter any of the microbiological parameters evaluated. These results are similar to those of Newbold et al. (2004) and Benchaar, Duynisveld, and Charmley (2006), who also found no influence of natural additive use on protozoa numbers.

4 CONCLUSION

The results suggest that the use of a mixture of natural additives for dietary supplementation in grazing cattle did not modify the animals' body weight gain, but did alter food intake and digestibility. An increase in the concentration of rumen ammoniacal nitrogen was also recorded, as well as in propionic and isovaleric volatile fatty acids. No marked effects were observed in the microbiological population of the rumen. These results suggest that doses above 1500 mg/animal/d are high for livestock grazing on temperate pasture, and that studies conducted using doses below this value are required.

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TABLE 1. Doses of the natural additive mix supplemented in the experimental diets

Natural additive	Experimental diets				
	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]
Clove essential oil[6]	0	500	1000	1500	2000
Cashew oil[7]	0	250	500	750	1000
Castor oil[7]	0	250	500	750	1000
Eugenol/thymol/vanillin microencapsulated[7]	0	500	1000	1500	2000
Total, mg/animal/day	0	1500	3000	4500	6000

[1]Control: 0 mg of NA/animal/d;
[2]NA15: 1500 mg NA/animal/d;
[3]NA30: 3000 mg of NA/animal/d;
[4]NA45: 4500 mg of NA/animal/d and
[5]NA60: 6000 mg of NA/animal/d.
[6] Product were obtained from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil).
[7] Products were obtained from Safeeds® (Cascavel, Paraná, Brazil).

TABLE 2. Ingredients and chemical composition of diets

Ingredients	Chemical composition							
	DM[1]	CP[2]	OM[3]	EE[4]	NDF[5]	ADF[6]	ME[7*]	Diet, %
Forage, % DM								
Oat + ryegrass	22.8	11.2	67.1	1.8	66.4	39.6	250.9	-
Concentrate, % DM								
Cracked corn	88.9	10.0	99.1	3.5	17.7	4.4	325.38	94.5
Soybean meal	88.6	49.7	93.7	1.3	13.7	5.9	260.3	0.75
Salt	98.0							2.5
Limestone	98.0							1.94
Dicalcium phosphate	98.0							0.65
Yeast[8]	98.0	30.0	98.0					-
Diet (%)	89.1	19.6	94.6	2.83	16.1	4.65	298.6	

[1]DM dry matter;
[2]CP crude protein;
[3]OM organic matter;
[4]EE ether extract;
[5]NDF neutral detergent fiber;
[6]ADF acid detergent fiber;
[7]ME metabolizable energy; *Values expressed in Mcal/kg DM;
[8]BIOSAF®, Saccharomyces cerevisiae from strain Sc 47, at a concentration of 1×10^{10} cfu/g of product.

TABLE 3. Animal performance, feed intake and in vivo digestibility of steers with natural additives in the diet.

Items	Experimental diet					SEM[6]	P < value		
	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]		L	Q	0 vs NA
Performance, kg									
Initial weight	410.8	411.0	410.3	411.9	411.4	6.96	0.966	0.999	0.723
Final weight	494.3	485.3	477.4	482.5	476.0	7.32	0.453	0.726	0.158
Average daily gain	1.06	0.94	0.85	0.89	0.82	0.04	0.068[a]	0.156	0.831
Intake kg/d									
Dry matter	10.64	11.31	9.61	9.68	9.40	0.186	0.002	0.011[b]	0.494
Dry matter forage	8.88	9.55	7.85	7.92	7.64	0.186	0.002	0.011[c]	0.949
Crude protein	1.24	1.31	1.13	1.13	1.10	0.209	0.002	0.009[d]	0.586
Neutral detergent fiber	6.74	7.18	6.05	6.11	5.92	0.124	0.002	0.011[e]	0.493
Ether extract	0.39	0.42	0.36	0.39	0.35	0.006	0.002	0.011[f]	0.504
Non fibrous carbohydrate	1.88	2.01	1.67	1.69	1.63	0.03	0.002	0.010[g]	0.517
Apparent digestibility g/kg DM									
Dry matter	581.1	589.8	585.6	542.3	593.5	0.655	0.785	0.788	0.822
Crude protein	843.3	624.8	583.2	519.0	583.7	2.829	0.001	0.001[h]	0.001
Neutral detergent fiber	590.8	581.9	572.3	580.2	611.8	0.488	0.305	0.068[i]	0.524
Ether extract	761.6	814.2	802.8	766.4	783.9	0.659	0.969	0.224	0.110
Non fibrous carbohydrate	331.6	563.7	641.5	418.0	534.8	3.066	0.235	0.05[j]	0.017

[1]Control: 0 mg of NA/animal/d.
[2]NA15: 1500 mg NA/animal/d.
[3]NA30: 3000 mg of NA/animal/d.
[4]NA45: 4500 mg of NA/animal/d.
[5]NA60: 6000 mg of NA/animal/d.
[6]Standard error of means.
[a]$\hat{Y}$=1.06-0.02X (r^2=0.260).
[b]$\hat{Y}$=10,69+0.15X-0.76X^2 (r^2=0.483).
[c]$\hat{Y}$=9.40-0.19X-0.02X^2 (r^2=0.359).
[d]$\hat{Y}$=1.31-0.03X-0.001X^2 (r^2=0.444).
[e]$\hat{Y}$=7.16-0.22X-0.007X^2 (r^2=0.430).
[f]$\hat{Y}$=0.20-0.007X (r^2=0.432).
[g]$\hat{Y}$=2.01-0.06X-0.002X^2 (r^2=0.437).
[h]$\hat{Y}$=108.84-29.40X+3.86X^2 (r^2=0.836).
[i]$\hat{Y}$=62.39-3.75X+0.69X^2 (r^2=0.286).
[j]$\hat{Y}$=12.99+27.50X-4.08X^2 (r^2=0.226).

TABLE 4. Ruminal pH, concentration of ruminal ammoniacal nitrogen and concentration of volatile fatty acids (VFA) of steers with natural additives in the diet

Items	Experimental diet					SEM[6]	P < value		
	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]		L	Q	0 vs NA
pH	7.76	7.79	7.73	7.63	7.82	0.034	0.880	0.656	0.891
Ammonia nitrogen, mg/dL	3.72	6.2	17.75	13.93	10.81	2.972	0.018	0.001[a]	0.035
VFA concentration mmol/dL									
Total	43.76	43.98	53.60	57.88	49.86	1.572	0.415	0.714	0.339
Acetic	32.04	29.62	35.59	35.02	33.74	1.992	0.691	0.925	0.170
Propionic	6.16	4.49	6.82	7.56	6.30	0.501	0.549	0.832	0.056
Isobutyric	0.59	0.55	0.63	0.87	0.54	0.042	0.580	0.232	0.206
Butiryc	6.40	5.15	7.43	6.45	4.71	0.440	0.969	0.949	0.218
Isovaleric	0.92	0.82	1.19	1.31	0.99	0.073	0.271	0.142	0.053
Valeric	0.39	0.35	0.42	0.49	0.39	0.033	0.594	0.801	0.256

[1]Control: 0 mg of NA/animal/d.
[2]NA15: 1500 mg NA/animal/d.
[3]NA30: 3000 mg of NA/animal/d.
[4]NA45: 4500 mg of NA/animal/d.
[5]NA60: 6000 mg of NA/animal/d.
[6]Standard error of means.
[a]$\hat{Y} = -11.44+15.24X-2.15X^2$ ($r^2=0.808$).

TABLE 5. Ruminal fluid parameters of steers with natural additives in the diet

Ruminal fluid parameters	Experimental diet					SEM[6]	P <	
	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]		value	0 vs NA
Macroscopic								
Ruminal movements	2.2ab	2.2ab	2.4a	1.8ab	1.6b	0.122	0.042	0.595
Color[7]	2.4	2.6	2.8	2.6	2.6	0.153	0.479	0.592
Odor[8]	1.0	1.0	1.0	1.0	1.0	0.001	0.999	0.999
Consistency[9]	1.8ab	1.2b	1b	1b	2.4a	0.165	0.002	0.911
Sedimentation and flotation[10]	1.4b	2a	1.8a	1.2b	1.6ab	0.115	0.002	0.453
Redox potential[11]	1.6a	1.8a	1b	1.2ab	1.2ab	0.114	0.035	0.452
Microbiological protozoa								
Total count, x10^3/mL	212.9	210.0	276.9	287.5	223.5	29.011	0.640	0.601
Viable, %	66	72	76	80	84	3.830	0.106	0.402
Density[12]	1.8	2	1.8	1.2	1.6	0.138	0.074	0.750
Great[13]	2.0	1.8	1.0	1.8	1.6	0.190	0.092	0.456
Medium[14]	2.8	2.8	2.8	3	3.2	0.140	0.355	0.915
Small[15]	3.0	3.4	3.0	3.0	3.0	0.099	0.214	0.480

[1]Control: 0 mg of NA/animal/d.
[2]NA15: 1500 mg NA/animal/d.
[3]NA30: 3000 mg of NA/animal/d.
[4]NA45: 4500 mg of NA/animal/d.
[5]NA60: 6000 mg of NA/animal/d.
[6]Standard error of means.
[a-b]Different letters on the same line are different (P < 0.05) by Kruskal-Wallis test.
[7]Color (1 - olive green, 2 - brownish green, 3 - yellowish brown color, 4 - grey and 5 - darker greenish).
[8]Odor (1 - aromatic, 2 - acid and 3 - putrid).
[9]Consistency (1 - viscous, 2 - viscous or frothy bloat and 3 - lightly viscous).
[10]Sedimentation and flotation time (1 - active (0 to 4 min), 2- normal (4 to 8 min) and 3 - reduced (greater than 8 min)).
[11]Potential redox (1 - active (0 to 3 min); 2 - normal (3 to 5 min) and 3 - reduced (greater than 5 min)).
[12, 13, 14, 15]Microbiological protozoa (1 - absent, 2 - little, 3 - normal, 4 - abundant).

CAPÍTULO III

(Meat Science)

Carcass characteristics and meat evaluation of cattle finished in temperate pasture and supplemented with natural additives

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ABSTRACT

Forty crossbred steers were supplemented with a natural additive blend containing clove essential oil, cashew oil, castor oil and a microencapsulated blend of eugenol, thymol and vanillin for 80 days. Carcass characteristics, drip loss and antioxidant activity were evaluated 24 h *post mortem* on *Longissimus thoracis*, and the effects of aging (14 days) were evaluated for water losses (thawing/aging and cooking), texture, color and lipid oxidation. The use of the natural additive blend did not modify ($P > 0.05$) carcass characteristics but did, however, modify body composition ($P < 0.05$). Natural additive treatments did not affect ($P > 0.05$) drip losses, although they affected ($P < 0.05$) thawing/aging and cooking losses, texture, color, antioxidant activity and lipid oxidation. Aging affected ($P < 0.05$) thawing/aging and cooking loss, texture, color and lipid oxidation. Based on this study's findings the blend of natural additives has potential use in animal feed and could improve meat stability.

Keywords:

Cashew oil

Castor oil

Clove oil

Natural plant extract

Meat quality

1. Introduction

The use of synthetic additives and growth promoters in cattle nutrition is known to improve performance, feed intake and efficiency (Duffield, Merrill, & Bagg, 2012). However, there is also concern about these products for human health in relation to the possible effects on consumer health for certain food or nutrient residues in final products. As a result, some countries have banned the use of antimicrobial growth promoters in animal feeds (Schäberle & Hack, 2014).

Since Brazil is the largest exporter of beef (FAPRI, 2017), there is a need to serve large markets by producing safe, healthy and sustainable food. Natural additives and mixtures of natural additives are widely accepted by consumers as being authentic and safe (Jiang & Xiong, 2016). Some studies report that these compounds possess antioxidant activity extending up to the meat (Kempinski et al., 2017; Monteschio et al., 2017; Rivaroli et al., 2016; Vital et al., 2018).

A considerable amount of effort has been devoted towards developing natural alternatives to modulate rumen fermentation to replace the synthetic additives, including yeasts, organic acids, plant extracts, probiotics, antibodies and plant secondary metabolites (Cruz et al., 2014; Fugita et al., 2018; Prado et al., 2015a; Valero et al., 2014). The secondary metabolites are naturally occurring chemical compounds in plants, and are primarily involved in plant defense against pathogens to ensure survival of the plant structures and reproductive elements (Demirtaş, Öztürk, & Pişkin, 2018).

Two classes of natural alternatives are vegetable and essential oils. The essential oil of clove is rich in eugenol, which is a phenylpropanoide that has been shown to have positive effects on meat quality (Ornaghi et al., 2017; Rivaroli et al., 2016). Similar effects have been reported for castor and cashew oils; these effects are attributed to terpenoids and phenolic compounds (Cruz et al., 2014; Prado et al., 2015a; Valero et al., 2016; Valero et al., 2014).

Another alternative marketed by some companies as an option for large-scale production and standardization of product uniformity is the microencapsulation technique. The oils can be microencapsulated in the primitive or synthetic form, preserving them from volatilization (Soltan, Natel, Araujo, Morsy, & Abdalla, 2017). They are generally used in the form of mixtures to gain several positive characteristics from each compound (Guerrero et al., 2018; Monteschio et al., 2017). For Burt (2004) additive and synergistic effects have been observed between the components of the oils when used as a blend.

Little is known about the ideal amount of microencapsulated oil compounds to be fed to grazing animals, and there are limited data regarding use and its impacts on meat quality. Research conducted by our work group on animals finished in feedlot has shown that levels above 1500 mg/animal/day can improve the antioxidant activity of meat, in studies accomplished by our work group on animals in feedlot (Monteschio et al., 2017). Including natural compounds with antioxidant activity can improve the quality of the meat through oxidative stability *in vivo*; these compounds are potent free radical scavengers, liposoluble and have antioxidant functions, all of which favor oxidative stability of muscle tissues and oxidation processes in the body (Amorati, Foti, & Valgimigli, 2013).

The purpose of this study was to test the synergism and dose of compounds (vegetables and essential oils), and investigate changes in the response of the finished animals to pasture and consequently the quality of the carcass and meat. To accomplish this, a mixture of the above-mentioned additives (clove essential oil, castor and cashew vegetable oils and microencapsulated essential oil mixtures) were tested at increasing dose.

2. Material and methods

2.1. Study site, animals and diets

The experimental procedures were reviewed and approved by the respective institutional animal care and use committees registered under case nº 9827130218. The study was carried out in a rural property in the Campina da Lagoa, Paraná, Brazil (24°35'34.4"S 52°36'38.3"W).

Forty 20-month old crossbred steers (Bons Mara x Nellore) with an initial body weight 416.9 ± 5.5 kg, were kept in a pasture of white oat (*Avena sativa*) consortium with ryegrass (*Lolium perene*), covering an area of 70 ha with continuous grazing. The cattle were immunologically castrated using Bopriva® (Zoetis, New Jersey, USA). The steers were sent daily to the corral where they were supplied with the concentrate containing natural additives (NA).

Steers were allocated to five natural additive (NA) treatments in a completely randomized design comprising based on the different doses of the NA blends tested (Table 1). The five experimental diets (based on previous studies) were: CON – without natural additives (mixture of clove essential oil, cashew oil, castor oil and microencapsulated principle blend); AN15 – natural additives (1500 mg/animal/day); AN30 – natural additives (3000 mg/animal/day); AN45 – natural additives (4500 mg/animal/ day); and AN60 – natural additives (6000 mg/animal/day). The natural additive is a mixture of clove essential oil, cashew oil, castor oil and microencapsulated principle blend in a ratio 25, 12.5, 12.5 and 25% respectively. Increasing dose levels were tested (0, 1500, 3000, 4500 and 6000 mg).

Table 1

Doses of the natural additive mix supplemented in the experimental diets

Natural additives	Experimental diet[1]				
	CON	NA15	NA30	NA45	NA60
Liquid, mg					
Clove essential oil	0	500	1000	1500	2000
Cashew oil	0	250	500	750	1000
Castor oil	0	250	500	750	1000
Microencapsulated principle blend, mg					
Eugenol/thymol/vanillin	0	500	1000	1500	2000
Total	0	1500	3000	4500	6000

[1]Experimental diet: CON: 0 mg of NA/animal/day; NA15: 1500 mg NA/animal/day; NA30: 3000 mg of NA/animal/day; NA45: 4500 mg of NA/animal/day; NA60: 6000 mg of NA/animal/day.

These concentrations were chosen according to the previous studies (Monteschio et al., 2017; Rivaroli et al., 2017) showed that the most adequate concentrations of the essential oils in the animal diets is between 1500 and 5000 mg/animal/day.

The concentrate from each treatment was provided once daily (0900 h) in individual pens (with latches) in the amount of 1.77 kg DM $animal^{-1}$ (composition g kg^{-1}, as fed: 1672.7 g cracked corn, 13.3 g soybean meal, 46 g mineral salt, 34.3 g limestone, 11.7 g dicalcium phosphate, and 4 g yeast), with only the amount of additives changed according to the dosages displayed in Table 1.

The clove essential oil predominantly contained 845 g kg^{-1}, 133 g kg^{-1} and 13 g kg^{-1} of eugenol, carofilene, and eugenyl acetate, respectively (Biondo et al., 2017); the cashew oil predominantly contained 750 g kg^{-1} anarcardic acid, 153 g kg^{-1} cardol, and 41 g kg^{-1} cardanol; and the castor oil predominantly contained 895 g kg^{-1} ricinoleic acid, 42 g kg^{-1} linoleic acid, and 30 g kg^{-1} oleic acid. Clove essential oil were obtained from Ferquima (Vargem Grande Paulista, São Paulo, Brazil). The cashew oil, castor oil and microencapsulated blend (eugenol, thymol and vanillin active principles) were obtained from Safeeds (Cascavel, Paraná, Brazil). The liquid textured oils were first added one at a time until completely homogenized with the microencapsulated compounds added later with the concentrate in a commercial mixer every two weeks when the diets were prepared.

2.2. Experimental procedure and sampling

Steers were adapted for 14 days and then spent 80 days in the study which was divided into four 20-day periods. For performance evaluation, the animals were weighed on a livestock scale kit (Toledo MGR 3000 Junior, Brazil) at the beginning and end of the experiment after 14 h fasting.

The steers were slaughtered in a commercial slaughterhouse (Campo Mourão, Paraná, Brazil) at approximately 23 months of age (average body weight of 494.1 ± 9.1 kg), following the slaughtering standards of the State Inspection Service Brazilian Legislation.

2.3. Carcass characteristics

After bleeding, skinning, evisceration and washing, the carcasses were divided medially from the sternum and spine, resulting in two similar halves, which were weighed to calculate the hot carcass weight (HCW). The percentage of the hot carcass yield (HCY) was defined as the HCW divided by the live weight 14 hours before slaughter. Next, the half-carcasses were identified and stored in a chilling chamber at 4° C for 24 h period.

At 24 h *post mortem* chilling, the left side of each carcass was fabricated to remove a rib section encompassing the 6^{th} to 13^{th} ribs; each rib section was labeled, vacuum packaged and then transported to the laboratory. Upon arrival at the meat laboratory, rib sections were dissected and separated for each analysis.

The subcutaneous fat thickness (SFT) was measured with electronic digital caliper (Stainless hardened LT-4237-000, China) at a point ¾ of the length of the *Longissimus thoracis* (*LT*) muscle from the bone end between the 12th and 13th ribs. The muscle area (MA) was measured on a transverse cut with a compensating planimeter inch placed over the loin between the 12th and 13th ribs by using a grid expressed in square centimeters (planimeter). The pH was determined with a pH metre (Hanna instruments HI99163, Italy). The electrode was calibrated and inserted into the muscle between the 12th and 13th ribs at and 24 h post slaughtering .

2.4. Body composition

The 6th beef rib was removed and weighed. The rib section was dissected into muscle, fat, bone and tissue others, and each were weighed. Results from rib dissection were used to calculate carcass composition according to Robelin and Geay (1975).

2.5. Storage of meat

Sixteen steaks were cut from the rib section for different analyzes. The steak (2 or 2.5 cm thick) were removed from the *LT* muscle and vacuum packaged after dissection. One steak was immediately frozen at -20° C (day 2 post slaughter) and the other steaks were aged for 7 and 14 d and frozen at -20° C. The vacuum-package 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. Steaks aged for 7 and 14 days were chilled at 4 ± 1º C, simulating typical Brazilian market conditions with artificial, cold white light from 50/50 siliconized Light Emitting Diode (LED) lighting (4.8 W) for 12 hours.

2.6. Water loss and texture

Drip loss was measured using the method described by Honikel (1998). A steak (7th rib) of each animal was taken 24 h *post mortem*, placed in a clear screw top jar (700 mL) suspended by a polyester fabric (tulle) 4 mm thick, and kept at 4° C. After 24 h, the sample was removed from the jar, dried on absorbent paper, and reweighed. Amount of drip at 48 h *post mortem* was expressed as a percentage.

For thawing and aging losses, 8^{th} rib samples were used, the steaks were thawed at 4° C for 12 h. They were then weighed and the thawing losses were calculated as the percentage difference between the fresh and thawed weights.

For cooking loss, the steaks (8^{th} rib) were weighed and wrapped in aluminum foil. Each sample was cooked in a heated grill (Philco Jumbo Inox, Brazil) at 200 °C until an internal temperature of 72° C was reached, which was monitored using an internal thermocouple (Incoterm 9791, 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 loss calculated as the percentage of difference in weight before and after cooking.

To determine the texture, the standard procedure was adopted as proposed by Wheeler et al. (1997). Samples from the cooking loss analyzes were filleted into ten rectangular subsamples parallel to the fiber direction of 2.5 cm in length and 1 cm diameter. The shear force was determined perpendicularly to the orientation of the muscle fibers with the Warner-Bratzler Shear blade adapted in the texture analyzer (Stable Micro Systems TA-XT2i, United Kingdom). The velocities used were 1.99 mm/s in the pre-test, test and in the post-test. The results were expressed in Newtons.

2.7. Instrumental color

Instrumental color measurements was based on the Commission International de I'Eclairage and were recorded for L* (measures darkness to lightness; lower L* indicates a darker color), a* (measures redness; greater a* value indicates a redder color), and b* (measures yellowness; greater b* value indicates a more yellow color). The equipment used was portable Minolta chromameter (Minolta CM-700, Japan) with a 50 mm diameter measurement area using a D65 illuminant, which was calibrated using the white ceramic disk provided by the manufacturer. Color readings were determined at 1, 7 and 14 days *post-mortem* on the LT muscle surface of the 9th rib. Values were recorded from 6 locations to obtain a representative reading. The color was analyzed in the samples after 30 minutes of exposure to oxygen for myoglobin reaction with atmospheric oxygen.

2.8. Phenolic compounds, beef antioxidant activity and lipid oxidation

The steaks were collected from the 10th rib in the *LT* muscle and extracted (1:1 w/v with methanol) in ultra-turrax equipment (IKA T10, United States) at 6000 rpm for 10 seconds, followed by centrifugation (4.000 rpm, 15 min) and filtration (filter paper (grammage – 80 g/m^2, thickness – 205 μm, pores – 14 μm).

The total phenolic content (TPC) was determined as methodology described by Vital et al. (2016), with modifications. Meat extracts (125 μL) was transferred to 5 mL (PVC) tubes, 125 μL Folin-–Ciocalteu and 2250 μL Sodium Carbonate (Na_2CO_3) were added and homogenized. After 30 min of rest in the dark the UV-visible spectrophotometer (Thermo Scientific Evolution 201, Malaysia) reading at 725 nm was performed. The TPC were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to Vital et al. (2016). Meat extract (150 μL) were mixed with 2850 μL of a methanolic solution containing DPPH (60 μM) and reacted for 30 min (protected from light). The absorbance at 515 nm was measured against pure methanol using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, Malaysia). 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.

The 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay was performed based on the method described by Vital et al. (2016). The ABTS solution was prepared by reacting the stock solution of 7 mM ABTS (5 mL) with 140 mM potassium persulfate (88 µL), and then allowing the resting solution to be protected from light at room temperature for 12 – 16 h before use. The 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 using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, Malaysia). 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.

The ferric reducing antioxidant power (FRAP) assay was evaluated using the methodology described by Vital et al. (2016). In this procedure, the meat extracts (250 µL) were mixed with 50 mM sodium phosphate buffers pH 7.0 and 1% potassium ferricyanide, 1.25 ml of each solution and subsequently incubated at 50° C for 20 min. Subsequently, 1.25 mL of 10% trichloroacetic acid was added, and the solution centrifuged at 4000 rpm for 10 min. The top layer of the solution (2.5 mL) was mixed with 0.1% ferric chloride (500 mL) and the samples measured against a white at 700 nm wavelength using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, Malaysia). 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.

The method used to measure lipid oxidation was thiobarbituric acid reactive substances (TBARS) described by Vital et al. (2016). Approximately 5.0 ± 0.2 g of meat were weighed and homogenized with 25 mL of 7.5% trichloroacetic acid solution (TCA) in ultra-turrax equipment (IKA T10, United States) at 6000 rpm for 15 seconds. The supernatant was filtered on filter paper (grammage – 80 g/m^2, thickness – 205 µm, pores – 14 µm). Aliquots of 4 mL were mixed with 5 mL of thiobarbituric acid solution (0.02 M TBA) and placed in a boiling bath (100° C) for 45 minutes, then cooled and read at a wavelength of 538 nm using a UV-visible spectrophotometer (Thermo Scientific Evolution 201, Malaysia). The results were expressed as mg of malonaldehyde per kg of meat.

2.9. Statistical analyses

The experimental design was completely randomized with five treatments (finishing diets) and eight replications per treatment. All studied variables were tested for normality, with those exhibiting a normal distribution submitted to variance analysis (ANOVA) via an adjusted regression model. On the statistical design the finishing diet was considered as fixed effect, on carcass characteristics, drip losses and antioxidant activity, the effect of aging (1, 7 and 14 days) was also considered as fixed effect and studied the interaction between diet and aging days. However, there was no interaction effect among diets and aging days. Orthogonal contrast was used to evaluate the effects of the control treatment versus natural additives. Differences between means were compared using the Tukey test

($P < 0.05$). The statistical program used was the SPSS v.21 (IBM Corporate Headquarters in Armonk, NY).

3. Results and discussion

3.1 Carcass characteristics

Natural additives may have different effects on metabolism and consequently on carcass characteristics and the quality of the meat produced, due to the complex digestive system of cattle (Monteschio et al., 2017; Rivaroli et al., 2016; Souza et al., 2019). Different responses will depend on the dose used and the finished system (Ornaghi et al., 2017; Rivaroli et al., 2017; Souza et al., 2018; Souza et al., 2019). Adding natural additives to the diets of crossbred steers finished in a pasture system did not affect ($P > 0.05$) hot carcass weight (HCW), hot carcass yield (HCY), subcutaneous fat thickness (SFT), muscle area (MA) or pH value (Table 2). The mean values of HCW and HCY were 262.7 kg and 52.9%, respectively.

No differences in carcass weight and dressing percentages were observed in bulls receiving natural additives in the diet and finished in a feedlot. Valero et al. (2014) evaluated the effect of propolis and essential oils additives in the diets and Rivaroli et al. (2017) evaluated the effect of the mix consisted of seven plant extracts of oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange. However, no information on the influence of natural additives on carcass characteristics was found on animals finishing on pasture. Usually this characteristic is related to the live weight of animals at slaughter, and no differences were observed in this experiment (data not shown). Feeding for a short time during finishing, even with the use of additives, does not alter these variables, which would be more susceptible to age, gender, genetic variations or drastic modifications to protein and energy synthesis (Leão et al., 2013).

Table 2

Carcass characteristics and body composition for crossbred steers finished on pasture along with receiving a mix of natural additives in the diet

Variables	Experimental diet[1]					SEM[2]	P *value*		
	CON	NA15	NA30	NA45	NA60		L	Q	0 *vs* Oil
Carcass characteristics									
Hot carcass weight, kg	272	261	257	258	264	4.27	0.105	0.153	0.222
Hot carcass yield, kg	53	52	53	53	53	0.23	0.697	0.419	0.746
Subcutaneous fat thickness, mm	4.2	4.5	3.8	3.9	4	0.17	0.314	0.611	0.706
Muscle area, cm^2	83	82	82.3	79.5	78.3	0.12	0.168	0.343	0.467
pH	5.65	5.64	5.64	5.61	5.78	0.21	0.882	0.139	0.629
Body composition, %									
Bone	13.0	13.3	14.4	13	13.2	1.70	0.129	0.274	0.395
Muscle	62.7	65	65.7	66.3	62.5	0.31	0.482	0.048	0.055
Fat	19.9	18.3	18	18	21.7	0.49	0.943	0.015	0.488
Outhers tissues[2]	2.2	2.0	2.0	2.0	2.4	0.44	0.944	0.015	0.220

[1]Experimental diet: CON: 0 mg of NA/animal/day; NA15: 1500 mg NA/animal/day; NA30: 3000 mg of NA/animal/day; NA45: 4500 mg of NA/animal/day; NA60: 6000 mg of NA/animal/day. [2]Standard error of means. [2]Unidentified tissues.

The mean value of subcutaneous fat thickness (4.1 mm) observed for the treatments met the standards required by the Brazilian slaughter industry (3 – 6 mm), but individual mean values did not differ ($P > 0.05$) among treatments. Intermediate fat deposition may be related primarily to diet and to the genetic group (Rotta et al., 2009). Other authors also did not find differences in fat thickness with the addition of natural additives such as propolis and various essential and vegetable oils (Valero et al., 2014; Zawadzki et al., 2011).

Longissimus muscle area (LMA) measurements are indicative of muscle development. There were no differences ($P > 0.05$) between treatments. The rate of muscle growth is dependent on protein turnover (Climaco et al., 2011). LMA does not have a high correlation with the proportion of carcass muscle. However, when considered together with other parameters, it can predict the degree of yield in boneless cuts (Cañeque & Sañudo, 2005).

The drop in pH is related to biochemical changes that occur in the transformation of muscle into meat. The influence of pH is of practical importance, as it relates to the storage and processing of meat. Although there were no differences ($P > 0.05$) between treatments, the mean of 5.6 observed in this study is considered excellent, since crossbred animals finished on pasture typically have higher values (Monteschio et al., 2017; Rivaroli et al., 2017). The absence of a muscle pH effect agrees with past findings evaluating similar production conditions (Cruz et al., 2014; Ornaghi et al., 2017) and implies good handling practices before slaughter (Cañeque & Sañudo, 2005; Climaco et al., 2011).

3.2 Body composition

There was a quadratic effect ($P < 0.05$) from feeding natural additives in the diet on muscle and fat composition. Muscle and fat growth seems to vary widely, as opposed to bone growth (Cañeque & Sañudo, 2005). Growth patterns of protein and fat deposits in the body are influenced by dietary energy and protein intake (Guerrero et al., 2016; Purchas, Fisher, Price, & Berg, 2002).

When more true protein is formed in the rumen, there is a greater availability of amino acids that can be absorbed in the intestine and a greater availability of substrate for muscle synthesis. However, protein synthesis is dependent on the carbohydrates and nitrogen available in the diet (Maggioni et al., 2009). There was a quadratic effect ($P < 0.05$) on the intake of non-fibrous carbohydrate (CON: 1.88, NA15: 2.01, NA30: 1.67, NA45: 1.69 and NA60: 1.63 kg/d) and crude protein (CON: 1.24, NA15: 1.31, NA30: 1.13, NA45: 1.13 and NA60: 1.10 kg/d) (data not shown). These data may explain the quadratic effect on the amount of carcass muscle.

Animals with high capacity for protein deposition (lean tissue), late maturing cattle, reach the maximum protein growth later; as observed in this experiment (Prado et al., 2015b; Prado et al., 2015c). Increased protein deposition in muscle tissue is a result of the synthesis and degradation of myofibril proteins. Therefore, an increase in muscle mass involves either increased synthesis or decreased degradation, or both processes (Therkildsen, 2005). These results can also be explained by the greater synthesis of volatile propionic fatty acid (0.59 CON *vs* 0.64 mmol/dL NA, $P < 0.05$) and isovaleric (6.16 CON *vs* 6.29 mmol/dL NA, $P < 0.05$) in the rumen of animals receiving natural additives in the diet (data not shown). Propionic is the major glycogenic and isovaleric fatty acid is indicative of proteolysis and deamination of food protein, resulting in liquid energy available for deposition of lean tissue. These factors led to an increase in the animals' energy efficiency for meat production (Purchas, Simcock, Knight, & Wilkinson, 2003).

3.3 Water loss and texture

The data for water losses (drip, thawing, aging, and cooking) and texture are shown in table 3. Several antioxidant substances that are supplied in the feed are absorbed and incorporated into the cell, protecting the integrity of cell membranes and reducing the effects of storage. Several studies have shown that the use of natural additives in the feedlot (Cruz et al., 2014; Monteschio et al., 2017; Valero et al., 2014) did not influence ($P > 0.05$) water losses of meat.

Table 3

Water losses and texture of meat for crossbred steers finished on pasture along with receiving a mix of natural additives in the diet

Variables	Experimental diet[1]					SEM[2]	P < *value*		
	CON	NA15	NA30	NA45	NA60		L	Q	0 *vs* NA
Losses, %									
Drip									
2	1.4	1.3	1.6	1.6	1.5	0.041	0.814	0.895	0.604
Thawing and aging									
1	6^{A}	8.71	8.16	8.2^{A}	7.6^{A}	0.281	0.409	0.048	0.007
7	11.8^{B}	10.3^{AB}	11.6^{B}	12.6^{AB}	10.8^{B}	0.455	0.741	0.829	0.981
14	12.1^{B}	12.2^{B}	12.6^{B}	14.4^{B}	11.4^{B}	0.610	0.880	0.669	0.732
SEM	0.811	0.612	0.585	1.051	0.504				
P < value	0.004	0.050	0.002	0.035	0.001				
Cooking									
1	30^{A}	31	31.4	31.1	30.2	0.536	0.397	0.546	0.660
7	36.7^{B}	33.1	32.2	34	36.4	0.604	0.942	0.012	0.012
14	34^{AB}	30.2	34	34.5	33	0.941	0.773	0.947	0.549
SEM	0.845	1.168	0.887	0.784	1.131				
P < *value*	0.004	0.606	0.503	0.187	0.068				
Texture, N									
Shear Force									
1	61^{A}	57.5^{A}	61.7^{A}	67.3^{A}	62.2^{A}	0.198	0.297	0.585	0.678
7	39.3^{B}	37.7^{B}	38.6^{B}	41.7^{B}	46.3^{AB}	0.157	0.102	0.140	0.605
14	30.7^{C}	32^{B}	34.3^{B}	35.1^{B}	38.2^{B}	0.133	0.048	0.143	0.034
SEM	0.340	0.272	0.298	0.381	0.373				
P < *value*	0.001	0.001	0.001	0.001	0.009				

[1]Experimental diet: CON: 0 mg of NA/animal/day; NA15: 1500 mg NA/animal/day; NA30: 3000 mg of NA/animal/day; NA45: 4500 mg of NA/animal/day; NA60: 6000 mg of NA/animal/day. [2]Standard error of means. $^{A-B}$Different letters on the same column are different ($P < 0.05$).

There were no differences in drip loss amongst treatments. The estimated weight loss is approximately 2% (Françozo et al., 2013; Monteschio et al., 2017; Souza et al., 2019), so the losses found in this study are within normal limits. In general, when the pH is adequate; faster cooling results in longer shelf life and less water loss.

Meat is frozen to increase its shelf life. Ageing also increases the shelf life and increases the tenderness of the meat through enzymatic processes. Freezing begins with the crystallization of water in extracellular spaces, due to a lower concentration of solutes than in the intracellular fluid. Water crystals can damage the structure of muscle fiber. Ageing induces proteolysis. This explains the exudation observed in these processes. The use of antioxidants does not seem to diminish the effects of thawing; however, they can help to delay the effects of ageing.

There was a quadratic effect ($P < 0.05$) observed on losses from thawing and ageing on the first day of storage of the meat, and the CON treatment lost less liquid than treatments with natural additives in the diet. Also, the proportion of water was lower in fat-rich meat. This effect is different from what has been found in other studies (Eiras et al., 2014; Monteschio et al., 2017). On the other days of storage there were no differences ($P > 0.05$) among treatments. Differences were observed between storage days in all treatments ($P < 0.05$), which is an expected result due to changes caused by water crystallization and proteolysis.

Regarding the effects of natural additives on cooking losses, there was a quadratic effect ($P < 0.05$) among the treatments on day seven of storage, with the CON treatment losing more liquids. Differences between days of storage were only observed with the CON treatment.

Shear force was similar among treatments on day 1 and 7 of storage. On day 14 a linear effect ($P < 0.05$) was observed, and the meat of the CON was tender. These changes may be related to the greater amount of muscle present in the carcasses of animals that received natural additives in the diet. When there is greater muscle deposition, there is an increase in the activity of calpastatin, reducing *post-mortem* muscle proteolysis (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010).

3.4 Instrumental color

A linear effect (*P < 0.05*) on meat lightness (L*) was observed (Table 4). The meat of animals receiving the CON treatment was clearer and potentially more attractive to the consumer on day 1 of storage. After 7 and 14 days of storage, L* values for meats were similar between the treatments. Differences in L* were observed between days of ageing (*P < 0.05*), i.e., the meat become clearer, which is an expected behavior resulting from cell membrane lesions causing greater light reflection (Page, Wulf, & Schwotzer, 2001).

Table 4
Color of meat of crossbred steers finishing in pasture system receiving levels of a mix of natural additives in the diet

Variables	Experimental diet¹					SEM²	P < *value*		
	CON	NA15	NA30	NA45	NA60		L	Q	0 *vs* NA
Color									
Lightness, L*									
1	35.2^{A}	33.4^{A}	35^{A}	33^{A}	33.2^{A}	0.305	0.051	0.146	0.382
7	37.5^{AB}	35.1^{A}	36^{A}	35.5^{B}	35.2^{AB}	0.406	0.153	0.269	0.047
14	40.0^{B}	38.7^{B}	39.8^{B}	39.7^{C}	38.4^{B}	0.467	0.583	0.818	0.439
SEM²	0.615	0.614	0.691	0.704	0.806				
P < *value*	0.030	0.001	0.001	0.001	0.018				
Redness, a*									
1	15.5	15	13.2	15.6	14.5	0.281	0.631	0.666	0.744
7	14.5	14.7	14.6	15	14.2	0.250	0.717	0.713	0.912
14	14.5	14.5	14.1	15.1	13.7	0.301	0.708	0.833	0.866
SEM²	0.279	0.351	0.262	0.391	0.540				
P < *value*	0.327	0.849	0.065	0.722	0.824				
Yellowness, b*									
1	12.2^{A}	11.7^{A}	12.5	11.8	11.3	0.241	0.260	0.413	0.419
7	12.7^{AB}	12^{AB}	12.8	12.2	12	0.227	0.254	0.526	0.167
14	14^{B}	13.1^{B}	13.7	13.8	12.5	0.285	0.335	0.039	0.407
SEM²	0.291	0.245	0.291	0.292	0.444				
P < *value*	0.034	0.034	0.219	0.010	0.578				

[1]Experimental diet: CON: 0 mg of NA/animal/day; NA15: 1500 mg NA/animal/day; NA30: 3000 mg of NA/animal/day; NA45: 4500 mg of NA/animal/day; NA60: 6000 mg of NA/animal/day. [2]Standard error of means. [A-B]Different letters on the same column are different (*P < 0.05*).

The mean L* value observed was estimated to be 33.96. Thus, the meat was slightly darker than that considered to be attractive (L* ≈ 38) for the consumer (Page et al. 2001). The L* of meat was affected by low fat deposition, high levels of carotenoids and the normal oxidation processes (Realini, Duckett, Brito, Dalla Rizza, & Mattos, 2004). The low amount of total lipids in muscle was affected by breed, age, sexual condition and the finishing system used. These results can be explained by diet, as reported by Abril et al. (2001); which report negative correlations between the content of yellowness (b*) and the variable L*.

No changes were observed in redness (a*) among treatments or among days of storage (*P > 0.05*). The a* value is related to the myoglobin content in the muscle. As the concentration of myoglobin in muscle tissue increases, the meat becomes darker. The levels of a* are usually between 11.1 and 23.6 (Page et al., 2001). The mean values found in this study are considered normal.

The b* values ranged from 9.7 and 11.4 (Page et al., 2001). However, greater values were found. Grass contains large amounts of carotenoids, which stimulate the increase of myoglobin in the muscles. Carotenoids pigments vary between yellow and dark red (Dian, Chauveau-Duriot, Prado, & Prache, 2007; Zawadzki, Prado, & Prache, 2013). The b* level was not modified by the treatments (*P > 0.05*), only by the days of storage in CON and NA15 treatments. This increase may be associated with ageing, because the value of b* increases with increased oxidation, since the pigments of the heme group present in the meat are sensitive to oxidation (Mancini & Hunt, 2005).

3.5 Phenolic compounds, beef antioxidant activity and lipid oxidation

Products with antioxidant activities may be supplemented in the animals' diet, and could be transferred to the muscle, not only to prevent or reduce oxidation in muscle nutrient delivery but also to improve meat quality (Falowo, Fayemi, & Muchenje, 2014). On day 1 of storage, there were lower numbers of phenolic compounds (TPC) and lower amounts of antioxidant activity for CON compared to other treatments (Table 5).

Table 5
Antioxidant activity of meat of crossbred steers finishing in pasture system receiving levels of a mix of natural additives in the diet

Variables	Experimental diet[1]					SEM[2]	*P < value*		
	CON	NA15	NA30	NA45	NA60		L	Q	0 *vs* NA
Antioxidant activity									
TPC[3], mg GAE[4] g meat^{-1}									
1	101.1	132.3	127.5	118.2	118.2	2.525	0.702	0.106	0.001
7	206.3	201.7	196.4	203.4	188.1	2.509	0.049	0.135	0.160
14	257.6	250.1	248.2	243.2	253.3	4.519	0.636	0.633	0.440
DPPH[5], %									
1	14.7	17.8	16.7	15.7	16.1	0.280	0.428	0.368	0.003
7	16	16.1	15.5	16	15.6	0.144	0.332	0.623	0.482
14	18.3	17	17.4	17.4	18	0.235	0.851	0.211	0.105
ABTS[6], %									
1	22.7	25.2	24.1	20.3	22.1	0.464	0.026	0.081	0.763
7	27.7	27.3	26.8	27.6	25.1	0.452	0.132	0.236	0.340
14	31.8	31	34.3	31.2	33.1	0.538	0.484	0.761	0.553
FRAP[7], %									
1	44	61	55	51.8	55.5	1.686	0.981	0.728	0.018
7	65.5	65	68.6	71.2	62.7	1.448	0.954	0.388	0.713
14	81.5	77	76.7	73.4	76.5	1.910	0.311	0.460	0.250
TBARS[8], mg MDA[9] kg meat^{-1}									
1	0.261^{A}	0.321^{A}	0.286^{A}	0.344^{A}	0.366^{A}	0.166	0.081	0.223	0.042
7	0.442^{B}	0.438^{B}	0.430^{B}	0.407^{AB}	0.486^{AB}	0.123	0.609	0.319	0.977
14	0.506^{B}	0.590^{C}	0.510^{B}	0.477^{B}	0.528^{B}	0.015	0.564	0.823	0.844
SEM[2]	0.026	0.030	0.029	0.017	0.028				
P < value	0.001	0.001	0.001	0.003	0.042				

[1]Experimental diet: CON: 0 mg of NA/animal/day; NA15: 1500 mg NA/animal/day; NA30: 3000 mg of NA/animal/day; NA45: 4500 mg of NA/animal/day; NA60: 6000 mg of NA/animal/day. [2]Standard error of means. [3]Total phenolic content. [4]Gallic acid equivalents. [5]2,2-diphenyl-1-picrylhydrazyl. [6]2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). [7]Ferric reducing antioxidant power. [8]Thiobarbituric acid reactive substances. [9]Malondialdehyde. [A-B]Different letters on the same column are different (*P < 0.05*).

The antioxidant activity of the meat did not increase over its shelf life. The solid-liquid ratio of the meat was altered because a greater loss of liquids led to an increased concentration of the constituents. Therefore, no analyses were performed between storage days for TPC, DPPH, ABTS and FRAP variables. There were no statistical differences between treatments on days 7 and 14 of storage (*P > 0.05*). Higher values (*P < 0.05*) of lipid oxidation (TBARS) were observed for treatments with natural additives in the diet on the first day of storage.

Several researchers have reported the antioxidant effects of beef cattle pasture. Pastures are rich in vitamin A and vitamin E (Descalzo & Sancho, 2008). Antioxidants should be added to the feed at moderate levels because they can act as pro-oxidants in some situations. A balance is needed between the production and elimination of free radicals generated in the oxidation reaction, because antioxidant agents can function as pro-oxidants when consumed in high doses (Rivaroli et al., 2016). This could be associated to the residual presence of the additive in the meat (Monteschio et al., 2017). However, this hypothesis is only speculative because it has not been analyzed. It has also observed that, despite higher observed values, NA45 and NA60 treatments delay oxidation of meat during storage, so this pro-oxidant effect may not be relevant in ageing meat.

4. Conclusion

The inclusion of natural additives had no effect on carcass characteristics; however, it did modify body composition of muscle, fat and other tissues. There was greater muscle percentage compared with the control. Treatments had no effect on fat thickness, *Longissimus* muscle area, pH and drip losses. However, treatments affected thawing/aging and cooking losses, texture, color, antioxidant activity and lipid oxidation. Supplementation with natural additives generally increased water loss and texture, modified color, antioxidant activity and lipid oxidation in the meat. Aging affected thawing/aging and cooking loss, texture, color and lipid oxidation, which are expected effects. Thus, these compounds have potential use in animal feed and could improve meat stability.

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6. Conflict of interests

The authors declare no conflict of interests.

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CONSIDERAÇÕES FINAIS

O sistema de produção de bovinos de corte no Brasil é essencialmente em pastagens. Contudo, para melhorar os índices opta-se pela suplementação a pasto e pela inclusão de substâncias com capacidade de modular a fermentação ruminal. Estas substâncias, de modo geral, são antibióticos ionóforos. Todavia, mais recentemente as autoridades da União Europeia baniram o uso destas substâncias na alimentação animal. Deste modo, o mundo científico é desafiado, mais uma vez, a oferecer compostos alternativos para substituir essas drogas. Assim sendo, vários produtos considerados não invasivos à saúde estão sendo estudados como, por exemplo, leveduras, extratos de própolis, extratos vegetais e, também, os óleos essenciais originários dos vegetais. Nesse sentido, o objetivo do trabalho foi avaliar a adição de aditivos naturais sobre o desempenho e qualidade da carne dos animais. Foram testados níveis de inclusão de uma mistura contendo óleo essencial de cravo, óleo de mamona, óleo de caju e um blend de princípios ativos micros encapsulados de eugenol, timol e vanilina. No entanto, resultados com o uso desses compostos em animais a pasto são escassos. No geral, a inclusão dos aditivos naturais, durante 79 dias, na dieta dos animais terminados em pastagem de aveia e azevém, não modificou o desempenho animal (maior ganho em peso ao longo do período e maior ganho em peso diário). No entanto, a adição dos aditivos naturais promoveu um efeito quadrático na ingestão de forragem, menor digestibilidade da proteína e dos carboidratos não fibrosos, aumento nas concentrações de nitrogênio amoniacal ruminal, e nos ácidos graxos voláteis propiônico e isovalérico, podendo indicar capacidade na modulação da microbiota ruminal. Em consequência da ausência de diferença de peso vivo de abate, o peso de carcaça foi semelhante entre os animais e não houve diferenças nas demais características físicas de carcaça (rendimento de carcaça, espessura de gordura, área de olho de lombo e pH). Foi observada modificação na composição corporal, a composição percentual de tecido muscular nos animais suplementados com NA foi aumentada, devido a alteração na digestibilidade e absorção de proteínas da dieta. Na qualidade da carne dos animais pode-se observar que a adição de NA teve efeito discreto nas perdas de líquidos e na força de cisalhamento. A carne sem inclusão dos aditivos naturais, apesar de mais clara (L* maior) e com menor nível de oxidação, houve menor número de compostos fenólicos e uma menor atividade antioxidante com relação aos tratamentos com AN. O tempo de armazenamento afetou as perdas por descongelamento/armazenamento, perdas por cocção, textura, cor e oxidação lipídica, no entanto esses resultados são esperados devido ao processo de proteólise. Em conjunto, estes resultados sugerem que a mistura de aditivos naturais tem potencial para ser utilizado na alimentação animal e pode melhorar a estabilidade da carne, no entanto, ainda devem ser estudados com relação dose utilizada em bovinos terminados em pastagem.

APÊNDICES

(Normas das revistas científicas)

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MEAT SCIENCE

The official journal of the American Meat Science Association

AUTHOR INFORMATION PACK

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The qualities of **meat** – its **composition**, **nutritional value**, wholesomeness and **consumer** acceptability – are largely determined by the events and conditions encountered by the embryo, the live animal and the postmortem musculature. The control of these qualities, and their further enhancement, are thus dependent on a fuller understanding of the commodity at all stages of its existence – from the initial conception, growth and development of the organism to the time of slaughter and to the ultimate **processing**, preparation, distribution, cooking and consumption of its meat.

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The qualities of meat - its composition, nutritional value, wholesomeness and consumer acceptability - are largely determined by the events and conditions encountered by the embryo, the live animal and the postmortem musculature. The control of these qualities, and their further enhancement, are thus dependent on a fuller understanding of the commodity at all stages of its existence − from the initial conception, growth and development of the organism to the time of slaughter and to the ultimate processing, preparation, distribution, cooking and consumption of its meat.

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Another common problem in meat and food science is the lack of replication and also confounding. This is illustrated with two examples below taken from submitted papers:

Example 1
A total of thirty crossbred male lambs, single born in June were used in an experiment to compare three production systems (12 lambs allocated per system) and the subsequent effects not only on growth and carcase traits, but also meat quality traits. Lambs of the three production systems were weighed fortnightly. When a 35kg live weight target was achieved the lambs weighing >35kg were transported to an abattoir. Lambs were slaughtered after an overnight lairage without feed, but free access to water.

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Example 2

Hams were produced with five decreasing levels of phosphate in combination with 5 increasing levels of thyme. All formulations were applied to a **single batch** of pig meat. Each formulation produced one mixture which was vacuum stuffed into plastic casings to produce four ham 'replicates'. These were cooked in a water bath.

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Relevant references:

Granato, D., Calado, V., & Jarvis, B. (2013). Observations on the use of statistical methods in Food Science and Technology. Food Research International, 55, 137-145.
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