UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

INCLUSÃO DA COMBINAÇÃO DE *Baccharis dracunculifolia, Tamarindus indica* L., ÓLEO ESSENCIAL DE CRAVO E LÍQUIDO DA CASTANHA DE CAJU SOBRE O DESEMPENHO DE BOVINOS TERMINADOS EM CONFINAMENTO E SEUS EFEITOS SOBRE CEPAS BACTERIANAS DO RÚMEN

Autor: Venício Macêdo Carvalho
Orientador: Prof. Dr. Ivanor Nunes do Prado

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Estado do Paraná
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Tese apresentada, como parte das exigências para obtenção do título de DOUTOR EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá - Área de concentração Produção Animal.

MARINGÁ
Estado do Paraná
março – 2020

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

Carvalho, Venício Macêdo
C331i Inclusão da combinação de *Baccharis dracunculifolia, Tamarindus indica* L., óleo essencial de cravo e líquido da castanha de caju sobre o desempenho de bovinos terminados em confinamento e seus efeitos sobre cepas bacterianas do rúmen / Venício Macêdo Carvalho. -- Maringá, 2020.
92 f.: il., figs., tabs.

Orientador (a): Prof. Dr. Ivanor Nunes do Prado.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia, 2020.

1. Dietas alto grão. 2. Extrato natural. 3. Concentrações de ácidos graxos voláteis. 4. Óleo essencial. 5. Atividade antimicrobiana. I. Prado, Ivanor Nunes do, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 21.ed. 636.2085

Marinalva Aparecida Spolon Almeida-CRB 9/1094

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TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADO em 25 de março de 2020.

Prof. Dr. Robério Rodrigues Silva

Prof. Dr. Hilário Cuquetto Mantovani

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Orientador

“Our life always expresses the result of our dominant thoughts”.

(Soren Kierkegaard)

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less”.

(Marie Curie)

Aos meus pais, Ailde e Ernélia,

A minha irmã Aline e ao meu cunhado Glauber,

Aos meu tio Ailson (In memoriam),

Aos meus tios, Yovan e Itânia,

Aos meus avós,

A toda a minha família (primos, tios e tias)

DEDICO

AGRADECIMENTOS

A Deus, por ter me abençoado e me dar forças para seguir em frente.

A CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela bolsa de estudos.

A Universidade Estadual de Maringá, em especial ao PPZ – Programa de Pós-Graduação em Zootecnia, pela excelente capacitação profissional.

Aos professores do Programa de Pós-graduação em Zootecnia da UEM, pelos valiosos ensinamentos.

Ao Prof. Dr. Ivanor Nunes do Prado, pela oportunidade em tê-lo como orientador de Doutorado. Sinto muito orgulho em citá-lo como um dos principais responsáveis pela minha formação profissional. Sou muito grato pela confiança, amizade, conselhos, paciência, e pela sua preocupação com o desenvolvimento pessoal e profissional de todos os seus orientados. Minha eterna admiração e gratidão.

Ao Prof. Dr. Robério Rodrigues Silva, pela amizade, conselhos e orientação durante a Graduação e Mestrado.

Ao Prof. Dr. Hilário Cuquetto Mantovani, pelos ensinamentos durante a nossa estadia em Viçosa e pela contribuição com as correções do trabalho.

Ao Prof. Dr. Rodolpho Martin do Prado, pela contribuição com as correções do trabalho.

A Prof.ª Dra. Isolde Terezinha Previdelli, pela parceria com as análises estatísticas e ensinamentos.

Ao Prof. Dr. Benício Alves de Abreu Filho, Prof. Dr. Jurandir Fernando Comar e a Prof.ª Dra. Débora Cristina Baldoqui, pela parceria nas análises laboratoriais.

Aos professores da banca avaliadora, Prof. Dr. Robério Rodrigues Silva, Prof. Dr. Hilário Cuquetto Mantovani, Prof. Dr. Luiz Fernando Costa e Silva e Prof. Dr. Carlos Antônio Lopes de Oliveira, por todas considerações e avaliação para a finalização do trabalho.

Ao Departamento de Zootecnia, UEM, em especial ao Prof. Dr. Ricardo Souza Vasconcellos, Prof. Dr. Ferenc Istvan Bánkuti e Profa. Dra. Eliane Gasparino.

A Solange, funcionária do PPZ, por toda ajuda com a papelada, burocracia e por sempre nos atender com a maior força de vontade. Muito Obrigado!

Aos amigos Vicente Díaz e Edinéia Bonin, pelo companheirismo e dedicação. Declaro a minha imensa admiração e dedico amizade e carinho eternos.

Aos meus amigos da República BACEMA, Divaney, José Elton, Kariny, Kleves e Sillas, pelos momentos de descontrações e amizade que ficarão para sempre!

Ao Grupo de Pesquisa, em especial a todos que, direta ou indiretamente, contribuíram para a realização deste trabalho.

Aos amigos, Aylle, Aline, Cláudio, Célio, Cínthia, Edinéia, Gabriela, Maikon, Maribel, Melina, Murilo, Raquel, Tuan, Vanessa, Vicente, Washington e Zé Carlos, pelos momentos de descontrações e apoio nesta jornada.

Aos funcionários da Fazenda Experimental (FEI), pela dedicação com os projetos e conselhos quando tudo parecia que iria dar errado! Célio, Denilson, Zé Carlos e tantos outros que ajudaram imensamente na realização desse trabalho. Obrigado!

BIOGRAFIA

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No dia 25 de março de 2020, submeteu-se à Defesa de Tese junto ao Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá.

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RESUMO

Foram realizados dois experimentos para caracterizar os compostos fenólicos presentes no extrato vegetal de folhas e caules de baccharis (*Baccharis dracunculifolia),* sementes de tamarindo *(Tamarindus indica L.),* óleo essencial da folha de cravo (*Syzygium aromaticum*), líquido da castanha do caju (LCC) (*Anacardium occidentale*) e avaliar seus efeitos sobre o desempenho animal, eficiência alimentar, digestibilidade aparente, concentrações de ácidos graxos voláteis (AGV) no rúmen e comportamento ingestivo de bovinos mestiços (½ Angus x ½ Nelore) terminados em confinamento. Também foi verificado a atividade antimicrobiana *in vitro* desses extratos vegetais e óleos essenciais sobre cepas de bactérias ruminais Gram-negativas. No primeiro experimento, avaliou-se a atividade antimicrobiana dos extratos vegetais de folhas e caules de baccharis, sementes de tamarindo, óleo essencial da folha de cravo e LCC contra cinco cepas de bactérias ruminais Gram-negativas. Foram avaliados no meio de cultura contendo os extratos vegetais/óleos essenciais nas concentrações de 0,1; 0,2; 0,5 e 1,0 mg mL^{-1}. O crescimento das bactérias foi avaliado pelo monitoramento da densidade óptica (DO 600 nm) por espectrofotometria, nos intervalos de observação de 0, 8, 12 e 24 horas de incubação a 39° C. Os extratos inibiram o crescimento da *Prevotella albensis, Prevotella bryantti, Prevotella ruminicola, Treponema saccharophilum e Succinivibrio dextrinosolvens.* No segundo experimento, foram testados o MIX (baccharis, tamarindo, cravo e LCC), em três diferentes doses, sendo: 2, 4 e 6 g animal dia^{-1} *vs.* o tratamento controle (sem adição de material vegetal e óleos essenciais). Foram utilizados 32 machos não castrados (½ Angus x ½ Nelore), com peso corporal médio de 418 ± 4,51 kg e idade média de 24 ± 2,0 meses, distribuídos em um delineamento inteiramente ao acaso, com quatro tratamentos e oito repetições por tratamento. A dieta continha 30% de silagem de milho e 70% de concentrado (milho grão, glúten de milho, levedura, calcário, sal mineral e ureia). A ingestão de matéria seca e dos demais nutrientes, desempenho animal e o comportamento

ingestivo não foram influenciados (P > 0,05) pela adição do MIX na dieta, mas houve efeito do MIX (P < 0,05) sobre a digestibilidade e produção de AGV. A digestibilidades da matéria seca, fibra em detergente neutro e matéria orgânica foram maiores quando a mistura de 2 g animal dia^{-1} foi incluída na dieta. A adição do MIX proporcionou aumento (P < 0,05) na concentração molar de AGV propionato e diminuição na razão acetato: propionato. Este estudo sugere que a utilização de extratos vegetais/óleos essenciais de baccharis, tamarindo, cravo e LCC podem ser uma alternativa natural ao uso de antibióticos ionóforos na alimentação de ruminantes.

Palavras-chave: atividade antimicrobiana, bactérias ruminais, extratos naturais, óleos essenciais

ABSTRACT

Two experiments were carried out in order to characterize the phenolic compounds composition present in the plant extract of baccharis (*Baccharis dracunculifolia*) leaves and stems, tamarind (*Tamarindus indica* L.) seed, clove leaf (*Syzygium aromaticum*) essential oil, cashew nut (*Anacardium occidentale*) shell liquid (CNSL) and to study its effects on performance, feed efficiency, digestibility, volatile fatty acids concentrations (VFA) and ingestive behavior of crossbred bulls finished in feedlot. It was also verified the in vitro antimicrobial activity of these plant extracts and essential oils on ruminal strains of Gram-negative bacteria. The first experiment evaluated the plant extracts antimicrobial activity from plant extract of baccharis leaves and stems, tamarind seed, clove leaf essential oil, and CNSL against five strains of Gram-negative ruminal bacteria. There were evaluated in the culture medium containing the plant extracts / essential oils in the concentrations of 0.1; 0.2; 0.5 e 1.0 mg L^{-1}. The bacteria growth was evaluated by monitoring the optical density (OD 600 nm) in spectrophotometer, in the incubations intervals of 0, 8, 12 and 24 hours of incubation at 39°C. The extracts inhibited the *Prevotella albensis*, *Prevotella bryantti*, *Prevotella ruminicola*, Treponema saccharophilum e *Succinivibrio dextrinosolvens* growth. In the second experiment, three different doses of the MIX (*baccharis*, tamarind, clove and CNSL) was tested, being: 2, 4 and 6 g animal day^{-1} vs. the control treatment (without adding plant extracts and essential oils). A total of 32 (½ Angus vs. ½ Nellore) young bulls with a mean age of 24 ± 2.0 months and a mean body weight of 418 ± 4.51 kg were distributed in a completely randomized design with four treatments and eight replications per treatment. The diet had 30% corn silage and 70% concentrate (corn grain, corn gluten, yeast, limestone, mineral salt and urea). Dry matter and other nutrients intake, animal performance and intake behavior were not influenced ($P > 0.05$) by adding MIX to diet, but there was a MIX effect ($P < 0.05$) on dry matter digestibility and VFA production. The dry matter, neutral

detergent fiber and organic matter digestibility were higher when 2 g animal day^{-1} MIX was included in the diet. The MIX addition provided an increase ($P < 0.05$) in the propionate molar concentration and a decrease in the acetate: propionate ratio. This study suggests that the use of plant extracts/ essential oils from *Baccharis*, tamarind, Clove and CNSL can be a natural alternative to the use of ionophore antibiotics in the ruminants feeding.

Key words: antimicrobial activity, ruminal bacteria, natural extracts, essential oils

I - INTRODUÇÃO

1.0 Revisão bibliográfica

A pecuária de corte é importante segmento do agronegócio brasileiro, marcado por altas variações de preço e sazonalidade na produção de alimentos. Sabe-se que com o advento do crescimento da população mundial e melhorias no poder aquisitivo da população, aumenta a demanda por produtos de origem animal e, por conseguinte, é necessário melhorias no sistema de produção (FAO, 2018; Ickowitz et al., 2019).

Partindo deste pressuposto, a crescente demanda por carne bovina será atendida por meios de processos de intensificação do sistema de criação, pois a disponibilidade de pastagens para produção extensiva é limitada, além dos processos orientados pelas mudanças climáticas, como a desertificação, a expansão nas lavouras e a urbanização podem reduzir ainda mais a disponibilidade de pastagens no futuro (McAllister et al., 2020). Assim, o sistema de terminação em confinamento tem sido utilizado, por meio do encurtamento do ciclo de produção, com maior adensamento de grãos na dieta (>70%) sendo projetados para aumentar tanto a gordura de cobertura subcutânea quanto a intramuscular (marmoreio), de modo a garantir o fornecimento de proteína animal capaz de suprir as exigências do mercado consumidor (McAllister et al., 2020; Ornaghi et al., 2020; Tullo et al., 2019).

Neste cenário de produção animal, no qual se procura reduzir o ciclo de produção e aumentar a competividade do setor pecuário, o principal fator que onera a produção é a alimentação, com participação em cerca de 70% ou mais dos custos de produção. De maneira geral, na medida em que aumenta o incremento de grãos na dieta, perturbações no ambiente ruminal podem ocorrer, afetando negativamente o sistema de produção. Estes efeitos são ocasionados pelo acúmulo de ácidos graxos voláteis (AGV) no fluido

ruminal. Os AGVs são gerados durante a fermentação ruminal, podendo levar a ocorrência de distúrbios fermentativos, como a acidose (Matthews et al., 2019; Silva et al., 2019).

Na tentativa de eliminar ou reduzir os efeitos decorrentes da utilização de alto grão na dieta animal e proporcionar o maior aproveitamento do alimento ingerido, utiliza-se aditivos, para diminuir as perdas alimentares e aumentar os produtos finais da fermentação ruminal, aumentando assim a eficiência da utilização dos alimentos ingeridos (McAllister et al., 2020; Russell, 1987; Russell and Houlihan, 2003). Antibióticos ionóforos são os aditivos alimentares mais utilizados para a manipulação da fermentação ruminal em bovinos (Patra and Yu, 2012). Problemas quanto ao uso de antibióticos vêm sendo relatado há algumas décadas, em função dos possíveis danos à saúde animal e humano. Assim, torna-se necessário a busca por aditivos químicos e biológicos que sejam capazes de prover melhorias no ambiente ruminal, sem causar danos à saúde humana. Partindo deste ponto de vista, estudos com a utilização de aditivos de origem natural vêm sendo conduzidos em diversas instituições e têm demonstrado resultados promissores.

1.1. Aditivos alimentares na dieta de bovinos de corte

A utilização de antibióticos na nutrição de ruminantes se iniciou durante a década de 50, sendo difundida como promotores de crescimento, obtendo bons resultados para a época, em virtude das baixas condições sanitárias presentes. Com o passar dos anos, surgiram novas preocupações quanto ao uso destes produtos, em razão dos possíveis efeitos de resistência aos seus princípios farmacológicos, como a penicilina e a tetraciclina e os possíveis riscos à saúde humana (Kirchhelle, 2018; Russell and Houlihan, 2003).

Historicamente, a partir da década de 1970, deu início a utilização de uma nova classe de antibióticos alimentares, denominada de ionóforos. Essas moléculas, por sua vez, são produzidas por bactérias do gênero *Streptomyces* (Kirchhelle, 2018; Russel and Strobel, 1989; Wallace et al., 2002). No início da década de 1970, estas moléculas foram aprovadas pela Food and Drug Administration para adição às rações de ruminantes nos Estados Unidos (Russell, 1987). De acordo com McGuffey et al. (2001) são conhecidos mais de 120 tipos de ionóforos produzidos por actinomicetos. No entanto, apenas a monensina (Rumensin), lasalocida (Bovatec), salinomicina (Bio-cox, Sacox) e laidlomycin (Cattlyst) possuem aprovação para o uso em dietas de ruminantes (Novilla, 2018).

As razões pelas quais os ionóforos são incluídos na dieta animal se devem ao fato de atuarem como modulador da fermentação ruminal, promovendo redução da desaminação de aminoácidos da proteína da dieta, resultando em menor perda de amônia pela excreção urinária (Bergen and Bates, 1984; Novilla et al., 2017; Russell, 1987; Schelling, 1984).

1.2. Modo de ação dos ionóforos

O modo de ação dos ionóforos ocorre em função da capacidade que as moléculas possuem em facilitar o transporte de íons pelas membranas plasmáticas, promovendo alterações no gradiente de concentração (Novilla et al., 2017). Essas moléculas possuem alta capacidade de formar complexos lipossolúveis com cátions e são caracterizadas por possuírem em seu exterior envolto hidrofóbico, enquanto sua parte interna é hidrofílica, capacitando assim sua ligação com cátions. Os ionóforos são capazes de proteger e translocar íons, facilitando seu movimento através da membrana citoplasmática (Benarroch and Asally, 2020; Russell, 1987), impedindo que os gradientes iônicos sejam utilizados como força motriz para a absorção de nutrientes e geração de energia.

Quando avaliado o modo de ação nas bactérias ruminais, observa-se que os ionóforos atuam diretamente na inibição das bactérias Gram-positivas, e possuem pouca ou nenhuma atividade contra bactérias Gram-negativas Esse fato ocorre em função da estrutura das membranas celulares das bactérias. As bactérias Gram-negativas são constituídas por uma parede celular composta por peptideoglicanos e membrana externa constituída por lipopolissacarídeos, proteínas e lipoproteínas (Morais et al., 2006; Novilla, 2018). Nas bactérias anaeróbias Gram-positivas o envelope celular é constituído primariamente por uma camada espessa de peptideoglicanos e ácidos teicoicos.

As bactérias ruminais mantêm altas concentrações de potássio intracelular e baixas de sódio intracelular e, inversamente, o ambiente ruminal contém altas concentrações de sódio e baixas de potássio. Assim, o mecanismo de ação dos ionóforos está relacionado com a capacidade dessas moléculas de transportar íons através da bicamada lipídica e de dissipar o gradiente eletroquímico gerado pelo acúmulo de prótons na face externa da membrana plasmática (Novilla, 2018; Russell, 1987). Com isso, observa-se que a utilização de ionóforos causa alterações na microbiota ruminal, afetando negativamente as bactérias Gram-positivas, além de promover alterações no perfil de fermentação do rúmen, garantindo a maior estabilidade do pH ruminal e a redução da razão acetato/propionato (Rodrigues, 2016). Assim, seriam encontrados outros efeitos secundários, como modificações na produção de AGV, na produção de gás, maior

aproveitamento dos nutrientes ingeridos, com melhorias na digestibilidade e, consequentemente, no aumento da produção animal.

Nesse contexto, são notórios os efeitos promovidos pela inclusão de aditivos alimentares na dieta de bovinos de corte, possibilitando a utilização de dietas com alto teor de carboidratos prontamente fermentáveis, possibilitando que os animais expressem o seu potencial genético para ganho de peso e, consequentemente, reduzindo o ciclo de produção e a rotatividade do setor.

1.3. Restrições quanto ao uso dos ionóforos

Embora observado efeitos positivos na produção animal com o uso de ionóforos, existem algumas questões sanitárias, de segurança alimentar e restrições quanto ao seu uso, atrelado aos possíveis resíduos provocados por estas moléculas.

Há alguns anos, com a proibição do uso de ionóforos pela União Europeia (Directive 1831/2003/CEE, European Commission, 2003) (OJEU, 2003), a comunidade europeia têm restringido o uso de antibióticos e coccidiostáticos como aditivos alimentares para bovinos, por causa das preocupações quanto aos possíveis resíduos no produto e seleção de resistência cruzada aos antibióticos em bactérias comensais ou patogênicas do trato gastrointestinal de animais de produção. Desta forma, alguns princípios farmacêuticos não são mais encontrados e outros estão sendo gradativamente retirados do mercado (Herrera et al., 2009; Kirchhelle, 2018).

De acordo com Hao et al. (2014), serão necessárias políticas públicas de longo prazo para estabelecer a regulamentação internacional quanto ao uso de antibióticos em animais de produção. Os mesmos autores fazem analogia quanto à restrição imposta ao uso dos antibióticos e ionóforos, pois caso houvesse a proibição nos EUA, a taxa de eficiência alimentar poderia sofrer queda na ordem de 5% ou mais, e com isso, seriam necessárias a abertura de novas áreas destinadas para o plantio do milho e soja, para atender a demanda de produção de alimentos para os animais.

Assim, com a crescente modernização e a facilidade ao acesso à informação, as pessoas preocupadas com a saúde e bem-estar vêm levantando questões relacionadas ao uso dos antibióticos ionóforos na alimentação animal e os possíveis riscos relacionados ao desenvolvimento de resistência aos antibióticos. Neste sentido, para atender as exigências do mercado consumidor que busca por alimentos vegetais e animais saudáveis, estudos estão sendo realizados com o propósito de substituir os antibióticos e ionóforos por moléculas bioativas de origem vegetal.

1.4. Compostos naturais e sua utilização na alimentação de ruminantes

Considerando a dimensão territorial e a variação climática, o Brasil é um país de clima tropical que possui condições favoráveis ao desenvolvimento de diversas espécies arbóreas, e, com isso, ao considerar a diversidade de plantas existentes, toma-se um desafio à identificação e quantificação dos princípios ativos e a avaliação dos efeitos dos extratos dessas plantas como aditivos na alimentação animal.

Inicialmente, surgiram pesquisas com a utilização da própolis na alimentação de ruminantes, pelas suas propriedades antimicrobianas, anti-inflamatória, e por apresentar em sua composição os compostos flavonoides (Beecher, 2003; Williams et al., 2004). Desde então, resultados positivos quanto aos parâmetros de desempenho animal vêm sendo encontrados (Valero et al., 2014; Zawadzki et al., 2011), indicando a possibilidade real de inclusão destes produtos na alimentação animal com potencial substituto aos antibióticos e ionóforos comumente utilizados. No entanto, por se tratar de um produto usual tanto na alimentação animal quanto na alimentação humana, têm-se limitado a sua utilização. Assim, têm-se despertado o interesse dos pesquisadores em investigar os efeitos decorrentes da utilização de extratos vegetais e óleos essenciais na alimentação animal.

Os óleos essenciais são formados por misturas complexas de metabólitos secundários, lipofílicos voláteis (Benchaar and Greathead, 2011) que podem ser sintetizados por diversas partes das plantas, como folhas, flores, brotos, caules, sementes, raízes e são armazenadas em células secretoras, epidérmicas ou tricomas glandulares (Bakkali et al., 2008). Os extratos vegetais são derivados dos compostos metabólitos secundários das plantas, principalmente os terpenoides (monoterpenos e sesquiterpenos), podendo conter cerca de 20 a 60 componentes em diferentes concentrações (Bakkali et al., 2008) e desempenham funções importantes, como antissépticos e antimicrobianos (Calsamiglia et al., 2007).

As atividades antimicrobianas dos extratos naturais ocorrem em função dos compostos presentes, como, fenólicos, quinonas, saponinas, flavonoides, taninos, cumarinas, terpenoides e alcaloides (Cowan, 1999), sendo a ação antimicrobiana variável e dependente da configuração estrutural (Gyawali and Ibrahim, 2014), além de apresentarem rendimento e composição variável entre plantas de diferentes espécies e em diferentes partes da mesma planta (Benchaar and Greathead, 2011).

Quanto às formas de extração, utiliza-se o método por temperatura e destilação a vapor, extração com solvente, extração supercrítica de CO_2 (Benchaar and Greathead, 2011), sendo que o perfil químico dos derivados dos óleos essenciais irá diferir de acordo com o método de extração empregado (Bakkali et al., 2008).

Estudos têm sido realizados com o objetivo de caracterizar a ação dos extratos e óleos vegetais na inibição do crescimento bacteriano, e vem demonstrando resultados satisfatórios. Com isso, tem-se despertado o interesse dos nutricionistas de animais, pois se vislumbra uma alternativa que possa ser utilizada como aditivo alimentar e que atue como possível substituto aos antibióticos ionóforos comumente utilizados.

Assim, os óleos essenciais possuem características particulares, que lhes conferem maior atratividade de utilização. De maneira geral, observa-se ação antimicrobiana dos óleos essenciais frente aos microrganismos anaeróbios. O modo de ação destes produtos é variado, em função dos metabólitos secundários presentes e, acredita-se que a presença de oxigênio e enxofre na estrutura química potencializa o modo de ação destes produtos, sendo o grupo hidroxila responsável em interromper o transporte normal dos íons pela membrana citoplasmática e na inativação das enzimas microbianas (Benchaar and Greathead, 2011).

Ao contrário dos antibióticos ionóforos (monensina), que apresentam modo de ação no grupo das bactérias Gram-positivas, os extratos vegetais e óleos essenciais vem demonstrado eficácia em ambos os grupos de bactérias Gram-positivas e negativas. Este modo de ação tem sido evidenciado principalmente nos compostos ao qual estão presentes as estruturas fenólicas, notando-se amplo espectro de atividade (Lambert et al., 2001), em função de sua capacidade em penetrar a membrana das bactérias Gram-negativas (Helander et al., 1998).

2.0. Caracterização dos extratos naturais e óleos vegetais

2.1. Baccharis dracunculifolia

Nativa do Brasil, a baccharis (*Baccharis dracunculifolia*) é popularmente conhecida como “alecrim do campo” ou “vassourinha” e caracteriza-se por sua adaptabilidade às mais variadas condições climáticas e presença de compostos fenólicos, com propriedades antioxidantes e antimicrobianas (Bonin et al., 2020). Sabe-se que *Baccharis dracunculifolia* é a principal matéria-prima utilizada pelas abelhas (*Apis mellifera*) na

produção de própolis verde, cujos benefícios são bem relatados na literatura (Rodrigues et al., 2020).

Pesquisas com extratos da baccharis tem demonstrado a importância desta espécie arbórea e a sua diversidade de utilização, destacando-se as suas atividades antibacteriana, poder antioxidante, anti-inflamatória, antifúngica e antiviral (Hocayen et al., 2012; Lage et al., 2015).

Cazella et al. (2019) ao estudar a composição química e avaliar a atividade antimicrobiana do óleo essencial da parte aérea da *B. dracunculifolia,* identificaram trinta constituintes no óleo essencial, sendo suas principais classes formadas por sesquiterpenos oxigenados (60,8%), sesquiterpenos hidrocarbonetos (22,9%) e monoterpenos hidrocarbonetos (9,6%). O estudo da ação antimicrobiana demonstrou que os óleos foram altamente eficazes contra as bactérias patogênicas Gram-positivas e Gram-negativas, apresentando efeito bactericida sobre *Staphylococcus aureus, Bacillus cereus e Pseudomonas aeruginosa.*

Guimarães et al. (2012) ao avaliarem as propriedades antioxidantes do extrato da *B. dracunculifolia* e seus efeitos contra o estresse oxidativo em mitocôndrias isoladas de ratos, observaram que o extrato utilizado apresentou atividade antioxidante, eliminando os radicais livres e quelantes de ferro, evitou a oxidação de grupos tiol de proteínas mitocondriais e a depleção da Glutationa Peroxidase (GSH), demonstrando a importância deste produto como potencial agente protetor dos danos hepáticos ocasionados pela oxidação.

2.2. Tamarindus indica L.

Popularmente conhecida como tamarindo (*Tamarindus indica* L.), caracteriza-se como planta frutífera pertencente à família das leguminosas, nativa da África, capaz de se desenvolver em regiões tropicais e subtropicais, tendo a temperatura de 25º C como a ideal para o seu desenvolvimento (Reis et al., 2016). As diversas partes da planta (frutas, folhas e sementes) são compostas por fontes naturais de antioxidantes (Luzia and Jorge, 2011; Reis et al., 2016), despertando, assim, o interesse dos pesquisadores em investigar a utilização deste produto como potencial substituto aos antioxidantes.

Quanto à composição das sementes, destaca-se as proteínas (13 a 20%), óleo (4,5 a 16,2%). Entre os ácidos graxos presentes, o linoleico, oleico e o palmítico foram os mais abundantes (Rao and Mathew, 2012), tendo como constituintes fito-químico os

compostos fenólicos, como epicatequina, além do ácido tartárico, pectina, arabinose, xilose, galactose, glicose e triterpeno (Kuru, 2014).

Em relação à atividade antimicrobiana, estudos vêm sendo realizados e os resultados têm demonstrado efeitos variados contra cepas de bactérias Gram-positivas e Gram-negativas (Arshad et al., 2019; Daniyan and Muhammad, 2008; Tril et al., 2014).

Nunoi et al. (2019) ao realizarem o estudo com as sementes de tamarindo ao nível de 0, 30, 60 e 100% em substituição ao farelo de arroz na dieta de novilhos leiteiros, observaram que houve melhorias na eficiência de fermentação ruminal, com aumento na concentração de ácidos graxos totais e ácido propiônico, sendo que a ingestão e a digestibilidade não foram influenciados quando as sementes de tamarindo foram adicionadas na dieta. De acordo com os autores, a semente de tamarindo pode ser utilizada como fonte de energia alternativa e potencial substituto ao farelo de arroz, podendo ser substituído em até 100%.

2.3. Líquido da castanha do caju

Nativo do Brasil, o líquido da casca da castanha de caju é um subproduto do processamento da castanha de caju e possui vários usos industriais (Akinhanmi et al., 2008; Lubi and Thachil, 2000), tendo como constituintes principais o cardanol, cardol e o ácido anacárdico, conferindo-lhes um amplo espectro de ação, sendo comumente utilizados como antimicrobianos, antioxidantes e antitumorais (Andrade et al., 2011; Watanabe et al., 2010).

O líquido da castanha do caju, popularmente conhecido como LCC, apresenta potencial de utilização na dieta animal, e seus efeitos na modulação do ambiente ruminal são bem documentados na literatura (Akinhanmi et al., 2008; Oh et al., 2017; Siddhuraju, 2007). Watanabe et al. (2010), após realizarem vários experimentos *in vitro* com a utilização do LCC, constataram que este produto apresenta potencial uso na dieta de ruminantes, podendo atuar como modulador da fermentação ruminal, aumentando a produção de propionato e reduzindo as emissões do metano, sem afetar a produção de ácidos graxos totais e a digestibilidade dos nutrientes.

2.4. Óleo essencial do cravo (Syzygium aromaticum)

O cravo da índia é uma planta arbórea, nativa da Indonésia, possui aroma, sabor e odor característico. Estudos demonstram a potencialidade do óleo essencial de cravo-da-índia como agentes antimicrobianos, antifúngico e anticarcinogênico (Chaieb et al., 2007).

Além disso, possui alta capacidade antioxidante, tendo o eugenol como composto majoritário (Biondo et al., 2017; Cortés-Rojas et al., 2014). O eugenol, a substância ativa, compõe 90-95% do óleo de cravo-da-índia e como aditivo alimentar é classificado pela FDA (Food and Drug Administration) como uma substância ‘‘Geralmente Considerada Como Segura’’ (Franklyne et al., 2019).

A maioria dos ingredientes químicos isolados e identificados no óleo essencial foram mono e sesquiterpeno, hidrocarbonetos normais e cíclicos e derivados fenólicos (Hossain et al., 2014; Pandey and Singh, 2011; Pandey et al., 2017). Esses compostos têm despertado o interesse dos pesquisadores, que vislumbram o potencial de utilização desse produto como aditivo biológico na dieta de ruminantes. Assim, há relatos na literatura da utilização desse óleo essencial associado a outros compostos vegetais, resultando em melhorias no desempenho animal, sem afetar a digestibilidade dos nutrientes e comportamento animal (Ornaghi et al., 2020, 2017; Souza et al., 2019), aumento da atividade antioxidante, com diminuição da oxidação lipídica da carne (Monteschio et al., 2017), e na modulação ruminal, com diminuição da concentração de acetato (Roy et al., 2015).

3.0. Conclusões e perspectivas

Os extratos naturais e óleos vegetais apresentam potenciais de utilização e substituição aos antibióticos ionóforos utilizados na alimentação animal, proporcionando melhorias aos animais e ao produto final (carne/leite). Além disso, podem contribuir para diminuir a emissão de gás metano, tornando-se adequados para reduzir o uso de antibióticos como promotores de crescimento, e atendendo as exigências do mercado consumidor global.

Assim, com a preocupação pelo surgimento de resistência a antibióticos na produção animal, tem se tornado prioridade expandir os estudos para inclusão dessas moléculas bioativas na dieta animal, além de avaliar os sinergismos entre esses compostos e sua aplicabilidade no sistema de produção.

Nesse sentido, pesquisas devem ser elaboradas visando elucidar a forma prática de utilização desses compostos, pois existem limitações quanto a utilização dos mesmos, por apresentarem baixa solubilidade em água e forte propriedade organolépticas.

4.0. Referências Bibliográficas

Akinhanmi, T.F., Atasie, V.N., Akintokun, P.O., 2008. Chemical composition and physicochemical properties of cashew nut *(Anacardium occidentale)* oil and cashew nut shell liquid. *J. Agric. Food Environ. Sci.*, 2, 1–10.

Andrade, T.D.J.A.D.S., Araújo, B.Q., Citó, A.M.D.G.L., Da Silva, J., Saffi, J., Richter, M.F., Ferraz, A.D.B.F., 2011. Antioxidant properties and chemical composition of technical Cashew Nut Shell Liquid (tCNSL). *Food Chem.*, 126, 1044–1048. https://doi.org/10.1016/j.foodchem.2010.11.122

Arshad, M.S., Imran, M., Ahmed, A., Sohaib, M., Ullah, A., Nisa, M. un, Hina, G., Khalid, W., Rehana, H., 2019. Tamarind: A diet-based strategy against lifestyle maladies. *Food Sci. Nutr.*, 7, 3378–3390. https://doi.org/https://bit.ly/2YfKndu

Bakkali, F., Idaomar, M., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – A review. *Food Chem. Toxicol.*, 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Beecher, G.R., 2003. Overview of dietary flavonoids: nomenclature, occurrence and intake. *J. Nutr.*, 133, 3248S-3254S. https://doi.org/https://doi.org/10.1093/jn/133.10.3248S

Benarroch, J.M., Asally, M., 2020. The Microbiologist's Guide to Membrane Potential Dynamics. *Trends Microbiol.*, 28, 304–314. https://doi.org/10.1016/j.tim.2019.12.008

Benchaar, C., Greathead, H., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Anim. Feed Sci. Technol.*, 166–167, 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024

Bergen, W.G., Bates, D.B., 1984. Ionophores: their effect on production efficiency and mode of action. *J. Aimal Sci.*, 58, 1465–1483.

Biondo, P.B.F., Carbonera, F., Zawadzki, F., Chiavelli, L.U.R., Pilau, E.J., Prado, I.N., Visentainer, J. V., 2017. Antioxidant Capacity and Identification of Bioactive Compounds by GC-MS of Essential Oils from Spices, Herbs and Citrus. *Curr. Bioact. Compd.*, 13, 137–143. https://doi.org/10.2174/1573407212666160614080846

Bonin, E., Carvalho, V.M., Avila, V.D., Santos, N.C.A. dos, Benassi-Zanqueta, É., Lancheros, C.A.C., Previdelli, I.T.S., Ueda-Nakamura, T., Abreu Filho, B.A. de, Prado, I.N., 2020. *Baccharis dracunculifolia*: Chemical constituents, cytotoxicity and antimicrobial activity. *LWT - Food Sci. Technol.*, 120, 1–10. https://doi.org/10.1016/j.lwt.2019.108920

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., Ferret, A., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. *J. Dairy Sci.*,. https://doi.org/10.3168/jds.2006-644

Cazella, L.N., Glamoclija, J., Soković, M., Gonçalves, J.E., Linde, G.A., Colauto, N.B., Gazim, Z.C., 2019. Antimicrobial activity of essential oil of *baccharis dracunculifolia* DC (Asteraceae) aerial parts at flowering period. *Front. Plant Sci.*, 10, 1–9. https://doi.org/10.3389/fpls.2019.00027

Chaieb, K., Zmantar, T., Ksouri, R., Hajlaoui, H., Mahdouani, K., Abdelly, C., Bakhrouf, A., 2007. Antioxidant properties of the essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. *Mycoses*, 50, 403–406. https://doi.org/10.1111/j.1439-0507.2007.01391.x

Cortés-Rojas, D.F., Souza, C.R.F., Oliveira, W.P., 2014. Clove *(Syzygium aromaticum)*: A precious spice. *Asian Pac. J. Trop. Biomed.*, 4, 90–96. https://doi.org/10.1016/S2221-1691(14)60215-X

Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12, 564–582. https://doi.org/10.1128/cmr.12.4.564

Daniyan, S.Y., Muhammad, H.B., 2008. Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. *African J. Biotechnol.*, 7, 2451–2453. https://doi.org/10.5897/AJB08.337

FAO, 2018. The State of Food Security and Nutrition in the World 2018 - Building climate resilience for food security and nutrition. FAO, Rome.

Franklyne, J.S., Andrew Ebenazer, L., Mukherjee, A., Natarajan, C., 2019. Cinnamon and clove oil nanoemulsions: novel therapeutic options against vancomycin intermediate susceptible *Staphylococcus aureus*. *Appl. Nanosci.*, 9, 1405–1415. https://doi.org/10.1007/s13204-019-01111-4

Guimarães, N.S.S., Mello, J.C., Paiva, J.S., Bueno, P.C.P., Berretta, A.A., Torquato, R.J., Nantes, I.L., Rodrigues, T., 2012. *Baccharis dracunculifolia*, the main source of green propolis, exhibits potent antioxidant activity and prevents oxidative mitochondrial damage. *Food Chem. Toxicol.*, 50, 1091–1097. https://doi.org/10.1016/j.fct.2011.11.014

Gyawali, R., Ibrahim, S.A., 2014. Natural products as antimicrobial agents. *Food Control*, 46, 412–429. https://doi.org/10.1016/j.foodcont.2014.05.047

Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H.I., Huang, L., Dai, M., Wang, Y., Liu, Z., Yuan, Z., 2014. Benefits and risks of antimicrobial use in food-producing animals. *Front. Microbiol.*, 5, 1–11. https://doi.org/10.3389/fmicb.2014.00288

Helander, I.M., Alakomi, H., Mattila-sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M., Wright, A. Von, 1998. Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *J. Agric. Food Chem.*, 8561, 3590–3595. https://doi.org/10.1021/jf980154m

Herrera, P., Kwon, Y.M., Ricke, S.C., 2009. Ecology and pathogenicity of gastrointestinal *Streptococcus bovis*. *Anaerobe*, 15, 44–54. https://doi.org/10.1016/j.anaerobe.2008.11.003

Hocayen, P.D.A.S., Campos, L.A., Pochapski, M.T., Malfatti, C.R.M., 2012. Avaliação da Toxicidade do extrato bruto metanólico de *Baccharis dracunculifolia* por meio do bioensaio com Artemia salina. *Insul. Rev. Botânica*, 0, 23–31. https://doi.org/10.5007/2178-4574.2012n41p23

Hossain, M.A., Harbi, S.R.A.L., Weli, A.M., Al-Riyami, Q., Al-Sabahi, J.N., 2014. Comparison of chemical constituents and antimicrobial activities of three essential oils from three different brands' clove samples collected from Gulf region. *Asian Pacific J. Trop. Dis.*, 4, 262–268. https://doi.org/10.1016/S2222-1808(14)60570-3

Ickowitz, A., Powell, B., Rowland, D., Jones, A., Sunderland, T., 2019. Agricultural intensification, dietary diversity, and markets in the global food security narrative. *Glob. Food Sec.*, 20, 9–16. https://doi.org/10.1016/j.gfs.2018.11.002

Kirchhelle, C., 2018. Pharming animals: a global history of antibiotics in food production (1935–2017). *Palgrave Commun.*, 4, 96. https://doi.org/10.1057/s41599-018-0152-2

Kuru, P., 2014. *Tamarindus indica* and its health related effects. *Asian Pac. J. Trop. Biomed.*, 4, 676–681. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0173

Lage, T.C. de A., Montanari, R.M., Fernandes, S.A., Monteiro, C.M. de O., Senra, T. de O.S., Zeringota, V., Matos, R. da S., Daemon, E., 2015. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). *Exp. Parasitol.*, 148, 24–29. https://doi.org/10.1016/j.exppara.2014.10.011

Lambert, R.J.W., Skandamis, P.N., Coote, P.J., Nychas, G.-J.E., 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.*, 91, 453 – 462. https://doi.org/10.2523/92513-ms

Lubi, M.C., Thachil, E.T., 2000. Cashew nut shell liquid (CNSL) - A versatile monomer for polymer synthesis. *Des. Monomers Polym.*, 3, 123–153. https://doi.org/10.1163/156855500300142834

Luzia, D.M.M., Jorge, N., 2011. Antioxidant activity, fatty acid profile and tocopherols of *Tamarindus indica* L. seeds. *Ciência e Tecnol. Aliment.*, 31, 497–501. https://doi.org/10.1590/s0101-20612011000200034

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P.W., Cotter, P.D., 2019. The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*, 10, 115–132. https://doi.org/10.1080/19490976.2018.1505176

McAllister, T.A., Stanford, K., Chaves, A. V, Evans, P.R., Eustaquio de Souza Figueiredo, E., Ribeiro, G., 2020. Nutrition, feeding and management of beef cattle in intensive and extensive production systems, in: Animal Agriculture. Elsevier, pp. 75–98. https://doi.org/10.1016/B978-0-12-817052-6.00005-7

McGuffey, R.K., Richardson, L.F., Wilkinson, J.I.D., 2001. Ionophores for Dairy Cattle: Current Status and Future Outlook. *J. Dairy Sci.*, 84, E194–E203. https://doi.org/10.3168/jds.s0022-0302(01)70218-4

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

Morais, J.A. da S., Berchielli, T.T., Reis, R.A., 2006. Aditivos. *Nutr. ruminantes*, 1, 539–570.

Novilla, M.N., 2018. Ionophores, in: Veterinary Toxicology. Elsevier, pp. 1073–1092. https://doi.org/10.1016/B978-0-12-811410-0.00078-7

Novilla, M.N., Mcclary, D., Laudert, S.B., 2017. Chapter 29 - Ionophores, Reproductive and Developmental Toxicology. Elsevier Inc. https://doi.org/10.1016/B978-0-12-804239-7.00029-9

Nunoi, A., Wanapat, M., Foiklang, S., Ampapon, T., Viennasay, B., 2019. Effects of replacing rice bran with tamarind seed meal in concentrate mixture diets on the changes in ruminal ecology and feed utilization of dairy steers. *Trop. Anim. Health Prod.*, 51, 523–528. https://doi.org/10.1007/s11250-018-1719-z

Oh, S., Suzuki, Yasuyuki, Hayashi, S., Suzuki, Yutaka, Koike, S., Kobayashi, Y., 2017. Potency of cashew nut shell liquid in rumen modulation under different dietary conditions and indication of its surfactant action against rumen bacteria. *J. Anim. Sci. Technol.*, 59, 1–7. https://doi.org/10.1186/s40781-017-0150-8

OJEU, 2003. Regulation (EC) No. 1831/2003 of European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition, Official Journal of European Union.

Ornaghi, M.G., Guerrero, A., Vital, A.C.P., Souza, K.A., Passetti, R.A.C., Mottin, C., Castilho, R.A., Sañudo, C., Prado, I.N., 2020. Improvements in the quality of meat from beef cattle fed natural additives. *Meat Sci.*, 163, 108059. https://doi.org/10.1016/J.MEATSCI.2020.108059

Ornaghi, M.G., Passetti, R.A.C., Torrecilhas, J.A., Mottin, C., Vital, A.C.P., Guerrero, A., Sañudo, C., del Mar Campo, M., Prado, I.N., 2017. Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Anim. Feed Sci. Technol.*, 234, 274–283. https://doi.org/10.1016/j.anifeedsci.2017.10.008

Pandey, A., Singh, P., 2011. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. *Asian J. Plant Sci. Res.*, 1, 69–80.

Pandey, A.K., Kumar, P., Singh, P., Tripathi, N.N., Bajpai, V.K., 2017. Essential oils: Sources of antimicrobials and food preservatives. *Front. Microbiol.*, 7, 2161.

Patra, A.K., Yu, Z., 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. *Appl. Environ. Microbiol.*, 78, 4271–4280. https://doi.org/10.1128/AEM.00309-12

Rao, Y.S., Mathew, K.M., 2012. Tamarind, in: Handbook of Herbs and Spices. Elsevier, pp. 512–533. https://doi.org/10.1533/9780857095688.512

Reis, P.M.C.L., Dariva, C., Barroso Vieira, G.Â., Hense, H., 2016. Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (*Tamarindus indica*), sweet variety. *J. Food Eng.*, 173, 116–123. https://doi.org/10.1016/j.jfoodeng.2015.11.001

Rodrigues, D.M., De Souza, M.C., Arruda, C., Pereira, R.A.S., Bastos, J.K., 2020. The Role of *Baccharis dracunculifolia* and its Chemical Profile on Green Propolis Production by *Apis mellifera*. *J. Chem. Ecol.*, 46, 150–162. https://doi.org/10.1007/s10886-019-01141-w

Rodrigues, P.H.M., 2016. Rumenology: Chapter 6 - Control and Manipulation of Ruminal Fermentation. Springer Nature. https://doi.org/10.1007/978-3-319-30533-2

Roy, D., Tomar, S.K., Kumar, V., 2015. Rumen modulatory effect of thyme, clove and peppermint oils in vitro using buffalo rumen liquor. *Vet. World*, 8, 203–207. https://doi.org/10.14202/vetworld.2015.203-207

Russel, J.B., Strobel, H.J., 1989. Effect of lonophores on ruminal fermentation. *Appl. Environ. Microbiol.*, 55, 1–6.

Russell, J.B., 1987. A Proposed Mechanism of Monensin Action in Inhibiting Ruminant Bacterial Growth : Effects on Ion Flux and Protonmotive Force. *J. Anim. Sci.*, 64, 1519–1525. https://doi.org/10.2527/jas1987.6451519x

Russell, J.B., Houlihan, A.J., 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiol. Rev.*, 27, 65–74. https://doi.org/10.1016/S0168-6445(03)00019-6

Schelling, G.T., 1984. Monensin mode of action in the rumen. *J. Anim. Sci.*, 58, 1518–1527.

Siddhuraju, P., 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. *LWT - Food Sci. Technol.*, 40, 982–990. https://doi.org/10.1016/j.lwt.2006.07.010

Silva, A.P. dos S., Zotti, C.A., Carvalho, R.F., Corte, R.R., Cônsolo, N.R.B., Silva, S. da L. e, Leme, P.R., 2019. Effect of replacing antibiotics with functional oils following an abrupt transition to high-concentrate diets on performance and carcass traits of Nellore cattle. *Anim. Feed Sci. Technol.*, 247, 53–62. https://doi.org/10.1016/j.anifeedsci.2018.10.015

Souza, K.A. de, Monteschio, J. de O., Mottin, C., Ramos, T.R., Pinto, L.A. de M., Eiras, C.E., Guerrero, A., Prado, I.N., 2019. Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedl. *Livest. Sci.*, 220, 190–195. https://doi.org/10.1016/j.livsci.2018.12.026

Tril, U., Fernández-López, J., Álvarez, J.Á.P., Viuda-Martos, M., 2014. Chemical, physicochemical, technological, antibacterial and antioxidant properties of rich-fibre powder extract obtained from tamarind (*Tamarindus indica* L.). *Ind. Crops Prod.*, 55, 155–162. https://doi.org/10.1016/j.indcrop.2014.01.047

Tullo, E., Finzi, A., Guarino, M., 2019. Review: Environmental impact of livestock farming and Precision Livestock Farming as a mitigation strategy. *Sci. Total Environ.*, 650, 2751–2760. https://doi.org/10.1016/j.scitotenv.2018.10.018

Valero, M.V., Prado, R.M., Zawadzki, F., Eiras, C.E., Madrona, G.S., Prado, I.N., 2014. Propolis and essential oils additives in the diets improved animal performance. *Acta Sci. - Anim. Sci.*, 36, 419–426. https://doi.org/10.4025/actascianimsci.v36i4.23856

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. *Asian-Australasian J. Anim. Sci.*, 15, 1458–1468.

Watanabe, Y., Suzuki, R., Koike, S., Nagashima, K., Mochizuki, M., Forster, R.J., Kobayashi, Y., 2010. In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. *J. Dairy Sci.*, 93, 5258–5267. https://doi.org/10.3168/jds.2009-2754

Williams, R.J., Spencer, J.P.E.E., Rice-Evans, C., 2004. Flavonoids: antioxidants or signalling molecules? *Free Radic. Biol. Med.*, 36, 838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Zawadzki, F., Prado, I.N., Marques, J.A., Zeoula, L.M., Rotta, P.P., Sestari, B.B., Valero, M. V., Rivaroli, D.C., 2011. Sodium monensin or propolis extract in the diets of feedlot-finished bulls: Effects on animal performance and carcass characteristics. *J. Anim. Feed Sci.*, 20, 16–25. https://doi.org/10.22358/jafs/66153/2011

II - OBJETIVOS GERAIS

Caracterizar os compostos fenólicos presentes no extrato vegetal de folhas e caules de baccharis (*Baccharis dracunculifolia*), sementes de tamarindo (*Tamarindus indica* L.), óleo essencial da folha de cravo (*Syzygium aromaticum*), líquido da castanha do caju (LCC) (*Anacardium occidentale*) e avaliar a atividade antimicrobiana *in vitro* desses extratos vegetais e óleos essenciais sobre cepas de bactérias ruminais Gram-negativas. Também foi verificado os efeitos sobre o desempenho, eficiência alimentar, digestibilidade, concentrações de ácidos graxos voláteis (AGV) e comportamento ingestivo de bovinos mestiços terminados em confinamento.

III - *In vitro* antimicrobial activity of baccharis, tamarind, cashew nut shell liquid, and clove oil against Gram-negative ruminal bacteria

Journal: Animal Feed Science and Technology

Abstract

This study aimed to evaluate the *in vitro* antibacterial activity of *Baccharis dracunculifolia* and *Tamarindus indica* L. aqueous extract, cashew nut shell liquid (CNSL) natural extracts, and clove essential oil (EO) against five species of Gram-negative ruminal bacteria. Cultures were grown in anaerobic media containing 0.1, 0.2, 0.5 and 1.0 mg mL^{-1} of the extracts or oils. Growth was evaluated by monitoring the optical density (OD 600 nm) at intervals of 0, 8, 12 and 24 hours of incubation at 39 °C. The baccharis and tamarind aqueous extract, and CNSL natural extract inhibited the growth of *Prevotella albensis*, *Prevotella bryantii*, *Treponema saccharophilum* and *Succinivibrio dextrinosolvens*. For *Prevotella ruminicola* and *Succinivibrio dextrinosolvens*, the addition of the clove leaf EO of 1.0 mg mL^{-1} resulted in a greater impact on growth dynamics, with a reduction in optical density in all intervals of observations. The findings of this research establish the efficacy of natural additives aqueous extracts of baccharis and tamarind, CNSL, and clove essential oil, in antimicrobial activity *in vitro* against the Gram-negative ruminal bacteria analyzed.

Keywords:

Antibacterial activity, natural extracts, essential oil, rumen bacteria

Abbreviations:

Cashew nut shell liquid (CNSL); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Essential oil (EO); Gas chromatography coupled to mass spectrometry (GC-MS); High-resolution mass spectrometry (HRMS); Hydro ethanolic lyophilized *Baccharis dracunculifolia* (HBDL); Nuclear magnetic resonance (NMR); Optical density (OD); Ultra-high-performance liquid chromatography (UHPLC).

1. Introduction

Ruminant nutritionists are studying the effects of natural additives in the diets of animals to reduce the preventable losses during the fermentation process (Benchaar and Greathead, 2011; Monteschio et al., 2017; Ornaghi et al., 2017; Souza et al., 2019; Wallace et al., 2002). These compounds are potential rumen fermentation modulators, and without causing risk to human and animal health.

Tropical plants are rich in secondary metabolites, such as phenolic and flavonoids compounds. Biological activities such as antibacterial and anti-inflammatory activity in ruminants can improve dry matter digestibility, reduce methane emissions and increase propionate production (Olagaray and Bradford, 2019). Among some of the natural compounds tested *in vitro* an *in vivo*, the baccharis (*Baccharis dracunculifolia*) (Bonin et al., 2020; Campos et al., 2016; Zuccolotto et al., 2019), tamarind (*Tamarindus indica* L) (Arshad et al., 2019; Souza et al., 2018; Wang et al., 2017), cashew (*Anacardium occidentale*) (Cruz et al., 2014; Valero et al., 2014, 2016), and clove (*Syzygium aromaticum*) (Cortés-Rojas et al., 2014; Pandey and Singh, 2011; Passone et al., 2012) are easily available. Research concerning the effects of plant extracts and essential oils (EO) on ruminal bacteria growing is still limited. Bioactive

compounds extracted from plants have the capacity to affect the integrity of the cell envelope in Gram-negative bacteria.

The hypothesis of this study was that the inclusion of extracts of leaves and stems of Baccharis, tamarind seeds, cashew and cloves oils could reduce the activity of the main Gram-negative bacteria of rumen.

Thus, this study was carried out to evaluate the main class of secondary metabolites present in the seed tamarind extract by ^{1}H NMR, and the chemical composition clove oils by GC-MS. Furthermore, the antimicrobial effect *in vitro* action against principal species of Gram-negative ruminal bacteria of baccharis extract, seed tamarind extract, cashew nut shell liquid and clove oil was also determined.

2. Materials and methods

2.1. Ethics

All experimental procedures were conducted under the surveillance of the Animal Care and Use Committee of the Universidade Estadual de Maringá, Brazil (protocol number 1103290719) and met the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

2.2. Origin of vegetable extracts and essential oils

The baccharis (*Baccharis dracunculifolia*) leaves and stems of the upper part was collected in Maringá, city, Paraná state, Brazil south (latitude 23º27'S and longitude 51º59' W) during the summer period. The region has a humid temperate climate with a temperate summer of 18 ºC, and an annual average rainfall of 1,114 mm. The extract of baccharis used in this study was the same used in our previous study (Bonin et al., 2020). Analysis of the chemical constituents of baccharis using UHPLC–HRMS/MS method indicated that the extracts contained

germacrene B, spathulenol, naringenin, kaempferol, artepillin C, α-pinene, hydroxycinnamic acid, apigenin, kaempferide, limonene, phenylethanol and β-caryophyllene (Bonin et al., 2020).

The tamarind (*Tamarindus indica* L.) seeds were collected in Nova Redenção city, Bahia state, Brazil northeast (latitude 12º49'S and longitude 41º03'W) during winter. This region has a tropical climate with a dry winter season, and an annual mean temperature of 23.5 ºC and an average annual rainfall of 805 mm.

The cashew nut shell liquid (CNSL) was purchased from Safeeds® (Cascavel city, Paraná state, Brazil south) and stored at -18 ºC. CNSL was chosen because it presents cardanol and cardol as the main constituents (Andrade et al., 2011; Das et al., 2004; Medeiros et al., 2020).

The essential oil (EO) from clove leaf (*Syzygium aromaticum*) was purchased from FERQUIMA® (Vargem Grande Paulista city, São Paulo state, Brazil, southeast) and stored at -18 ºC.

2.3. Preparation of natural extracts

The plant material collected from baccharis and tamarind seeds were partially dried in a forced ventilation oven (40 °C) until they reached a constant weight at 72 hours. Samples were then processed in a knife mill through a 1 mm sieve (Wiley TE-650/1). To prepare the extracts of baccharis and tamarind, 10 grams of the partially dried material were weighed, mixed with 100 ml of distilled water, placed under agitation every 15 minutes for 2 hours. After the stirring period, the extract was filtered using filter papers (Whatman Nº1, 90 mm), stored in closed flasks, overwrapped in aluminum foil, and kept stored at a temperature of 4 °C until the analyses. Extracts of baccharis, tamarind, the CNSL and the EO from clove leaf were diluted in Tween® 80 solution (5%), to make the following stock concentrations: 200; 400; 1000 and 2000 mg L^{-1}. These concentrations represent typical amounts of compounds from plant extracts and EO supplied to ruminants diets (Ornaghi et al., 2020; Rivaroli et al., 2020).

2.4. Chemical analysis

The chemical profile of the tamarind seeds was evaluated by nuclear magnetic resonance (NMR) analysis. A part of the crude extract of tamarind seeds (12.9 g) was suspended in methanol/water (1:1, 100 mL, v/v), and successively partitioned with *n*-hexane and ethyl acetate (3 x 20 mL, v/v). Ethyl acetate fraction was submitted to NMR analysis. ^{1}H NMR spectrum was recorded on a Bruker Avance III HD spectrometer (Bruker®, Billerica, MA, USA) operating at 500 MHz, using DMSO-d6 (Sigma-Aldrich) as solvent (Figure 1).

The identification of bioactive compounds of clove leaf oil was performed using gas chromatography coupled to a mass spectrometer (GC-MS, USA) (Biondo et al., 2017), on a Thermo-Finnigan equipment, model Focus DSQ II, fitted with J&W Scientific DB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The temperature of the ionization source was 250 °C, injector at 250 °C, split injection mode 1/10. The carrier gas used was helium (99.999%) at 1.0 mL/min. A volume of 1 µL of the sample dissolved in ethyl acetate at a ratio of 1:20 was injected. The equipment operated in electron impact (70 eV) using a SCAN mode with mass spectral range of 40-650 m/z. For the separation of chemical constituents of the clove essential oil, the initial column temperature was 60 °C, increased at a rate of 3 °C/min to 246 °C, which was held for 11 minutes, then increased at a rate of 30 °C/min until the column reach a final temperature of 290 °C. The comparison of the mass spectra obtained for each sample with the standard spectra (National Institute of Standards and Technology, NIST) was performed using the MS Search Program v.2.0 program spectral library (Figure 2).

2.5. Microorganisms

Five strains of Gram-negative ruminal bacteria were used: *Prevotella albensis* (DSM 11370), *Prevotella bryantii* (DSM 11371), *Prevotella ruminicola* (ATCC® 19189™),

Treponema saccharophilum (DSM 2985), *Succinivibrio dextrinosolvens* (ATCC® 19716™). All bacterial strains were cultivated in Hungate tubes under anaerobic conditions using Hobson's M2 medium (Hobson, 1969), and at 39 °C for 18 h. Stock cultures of bacteria were stored in a refrigerator at -80 °C in 15% (v/v) glycerol stock medium for further use. For every experiment, sub culturing was performed every 2 d using Hobson's M2 medium culture. The same procedure was repeated at least three times to remove any impurities coming from the glycerol.

2.6. Description and preparation of the culture medium

Ruminal contents (500 mL) were collected from three rumen-cannulated bulls (½ Zebu *vs.* ½ European) with a mean age of 24 ± 2.0 months and a mean body weight of 418 ± 4.51 kg, two hours after morning feeding. Ruminal contents were squeezed through four layers of cheesecloth and the pooled ruminal liquid sample was chilled to 5 °C with ice, placed in bottle thermos and immediately transported to the laboratory. The ruminal fluid was centrifuged at 12,000 × g for (25 min, 4 °C) and the supernatant was stored at −20 °C. Diet of the bulls consisted of corn silage (30% DM) and 70% concentrate (12.9% crude protein; 25.5% neutral detergent fiber and 3.3% ether extract), provided *ad libitum*.

The anaerobic medium contained (per L): glucose (2.0 g); maltose (2.0 g); sodium hydrogen carbonate (4.0 g); Bacto-casitone (10.0 g); yeast extract (2.5 g); cellobiose (2.0 g); mineral solution I (150 ml); mineral solution II (150 mL); clarified rumen Fluid (200 mL); sodium lactate solution (10 mL); resazurin Solution (1 mL); distilled water (up to 1000 mL) and cysteine-HCL (1.0 g). The mineral solution I consisted of 3 g of dipotassium phosphate, in 1 liter of distilled water and the mineral solution II consisted of 3 g of monopotassium phosphate, 6 g of ammonium sulfate, 6 g of sodium chloride, 6 g of sulfate of magnesium, 0.6 g of calcium chloride in 1 liter of distilled water. The pH of the medium (6.8) was adjusted using 2 N NaOH.

The culture medium was prepared under anaerobic conditions by boiling, adding a reducing agent (cysteine), and distributing the medium in Hungate glass tubes (9.0 ml), under flux of CO_2 and then sealed with rubber septa and plastic caps (Hobson, 1969; Hungate, 1966). Tubes were sterilized in an autoclave at 120 °C for 20 minutes. After this procedure, the tubes were removed from the autoclave, waited to cool at room temperature, and then stored in the dark.

2.7. Sample preparation and analysis

The assays were performed using six replicates per bacteria (*P. albensis, P. bryantii, P. ruminicola, T. saccharophilum* and *S. dextrinosolvens*), which were allocated in one of the following treatments: control (0.5 mL of the test bacteria + 0.5 mL of the culture medium); 0.1 mg mL^{-1} (0.5 mL of the test bacteria + 0.5 mL of plant extract/oil, at a concentration of 0.1 mg mL^{-1}); 0.2 mg mL^{-1} (0.5 mL of the test bacteria + 0.5 mL of plant extract/oil, at a concentration of 0.2 mg mL^{-1}); 0.5 mg mL^{-1} (0.5 mL of the test bacteria + 0.5 mL of plant extract/oil, at a concentration of 0.5 mg mL^{-1}); 1.0 mg mL^{-1} (0.5 mL of the test bacteria + 0.5 mL of plant extract/oil, in the concentration of 1.0 mg mL^{-1}). To verify if Tween® 80 solution (5%) could have antibacterial effect in the tested cultures, tubes were prepared with culture medium, the tested bacteria (0.5 mL) and Tween® 80 solution (0.5 mL) and compared to the control treatment.

All tubes contained 9.0 mL of culture medium. Cultivation was performed at 39 °C, and bacteria growth was evaluated by quantifying the optical density (OD) at 600 nm using an spectrophotometer (Thermo scientific, Genesys 10UV Scanning). Optical density was evaluated at 0, 8, 12, and 24 hours of incubation. Strains used in the current study reached the early stationary phase after 16 h of growth (data not shown).

2.8. Statistical Analysis

After exploratory analysis of longitudinal data, all response variables showed positive asymmetric distribution. Thus, the gamma model with log connection function of the mixed generalized linear models was used. This was based on generalized estimation equations (GEE), which are the marginal distribution of mixed models, without the denotation of random effects. In this case, the sample intra-unit covariance structure was incorporated. The AR (1) was considered: first order auto-regressive. The choice of this is due to the fact that it best represented the variation of the equally spaced data and, also, the covariance decreased on average between two observations as the time interval between them increased, as suggested by Diggle et al., (2002).

The adjusted model is as follows:

$Y= \beta o + \beta 1$ time $+ \beta 2$ concentration $+ \beta 3$ time* concentration $+ \varepsilon$(error)

For each Y ~ Gamma (link = log), "AR1" correlation structure wherein all models were adjusted in the R application using the geeglm package.

3. Results

3.1. Effect of Tween® 80 on bacterial growth

Tubes containing the culture medium, the bacteria and Tween®80 (5%; v/v) had similar growth compared to control (data not shown), indicating that 5.0 mL Tween®80 solution had no antimicrobial activity against the ruminal bacteria used in this study.

3.2. Baccharis dracunculifolia

The effects of baccharis extract on growth of Gram-negative ruminal bacteria are summarized in Table 1. Except for *P. ruminicola*, all cultures of ruminal bacteria reached cell high cell densities (> 1.0) after 8 h of growth, and turbidity remained high until the end of the

experiment. However, upon the addition of baccharis extracts, a drastic decrease ($P < 0.05$) in OD was observed for *P. albensis*, *P. bryantii* and *T. saccharophilum*. These cultures were highly susceptible to the baccharis extract and even concentrations as low as 0.1 mg mL^{-1} reduced the OD_{600nm} more than 70% after 8 h of growth. *P. ruminicola* showed little susceptibility to baccharis extracts and no differences in cell densities could be observed after 12 or 24 h of growth. The fibrolytic bacteria *Succinivibrio dextrinosolvens* initially showed a decrease of approximately 80% in the OD_{600nm} at the highest concentration of the baccharis extract tested (1.0 mg ml^{-1}). However, this effect diminished during growth, and after 12 and 24 h of incubation the reduction in OD_{600nm} was only 20% when compared to the controls.

3.3. Tamarindus indica L.

The ^{1}H NMR spectrum of ethyl acetate fraction of the tamarind seeds (Figure 1) showed characteristic signals of fatty acid, such as olefinic protons at δ_H 5.3, methylene protons in the region of δ_H 1.1 to 2.7, and terminal methyl group protons at δ_H 0.8 to 0.9 (Knothe and Kenar, 2004; Tsiafoulis et al., 2019). It was also observed signs at the region of δ_H 5.7 to 7.3 and 8.7 to 10.2, indicating the possible presence of phenolic and flavonoid compounds in the tamarind acetate fraction (Charisiadis et al., 2014; Rivero-Cruz et al., 2017).

The extracts obtained from *Tamarindus indica* L. were among the least effective against the ruminal bacteria tested in this study (Table 2). Except for *P. ruminicola*, all cultures of ruminal bacteria reached cell high cell densities (> 1.0) after 8 h of growth, and turbidity remained high until the end of the experiment. However, upon the addition of *Tamarindus indica* L. extracts (12 - 24 hours), a drastic and significant decrease ($P < 0.05$) in OD was observed for *P. albensis*, and *S. dextrinosolvens*. Consistent inhibitory effects were observed at the highest concentration of the extract (1.0 mg mL^{-1}) and at later incubation times as shown for *P. ruminicola* and *S. dextrinosolvens*. *P. ruminicola* incubated with 1.0 mg mL^{-1} of tamarind extract for 24 h showed

the highest reduction in OD_{600nm} (41%). For the remaining cultures, the decrease in OD_{600nm} after 24 h was much lower, and growth inhibition varied from none (*P. bryantii*) to about 25% (*T. saccharophilum*).

3.4. Cashew nut shell liquid (CNSL)

The CNSL extract affected the bacterial growth ($P < 0.05$) for all analyzed concentrations (Table 3). Except for *S. dextrinosolvens*, upon the addition of CNSL, all cultures of ruminal bacteria presented drastic and significant decrease ($P < 0.05$) in OD_{600nm} at intervals of 8 and 12 hours of incubation. The addition of 0.1, 0.2, 0.5 and 1.0 mg mL^{-1} resulted in a late decrease in the optical density for *S. dextrinosolvens*, the effect being evident at 12 and 24 hours of observation.

3.5. Clove leaf

The identification of bioactive compounds in clove leaf EO was performed by GC-MS, and showed that eugenol ($C_{10}H_{12}O_2$, *m/z* 164.0 [M^+]) and caryophyllene ($C_{15}H_{24}$, *m/z* 204.09 [M^+]) (Figure 2) are the main compounds present in this oil.

The effects of adding clove EO (Table 4) varied according on the concentration being used. The addition of 0.2, 0.5 and 1.0 mg mL^{-1} had no effect in reducing the OD_{600nm} for *P. albensis, P. bryantii* and *T. saccharophilum.* However, the 0.1 mg mL^{-1} concentration promoted decrease in OD_{600nm} after the 12-hours observation interval. For *P. ruminicola* and *S. dextrinosolvens*, the addition of 1.0 mg mL^{-1} resulted in a greater impact on growth dynamics, with a reduction in optical density in all intervals of observations. For the concentration 0.1, 0.2 and 0.5 mg mL^{-1}, when evaluated in *P. ruminicola*, a decrease in OD_{600nm} was observed only for the first 8 hours of evaluation, whereas, for *S. dextrinosolvens*, the effects of these concentrations were evident after the 8 hours of observation.

4. Discussion

4.1. Baccharis dracunculifolia

The *in vitro* antimicrobial activity of the *Baccharis dracunculifolia* extract demonstrated in this study may be associated with synergism between the main classes of identified secondary metabolites: terpenes (germacrene B, spathulenol, α-pinene, limonene and β-caryophyllene), flavonoids (naringenin, kaempferol, kaempferide and apigenin) and phenolic compounds (artepillin C and hydroxycinnmic acid) (Bonin et al., 2020; Frizzo et al., 2008; Lage et al., 2015; Paula et al., 2017; Salazar et al., 2018). Although the exact mechanism of action of these bioactive compounds has not yet been elucidated, exposure of anaerobic ruminal bacteria to these compounds as performed in the current work indicates that they can reduce cell growth and biomass production. Previous studies indicate that plant extracts can affect the integrity of the cell membrane in bacterial cells, increasing ion permeability and causing the dissipation of the membrane potential (Mirzoeva et al., 1997; Tarahovsky et al., 2014). The electrochemical potential across the cytoplasmic membrane is essential for anaerobic bacteria to carry out ATP synthesis and substrate uptake, which is essential for biomass production (Mirzoeva et al., 1997). Further studies should be carried out to address if baccharis extracts also affect the energetics of ruminal bacteria, specially the species involved in the breakdown of proteins in ammonia in the rumen environment.

4.2. Tamarindus indica L.

All microorganisms tested showed susceptibility to the seed extract of tamarind. The antibacterial activity of the tamarind seed extract can be attributed to polyphenols, such as catechin, procyanidin B2, caffeic acid, chloramphenicol, quercetin, apigenin and kaempferol,

that are commonly found in tamarind seeds (Abukakar et al., 2008; Razali et al., 2015, 2012; Siddhuraju, 2007; Sudjaroen et al., 2005).

The underlying mechanism of action of the antibacterial activity of the tamarind extract is not yet elucidated in the literature, but it is believed in the occurrence of synergic effects of the compounds. The flavonols, such as the quercetin, found in the tamarind extract, are hydrophobic compounds that can translocate across lipid bilayers, exerting antimicrobial activity in the cytoplasm of the cell. Several mechanisms of action have been attributed to procyanidin and catechins, such as the destabilization/permeabilization of the cytoplasmic membrane and direct effects on the microbial metabolism (Tarahovsky et al., 2014). Antibacterial activities have been reported for phenolic acids, like caffeic acid, both against Gram-positive and Gram-negative bacteria (Daglia, 2012).

4.3. Cashew nut shell liquid (CNSL)

The bioactive compounds the CNSL has been the subject of research, and scientific discoveries have attracted the interest of researchers, due to its antimicrobial (Boonsai et al., 2014; Stasiuk and Kozubek, 2010) and antioxidant properties (Andrade et al., 2011) and its potential to reduce methane emissions (Watanabe et al., 2010).

The results reported in the current study demonstrate the inhibitory activity of CNSL compounds against Gram-negative ruminal bacteria. Previous studies reported that CNSL is mainly effective on Gram-positive anaerobic bacteria (Kubo et al., 2003, 1993). However, the data reported in this study, demonstrate that the Gram-negative ruminal bacteria are also highly susceptible to the compounds present in CNSL extracts.

Kubo et al. (2003) indicated that the compounds present in the CNSL extract act mainly as a surfactant, causing physical damages to the bacterial cell membranes, thus triggering cell death. Corroborating with this claim, Stasiuk and Kozubek. (2010) suggested that phenolic

lipids (such as cardanol and cardol) interact with the cell membranes and DNA structures promoting cytotoxic effects on target cells.

4.4. Clove leaf

The results found can be associated with the presence of the bioactive compounds identified, the example of eugenol (hydrophobic nature), which allows it to penetrate the lipolysaccharide layer of the Gram-negative outer membrane causing changes the cell envelope (Devi et al., 2010). In general, it has been proposed that the synergism between eugenol and caryophyllene promotes an increase in membrane permeability (Gill and Holley, 2006; Hemaiswarya and Doble, 2009; Trombetta et al., 2005), compromising the maintenance of the electrochemical gradient and impairing the growth/survival of target organisms.

5. Conclusion

The present work suggests that aqueous natural extracts of baccharis, tamarind, and cashew nut shell liquid has antimicrobial activity *in vitro* against the Gram-negative ruminal bacteria analyzed in this study. In addition, the study contributes to new information about the efficacy of the extracts plant as antimicrobial agent on ruminant production.

Acknowledgements

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for the scholarship, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (400375/2014-1) and the Company Safeeds Nutrição Animal (safeeds@safeeds.com.br). The authors gratefully acknowledge the company for financing and providing the products used in this research which it was possible to develop this work. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not

imply recommendations or endorsement by the Department of Animal Science, Universidade Estadual de Maringá, Maringá, Paraná, Brazil.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Abukakar, M.G., Ukwuani, A.N., Shehu, R.A., 2008. Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. Asian J. Biochem. https://doi.org/https://bit.ly/2RoHlj0

Andrade, T.D.J.A.D.S., Araújo, B.Q., Citó, A.M.D.G.L., Silva, J., Saffi, J., Richter, M.F., Ferraz, A.D.B.F., 2011. Antioxidant properties and chemical composition of technical Cashew Nut Shell Liquid (tCNSL). Food Chem. 126, 1044–1048. https://doi.org/10.1016/j.foodchem.2010.11.122

Arshad, M.S., Imran, M., Ahmed, A., Sohaib, M., Ullah, A., Nisa, M. un, Hina, G., Khalid, W., Rehana, H., 2019. Tamarind: A diet-based strategy against lifestyle maladies. Food Sci. Nutr. 7, 3378–3390. https://doi.org/10.1002/fsn3.1218

Benchaar, C., Greathead, H., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166–167, 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024

Biondo, P.B.F., Carbonera, F., Zawadzki, F., Chiavelli, L.U.R., Pilau, E.J., Prado, I.N., Visentainer, .V., 2017. Antioxidant capacity and identification of bioactive compounds by GC-MS of essential oils from spices, herbs and citrus. Curr. Bioact. Compd. 13, 137–143. https://doi.org/10.2174/1573407212666160614080846.

Bonin, E., Carvalho, V.M., Avila, V.D., Santos, N.C.A. dos, Benassi-Zanqueta, É., Lancheros, C.A.C., Previdelli, I.T.S., Ueda-Nakamura, T., Abreu Filho, B.A. de, Prado, I.N., 2020. *Baccharis dracunculifolia*: Chemical constituents, cytotoxicity and antimicrobial activity. LWT - Food Sci. Technol. 120, 1–10. https://doi.org/10.1016/j.lwt.2019.108920

Boonsai, P., Phuwapraisirisan, P., Chanchao, C., 2014. Antibacterial Activity of a Cardanol from Thai *Apis mellifera* Propolis. Int. J. Med. Sci. 11, 327–336. https://doi.org/10.7150/ijms.7373

Campos, R., Bressan, J., Cristina, V., Jasinski, G., Zuccolotto, T., Eduardo, L., Cerqueira, L.B., 2016.

Baccharis (Asteraceae): Chemical Constituents and Biological Activities. Chem. Biodivers. 13, 1–17. https://doi.org/10.1002/cbdv.201500363

Charisiadis, P., Kontogianni, V., Tsiafoulis, C., Tzakos, A., Siskos, M., Gerothanassis, I., 2014. ^{1}H-NMR as a Structural and Analytical Tool of Intra- and Intermolecular Hydrogen Bonds of Phenol-Containing Natural Products and Model Compounds. Molecules 19, 13643–13682. https://doi.org/10.3390/molecules190913643

Cortés-Rojas, D.F., Souza, C.R.F., Oliveira, W.P., 2014. Clove *(Syzygium aromaticum)*: A precious spice. Asian Pac. J. Trop. Biomed. 4, 90–96. https://doi.org/10.1016/S2221-1691(14)60215-X

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

Daglia, M., 2012. Polyphenols as antimicrobial agents. Curr. Opin. Biotechnol. 23, 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Das, P., Sreelatha, T., Ganesh, A., 2004. Bio oil from pyrolysis of cashew nut shell-characterisation and related properties. Biomass and Bioenergy 27, 265–275. https://doi.org/10.1016/j.biombioe.2003.12.001

Devi, K.P., Nisha, S.A., Sakthivel, R., Pandian, S.K., 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. J. Ethnopharmacol. 130, 107–115. https://doi.org/10.1016/j.jep.2010.04.025

Diggle, P., Diggle, P.J., Heagerty, P., Liang, K.-Y., Heagerty, P.J., Zeger, S., 2002. Analysis of longitudinal data. Oxford University Press.

Frizzo, C.D., Atti-Serafini, L., Laguna, S.E., Cassel, E., Lorenzo, D., Dellacassa, E., 2008. Essential oil variability in *Baccharis uncinella* DC and *Baccharis dracunculifolia* DC growing wild in southern Brazil, Bolivia and Uruguay. Flavour Fragr. J. 23, 99–106. https://doi.org/10.1002/ffj.1862

Gill, A.O., Holley, R.A., 2006. Disruption of *Escherichia coli, Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. Int. J. Food Microbiol. 108, 1–9. https://doi.org/10.1016/j.ijfoodmicro.2005.10.009

Hemaiswarya, S., Doble, M., 2009. Synergistic interaction of eugenol with antibiotics against Gram

negative bacteria. Phytomedicine 16, 997–1005. https://doi.org/10.1016/j.phymed.2009.04.006

Hobson, P.N., 1969. Rumen Bacteria, in: Methods in Microbiology. Bucksburn, Aberdeen, Scotland, pp. 133–149. https://doi.org/10.1016/S0580-9517(08)70504-X

Hungate, R.E., 1966. The Rumen and its microbes, Academic P. ed, The rumen and its microbes. New York. https://doi.org/10.1016/B978-1-4832-3308-6.50005-X

Knothe, G., Kenar, J.A., 2004. Determination of the fatty acid profile by ^{1}H-NMR spectroscopy. Eur. J. Lipid Sci. Technol. 106, 88–96. https://doi.org/10.1002/ejlt.200300880

Kubo, I., Muroi, H., Himejima, M., Yamagiwa, Y., Mera, H., Tokushima, K., Ohta, S., Kamikawa, T., 1993. Structure-Antibacterial Activity Relationships of Anacardic Acids. J. Agric. Food Chem. 41, 1016–1019. https://doi.org/10.1021/jf00030a036

Kubo, I., Nihei, K.I., Tsujimoto, K., 2003. Antibacterial Action of Anacardic Acids against Methicillin Resistant *Staphylococcus aureus* (MRSA). J. Agric. Food Chem. 51, 7624–7628. https://doi.org/10.1021/jf034674f

Lage, T.C. de A., Montanari, R.M., Fernandes, S.A., Monteiro, C.M. de O., Senra, T. de O.S., Zeringota, V., Matos, R. da S., Daemon, E., 2015. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). Exp. Parasitol. 148, 24–29. https://doi.org/10.1016/j.exppara.2014.10.011

Medeiros, M.C., dos Santos, E. V., Martínez-Huitle, C.A., Fajardo, A.S., Castro, S.S.L., 2020. Obtaining high-added value products from the technical cashew-nut shell liquid using electrochemical oxidation with BDD anodes. Sep. Purif. Technol. 117099. https://doi.org/10.1016/j.seppur.2020.117099

Mirzoeva, O.K., Grishanin, R.N., Calder, P.C., 1997. Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria. Microbiol. Res. 152, 239–246. https://doi.org/10.1016/S0944-5013(97)80034-1

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

Olagaray, K.E., Bradford, B.J., 2019. Plant flavonoids to improve productivity of ruminants – A review.

Anim. Feed Sci. Technol. 251, 21–36. https://doi.org/10.1016/j.anifeedsci.2019.02.004

Ornaghi, M.G., Guerrero, A., Vital, A.C.P., Souza, K.A., Passetti, R.A.C., Mottin, C., Castilho, R.A., Sañudo, C., Prado, I.N., 2020. Improvements in the quality of meat from beef cattle fed natural additives. Meat Sci. 163, 108059. https://doi.org/10.1016/J.MEATSCI.2020.108059

Ornaghi, M.G., Passetti, R.A.C., Torrecilhas, J.A., Mottin, C., Vital, A.C.P., Guerrero, A., Sañudo, C., del Mar Campo, M., Prado, I.N., 2017. Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. Anim. Feed Sci. Technol. 234, 274–283. https://doi.org/10.1016/j.anifeedsci.2017.10.008

Pandey, A., Singh, P., 2011. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian J. Plant Sci. Res. 1, 69–80.

Passone, M.A., Girardi, N.S., Ferrand, C.A., Etcheverry, M., 2012. *In vitro* evaluation of five essential oils as botanical fungitoxicants for the protection of stored peanuts from *Aspergillus flavus* and *A. parasiticus* contamination. Int. Biodeterior. Biodegradation 70, 82–88. https://doi.org/10.1016/j.ibiod.2011.11.017

Paula, J.T., Sousa, I.M.O., Foglio, M.A., Cabral, F.A., 2017. Selective fractionation of supercritical extracts from leaves of Baccharis dracunculifolia. J. Supercrit. Fluids 127, 62–70.

Razali, N., Junit, S.M., Ariffin, A., Siti, N., Ramli, F., Aziz, A.A., 2015. Polyphenols from the extract and fraction of *T. indica* seeds protected HepG2 cells against oxidative stress. BMC Complement. Altern. Med. 15:438, 1–16. https://doi.org/10.1186/s12906-015-0963-2

Razali, N., Mat-Junit, S., Abdul-Muthalib, A.F., Subramaniam, S., Abdul-Aziz, A., 2012. Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica* L. Food Chem. 131, 441–448. https://doi.org/10.1016/j.foodchem.2011.09.001

Rivaroli, D.C., Campo, M.M., Sañudo, C., Guerrero, A., Jorge, A.M., Vital, A.C.P., Valero, M.V., Prado, R.M., Prado, I.N., 2020. Effect of an essential oils blend on meat characteristics of crossbred heifers finished on a high-grain diet in a feedlot. Anim. Prod. Sci. 60, 595–602. https://doi.org/https://doi.org/10.1071/AN18620.

Rivero-Cruz, J., Rodríguez de San Miguel, E., Robles-Obregón, S., Hernández-Espino, C., Rivero-Cruz, B., Pedraza-Chaverri, J., Esturau-Escofet, N., 2017. Prediction of Antimicrobial and Antioxidant Activities of Mexican Propolis by ^{1}H-NMR Spectroscopy and Chemometrics Data Analysis.

Molecules 22, 1184. https://doi.org/10.3390/molecules22071184

Salazar, G.J.T., de Sousa, J.P., Lima, C.N.F., Lemos, I.C.S., da Silva, A.R.P., Freitas, T.S. de, Coutinho, H.D.M., Silva, L.E. da, Amaral, W. do, Deschamps, C., 2018. Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation. Ind. Crop. Prod. 122, 591–595. https://doi.org/10.1016/j.indcrop.2018.06.052

Siddhuraju, P., 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT - Food Sci. Technol. 40, 982–990. https://doi.org/10.1016/j.lwt.2006.07.010

Souza, C.M., Oliveira, R.L., Voltolini, T. V., Menezes, D.R., dos Santos, N.J.A., Barbosa, A.M., Silva, T.M., Pereira, E.S., Bezerra, L.R., Santos, N.J.A., Barbosa, A.M., Silva, T.M., Pereira, E.S., Bezerra, L.R., 2018. Lambs fed cassava silage with added tamarind residue: silage quality, intake, digestibility, nitrogen balance, growth performance and carcass quality. Anim. Feed Sci. Technol. 235, 50–59. https://doi.org/10.1016/j.anifeedsci.2017.11.007

Souza, K.A. de, Monteschio, J. de O., Mottin, C., Ramos, T.R., Pinto, L.A. de M., Eiras, C.E., Guerrero, A., Prado, I.N., 2019. Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedl. Livest. Sci. 220, 190–195. https://doi.org/10.1016/j.livsci.2018.12.026

Stasiuk, M., Kozubek, A., 2010. Biological activity of phenolic lipids. Cell. Mol. life Sci. 67, 841–860.

Sudjaroen, Y., Haubner, R., Würtele, G., Hull, W.E., Erben, G., Spiegelhalder, B., Changbumrung, S., Bartsch, H., Owen, R.W., 2005. Isolation and structure elucidation of phenolic antioxidants from Tamarind (*Tamarindus indica* L.) seeds and pericarp. Food Chem. Toxicol. 43, 1673–1682. https://doi.org/10.1016/j.fct.2005.05.013

Tarahovsky, Y.S., Kim, Y.A., Yagolnik, E.A., Muzafarov, E.N., 2014. Flavonoid–membrane interactions: Involvement of flavonoid–metal complexes in raft signaling. Biochim. Biophys. Acta - Biomembr. 1838, 1235–1246. https://doi.org/10.1016/j.bbamem.2014.01.021

Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., Bisignano, G., 2005. Mechanisms of antibacterial action of three monoterpenes. Antimicrob.

Agents Chemother. 49, 2474–2478. https://doi.org/10.1128/AAC.49.6.2474-2478.2005

Tsiafoulis, C., Papaemmanouil, C., Alivertis, D., Tzamaloukas, O., Miltiadou, D., Balayssac, S., Malet-Martino, M., Gerothanassis, I., 2019. NMR-Based Metabolomics of the Lipid Fraction of Organic and Conventional Bovine Milk. Molecules 24, 1067. https://doi.org/10.3390/molecules24061067

Valero, M.V., Prado, R.M., Zawadzki, F., Eiras, C.E., Madrona, G.S., Prado, I.N., 2014. Propolis and essential oils additives in the diets improved animal performance. Acta Sci. - Anim. Sci. 36, 419–426. https://doi.org/10.4025/actascianimsci.v36i4.23856

Valero, M. V, Farias, M.S., Zawadzki, F., Prado, R.M., Fugita, C.A., Rivaroli, D.C., Ornaghi, M.G., Prado, I.N., 2016. Feeding propolis or essential oils (cashew and castor) to bulls: performance, digestibility, and blood cell counts. Rev. Colomb. Ciencias Pecu. 29, 33–42.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. Asian-Australasian J. Anim. Sci. 15, 1458–1468.

Wang, L., Nakanishi, T., Sato, Y., Oishi, K., Hirooka, H., Takahashi, K., Kumagai, H., 2017. Effect of feeding tamarind kernel powder extract residue on digestibility, nitrogen availability and ruminal fermentation in wethers. Asian-Australasian J. Anim. Sci. 30, 379–385. https://doi.org/10.5713/ajas.16.0137

Watanabe, Y., Suzuki, R., Koike, S., Nagashima, K., Mochizuki, M., Forster, R.J., Kobayashi, Y., 2010. In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. J. Dairy Sci. 93, 5258–5267. https://doi.org/10.3168/jds.2009-2754

Zuccolotto, T., Bressan, J., Lourenço, A.V.F., Bruginski, E., Veiga, A., Marinho, J.V.N., Raeski, P.A., Heiden, G., Salvador, M.J., Murakami, F.S., Budel, J.M., Campos, F.R., 2019. Chemical, Antioxidant, and Antimicrobial Evaluation of Essential Oils and an Anatomical Study of the Aerial Parts from *Baccharis* Species (Asteraceae). Chem. Biodivers. 16, e1800547. https://doi.org/10.1002/cbdv.20180054

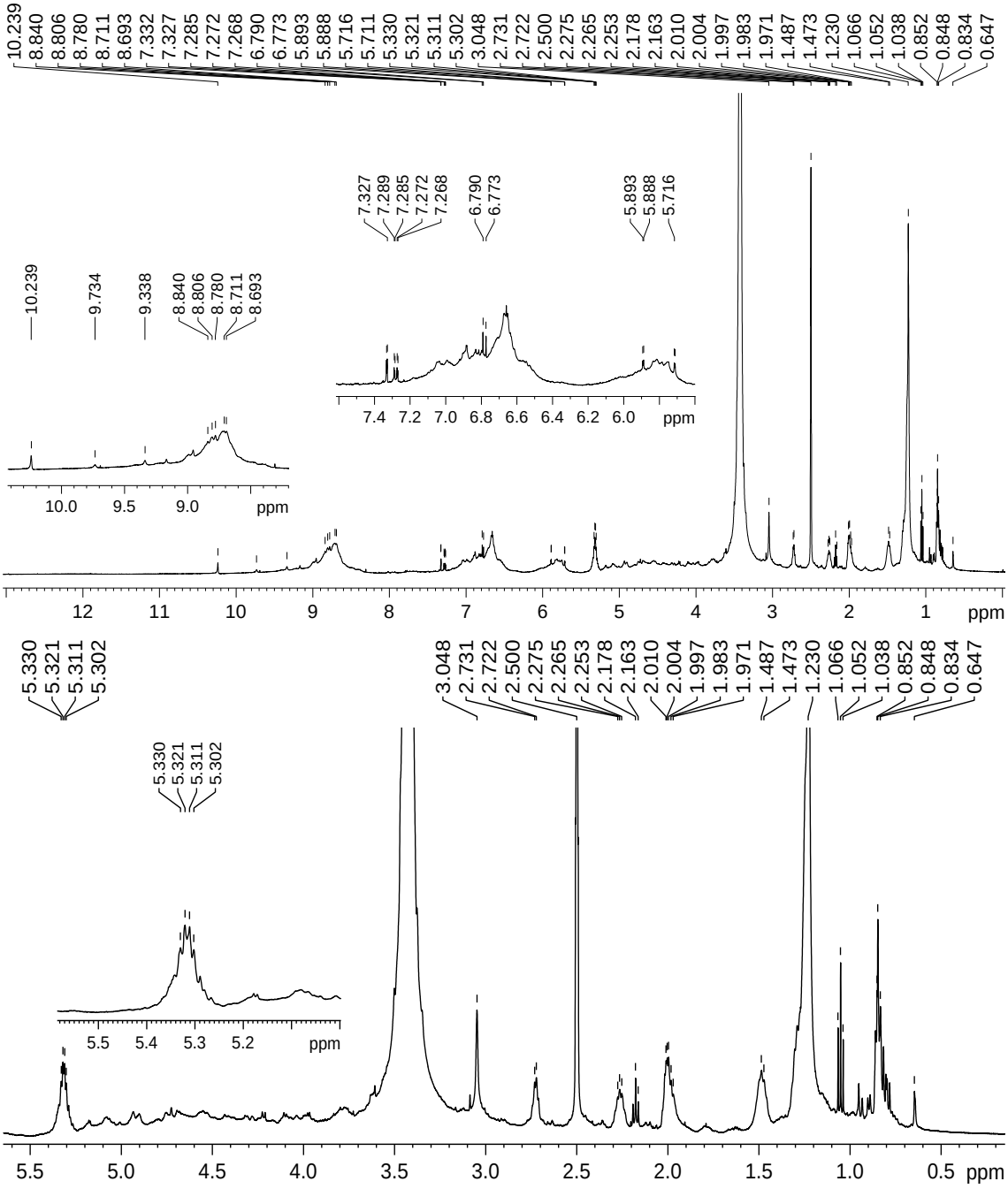

Figure 1. ^{1}H-NMR spectrum (DMSO-d_6, 500 MHz) of ethyl acetate fraction of tamarind.

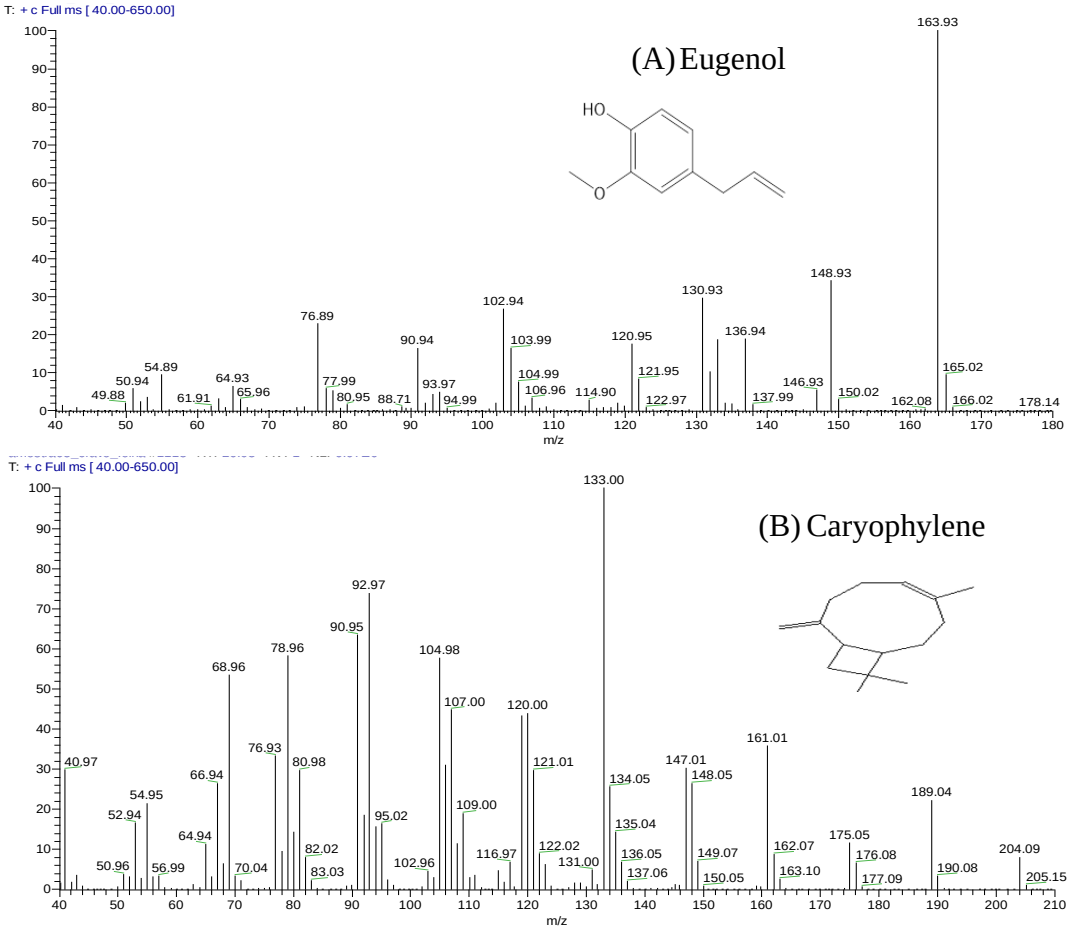

Figure 2. Chemical profile from essential oil of clove leaf, using gas chromatography coupled to mass spectrometry (GC-MS).

Table 1. Effect of *Baccharis dracunculifolia* extract against Gram-negative ruminal bacteria

Concentration mg mL^{-1}	Time (hours)			[1]SEM
	8	12	24	
	Prevotella albensis			
0.0	1.527^{A}	1.611^{A}	1.532^{A}	0.019
0.1	0.213^{bB}	0.202^{bC}	0.507^{aBC}	0.027
0.2	0.285^{cB}	0.456^{bB}	0.581^{aB}	0.027
0.5	0.266^{cB}	0.374^{bB}	0.468^{aC}	0.027
1.0	0.304^{bB}	0.367^{bB}	0.431^{aC}	0.027
SEM	0.027	0.027	0.027	
	Prevotella bryantii			
0.0	1.284^{cA}	1.374^{bA}	1.454^{aA}	0.016
0.1	0.338^{bD}	0.441^{aD}	0.506^{aBC}	0.022
0.2	0.429^{C}	0.487^{CD}	0.469^{C}	0.022
0.5	0.520^{B}	0.571^{B}	0.555^{B}	0.022
1.0	0.552^{aB}	0.525^{bBC}	0.426^{bC}	0.022
SEM	0.022	0.022	0.022	
	Prevotella ruminicola			
0.0	0.794^{AB}	0.990	0.968	0.046
0.1	0.874^{A}	0.937	1.050	0.065
0.2	0.604^{bB}	0.983^{a}	0.942^{a}	0.065
0.5	0.561^{bB}	1.133^{a}	0.953^{a}	0.065
1.0	0.911^{A}	0.966	1.061	0.065
SEM	0.065	0.065	0.065	
	Treponema saccharophilum			
0.0	1.398^{A}	1.378^{A}	1.430^{A}	0.020
0.1	0.328^{bC}	0.349^{bC}	0.461^{aC}	0.028
0.2	0.526^{B}	0.583^{B}	0.551^{BC}	0.029
0.5	0.555^{B}	0.579^{B}	0.621^{B}	0.029
1.0	0.544^{aB}	0.537^{aB}	0.459^{bC}	0.029
SEM	0.028	0.028	0.028	
	Succinivibrio dextrinosolvens			
0.0	1.045^{bB}	1.322^{aA}	1.117^{bA}	0.04
0.1	1.320^{aA}	1.086^{bB}	0.860^{cB}	0.056
0.2	1.184^{aAB}	1.038^{bB}	0.815^{cB}	0.057
0.5	0.980^{aB}	1.015^{aB}	0.836^{bB}	0.057
1.0	0.203^{bC}	0.985^{aB}	0.879^{aB}	0.057
SEM	0.057	0.057	0.057	

Different lowercase letters in the same line are significantly different and different uppercase letters in the same column are significantly different ($P < 0.05$). [1]SEM = standard error of the mean.

Table 2. Effect of *Tamarindus indica* L. extract against Gram-negative ruminal bacteria

Concentration mg mL^{-1}	Time, hours			^{1}SEM
	8	12	24	
	Prevotella albensis			
0.0	1.527	1.611^{A}	1.532^{A}	0.025
0.1	1.417	1.420^{B}	1.372^{B}	0.036
0.2	1.432	1.433^{B}	1.358^{B}	0.036
0.5	1.440^{a}	1.390^{abB}	1.310^{bB}	0.036
1.0	1.395	1.415^{B}	1.395^{B}	0.036
SEM	0.036	0.036	0.036	
	Prevotella bryantii			
0.0	1.284^{bB}	1.374^{bB}	1.454^{a}	0.029
0.1	0.757^{bBC}	1.382^{aB}	1.492^{a}	0.042
0.2	0.762^{bC}	0.833^{bC}	1.456^{a}	0.042
0.5	1.581^{aA}	1.556^{abA}	1.448^{b}	0.042
1.0	1.556^{A}	1.631^{A}	1.570	0.042
SEM	0.042	0.042	0.042	
	Prevotella ruminicola			
0.0	0.794^{bB}	0.990^{a}	0.968^{aA}	0.028
0.1	0.935^{aAB}	1.067^{a}	0.465^{bC}	0.039
0.2	0.978^{aA}	1.061^{a}	0.457^{bC}	0.039
0.5	0.993^{aA}	1.060^{a}	0.406^{bC}	0.039
1.0	0.978^{aA}	1.054^{a}	0.571^{bB}	0.039
SEM	0.039	0.039	0.039	
	Treponema saccharophilum			
0.0	1.398^{A}	1.378^{A}	1.430^{A}	0.020
0.1	0.665^{cBC}	1.191^{bC}	1.413^{aA}	0.040
0.2	0.395^{bD}	1.226^{aBC}	1.311^{aA}	0.040
0.5	0.725^{bB}	1.351^{aAB}	1.326^{aA}	0.040
1.0	0.537^{bCD}	1.029^{aD}	1.075^{aB}	0.040
SEM	0.040	0.040	0.040	
	Succinivibrio dextrinosolvens			
0.0	1.045^{bBC}	1.322^{aA}	1.117^{bA}	0.04
0.1	1.235^{aAB}	0.994^{bB}	0.810^{cB}	0.055
0.2	1.277^{aA}	1.068^{bB}	0.888^{cB}	0.056
0.5	1.13^{aABC}	1.05^{aB}	0.809^{bB}	0.056
1.0	0.964^{abC}	1.000^{aB}	0.856^{bB}	0.056
SEM	0.056	0.056	0.056	

Different lowercase letters in the same line are significantly different and different uppercase letters in the same column are significantly different ($P < 0.05$). ^{1}SEM = standard error of the mean.

Table 3. Effect of cashew nut shell liquid against Gram-negative ruminal bacteria

Concentration mg mL^{-1}	Time, hours			[1]SEM
	8	12	24	
	Prevotella albensis			
0.0	1.527^{A}	1.611^{A}	1.532^{B}	0.025
0.1	0.100^{cB}	0.350^{bB}	1.630^{aAB}	0.038
0.2	0.160^{cB}	0.450^{bB}	1.630^{aAB}	0.038
0.5	0.120^{cB}	0.430^{abB}	1.670^{aA}	0.038
1.0	0.100^{cB}	0.420^{bB}	1.500^{aB}	0.038
SEM	0.038	0.038	0.038	
	Prevotella bryantii			
0.0	1.284^{bA}	1.374^{abA}	1.454^{aB}	0.028
0.1	0.104^{cB}	0.677^{bB}	1.554^{aAB}	0.039
0.2	0.132^{cB}	0.777^{bB}	1.488^{aAB}	0.039
0.5	0.040^{cB}	0.529^{bC}	1.477^{aB}	0.039
1.0	0.169^{cB}	0.686^{bB}	1.623^{aA}	0.039
SEM	0.039	0.039	0.039	
	Prevotella ruminicola			
0.0	0.794^{bA}	0.990^{aA}	0.968^{aA}	0.034
0.1	0.117^{bB}	0.357^{aCD}	0.476^{aC}	0.048
0.2	0.053^{cB}	0.291^{bD}	0.947^{aA}	0.048
0.5	0.165^{cB}	0.614^{bB}	1.010^{aA}	0.048
1.0	0.032^{cB}	0.484^{bBC}	0.678^{aB}	0.048
SEM	0.048	0.048	0.048	
	Treponema saccharophilum			
0.0	1.398^{A}	1.378^{A}	1.430	0.039
0.1	0.152^{cB}	0.415^{bD}	1.577^{a}	0.056
0.2	0.170^{cB}	0.456^{bCD}	1.436^{a}	0.056
0.5	0.107^{cB}	0.627^{bBC}	1.463^{a}	0.056
1.0	0.150^{cB}	0.771^{bB}	1.557^{a}	0.056
SEM	0.056	0.056	0.056	
	Succinivibrio dextrinosolvens			
0.0	1.045^{bB}	1.322^{aA}	1.117^{bA}	0.041
0.1	1.223^{aA}	0.585^{cB}	0.742^{bB}	0.057
0.2	1.250^{aA}	0.972^{bB}	0.774^{cB}	0.058
0.5	1.010^{aB}	1.022^{aB}	0.676^{bB}	0.058
1.0	1.067^{aB}	1.006^{aB}	0.775^{bB}	0.058
SEM	0.058	0.058	0.058	

Different lowercase letters in the same line are significantly different and different uppercase letters in the same column are significantly different ($P < 0.05$). [1]SEM = standard error of the mean.

Table 4. Effect of clove essential oil against Gram-negative ruminal bacteria

Concentration mg mL^{-1}	Time, hours			[1]SEM
	8	12	24	
	Prevotella albensis			
0.0	1.527	1.611^{AB}	1.532^{A}	0.045
0.1	1.480^{a}	1.540^{aAB}	1.070^{bB}	0.064
0.2	1.460	1.550^{AB}	1.480^{A}	0.064
0.5	1.550	1.690^{A}	1.560^{A}	0.064
1.0	1.390	1.440^{B}	1.570^{A}	0.064
SEM	0.064	0.064	0.064	
	Prevotella bryantii			
0.0	1.284^{bC}	1.374^{ab}	1.454^{aA}	0.038
0.1	1.560^{aA}	1.540^{a}	0.910^{bB}	0.053
0.2	1.510^{AB}	1.520	1.520^{A}	0.053
0.5	1.440^{ABC}	1.530	1.480^{A}	0.053
1.0	1.340^{BC}	1.470	1.410^{A}	0.053
SEM	0.053	0.053	0.053	
	Prevotella ruminicola			
0.0	0.794^{bA}	0.990^{aA}	0.968^{aA}	0.027
0.1	0.206^{bB}	0.833^{Ab}	0.839^{aAB}	0.038
0.2	0.194^{bB}	0.862^{aAB}	0.915^{aAB}	0.038
0.5	0.222^{bB}	0.847^{AB}	0.875^{aAB}	0.038
1.0	0.205^{bB}	0.832^{aB}	0.798^{aB}	0.038
SEM	0.038	0.038	0.038	
	Treponema saccharophilum			
0.0	1.398^{BC}	1.378^{B}	1.430^{A}	0.037
0.1	1.670^{aA}	1.540^{aAB}	1.040^{bB}	0.052
0.2	1.580^{AB}	1.570^{AB}	1.590^{A}	0.052
0.5	1.380^{bC}	1.650^{aA}	1.610^{aA}	0.052
1.0	1.580^{AB}	1.530^{AB}	1.480^{A}	0.052
SEM	0.052	0.052	0.052	
	Succinivibrio dextrinosolvens			
0.0	1.045^{bA}	1.322^{aA}	1.117^{bA}	0.041
0.1	1.252^{aA}	1.001^{bB}	0.798^{cB}	0.056
0.2	1.183^{aA}	0.975^{bB}	0.819^{cB}	0.057
0.5	1.124^{aA}	0.964^{bB}	0.729^{cB}	0.057
1.0	0.096^{cB}	0.229^{bC}	0.845^{aB}	0.057
SEM	0.057	0.057	0.057	

Different lowercase letters in the same line are significantly different and different uppercase letters in the same column are significantly different ($P < 0.05$). [1]SEM = standard error of the mean.

IV - Effect of extracts from baccharis, tamarind, cashew nut shell liquid and clove on animal performance, feed efficiency, digestibility, rumen fermentation and feeding behavior of bulls finished in feedlot

Journal: Livestock Science

Abstract

There is growing public concern on the use of antibiotics and ionophores in livestock and emerging antimicrobial resistance. Plant extracts and essential oils are natural alternatives having antimicrobial and antioxidant properties. This study evaluated the effects of a mixture of baccharis (*Baccharis dracunculifolia*) leaves and stems, tamarind (*Tamarindus indica* L.) seed, cashew (*Anacardium occidentale*) nut shell liquid, and clove (*Syzygium aromaticum*) essential oil on animal performance, feed intake, feed efficiency, apparent digestibility, molar concentration of volatile fatty acids, and feeding behavior of bulls finished in feedlot and fed high-grain diets. A total of 32 bulls (½ Angus *vs.* ½ Nellore) with a mean age of 24 ± 2.0 months and a mean body weight of 418 ± 4.51 kg were distributed in a completely randomized design with four diets and eight replications per diet. The four experimental diets were as follows: CONT – basal diet; MIX2 – basal diet and 2 g/animal/d of extracts from baccharis, tamarind, cashew nut shell liquid and clove; MIX4 – basal diet and 4 g/animal/d of extracts from baccharis, tamarind, cashew nut shell liquid and clove; MIX6 – basal diet and 6 g/animal/d of extracts from baccharis, tamarind, cashew nut liquid shell liquid and clove. Animal performance, dry matter and other nutrients intake were similar among diets. The dry matter, neutral detergent fiber and organic matter digestibilities were greater when MIX2 was fed to bulls. There was an increase in propionate and a decrease of the acetate/propionato ratio when MIX6 was used. A similar effect was observed when orthogonal contrasts comparing the

control diets with the addition of MIX was used. Feeding behavior was similar among the four diets. Our results suggest that the inclusion of 2, 4 and 6 g/animal/d of extracts from baccharis, tamarind, cashew nut shell liquid and clove was able to modulate rumen fermentation parameters of bulls fed high-grain diet, which is promising to replace antimicrobial feed additives used in beef cattle production systems.

Keywords: cattle; essential oil; natural compounds; natural extract; plant extract; vegetable oil

1. Introduction

The use of antibiotics and ionophores on animal production systems is a common practice to prevent diseases and metabolic disorders, to increase animal performance, and to improve feed efficiency, particularly when animals are finished in feedlot (Fugita et al., 2018; Ornaghi et al., 2017; Valero et al., 2016). However, due to emerging antimicrobial resistance, their use has been banned in some countries. Recently there is growing interest on alternatives such as natural compounds, which are well accepted by consumers (Kempinski et al., 2017; Vital et al., 2018a, 2018b).

Tropical plants have the ability to survive extreme biotic and abiotic stressors, having genes to produce abundant secondary metabolites to protect the plant, seeds, fruits and leaves (Araújo et al., 2017; Shahidi and Ambigaipalan, 2015). Secondary metabolites, such as phenolic and flavonoids compounds, have antibacterial and anti-inflammatory activity in animals, potentially improving dry matter (DM) digestibility, reducing methane emission and increasing propionate production (Olagaray and Bradford, 2019).

Studies have been performed with compounds isolated from plants (Guerrero et al., 2018; Ornaghi et al., 2017; Rivaroli et al., 2016) or, in some cases, with mixtures of secondary plant metabolites (Cruz et al., 2014; Fugita et al., 2018; Monteschio et al., 2017; Souza et al., 2019;

Valero et al., 2014). Isolated compounds have demonstrated efficacy to be used for ruminants, but synergistic effect is still poorly understood, although highly desired. Mixtures of vegetable and essential oils and plant extracts have been combined to enhance their antimicrobial and antioxidant activity, and to improve animal performance, meat shelf-life and meat co-products (Fugita et al., 2018; Kempinski et al., 2017; Vital et al., 2018a).

Baccharis (*Baccharis dracunculifolia*) is an endemic plant in South America enriched in flavonoids and phenolic compounds having antioxidant and antimicrobial properties (Bonin et al., 2020; Campos et al., 2016), mainly artepelin C (Veiga et al., 2017). Artepelin C is a phenolic compound involved in the control of the apoptosis response and mitochondrial dysfunction, characteristics that can be observed during inflammatory and oxidative processes (Veiga et al., 2017).

Tamarindus indica L. belongs to the family Leguminosae and is cultivated in many tropical countries. *T. indica* L is a medicinal plant with high antioxidant activity (Razali et al., 2015) with many uses in human health, and is a potential additive for animal nutrition (Geron et al., 2015; Wang et al., 2017; Souza et al., 2018). Tamarind is composed by the secondary compounds as alkaloids, saponins and total phenolic (Abdallah and Muhammad, 2018). The main molecule in the tamarind seed is the 2-hydroxy-3′,4′-dihydoxyacetophenone; a flavonoid with powerful antibiotic and antifungal activity (Ali et al., 2017).

Cashew (*Anacardium occidentale*) is a tree whose nuts are mainly used to produce the cashew nut shell liquid (CNSL) that can be used in animal nutrition (Cruz et al., 2014; Valero et al., 2014; Prado et al., 2015). CNSL is enriched in flavonoids, such as catechin, and has great antioxidant activity. Catechin is a flavonoid involved in the prevention of cytotoxicity and lipid peroxidation (Caro et al., 2019).

Clove (*Syzygium aromaticum*) is enriched in phenolic compounds with antimicrobial and antioxidant properties. Eugenol composes 88% of clove oil (Monteschio et al., 2017; Ornaghi

et al., 2017; Souza et al., 2019) and inhibits bacteria by affecting the integrity of the cytoplamic membrane (Devi et al., 2010). Thus, the dose level employed from plant extracts and essential oil was based on previous studies (Ornaghi et al., 2020, 2017; Rivaroli et al., 2020; Souza et al., 2019).

The hypothesis of this work was that a mixture of natural plant extracts added in the diets of cattle finished in feedlot could improve animal performance. Thus, this study was realized to evaluate the effects of a mixture of baccharis (*Baccharis dracunculifolia*) leaves and stems, tamarind (*Tamarindus indica* L.) seed, cashew (*Anacardium occidentale*) nut shell liquid, and clove (*Syzygium aromaticum*) essential oil on animal performance, feed intake, feed efficiency, apparent digestibility, molar concentration of volatile fatty acids and feeding behavior of bulls finished in a feedlot and fed high-grain diets.

2. Materials and methods

All animal care and experimental procedures were conducted under the surveillance of the Animal Care and Use Committee of the Universidade Estadual de Maringá, Brazil (protocol number 6680060219) and met the guidelines of the National Council for the Control of Animal Experimentation (CONCEA).

2.1. Location, animals and diets

The experiment was conducted at the Rosa & Pedro Sector of the Experimental Farm Station at Iguatemi city from Universidade Estadual de Maringá, Paraná, South Brazil, from March to June in 2019. This region has a humid temperate climate with an annual average temperature of 18 ºC and an annual average rainfall of 1,114 mm.

A total of 32 (½ Angus *vs.* ½ Nellore) bulls with a mean age of 24 ± 2.0 months and a mean body weight of 418 ± 4.51 kg were distributed in a completely randomized design with four

diets and eight replications per diet. The bulls were housed in individual pens (10 m^2) where each animal was considered an experimental unit. The adaptation period lasted 14 d. The bulls were weighed on a 16-hour fast every 28 days using a trunk balance (Beckehauser Cia., Paranavaí, Paraná, Brazil). At the beginning of the acclimatization period, all bulls were treated for endoparasites and ectoparasites using Long-acting Injectable Ivermectin LA 3.5% (200 µg/kg BW; Ivomec Merial®, Paulínia, Brazil). The basal diet was composed by 700 g/kg concentrate and 300 g/kg corn silage fed *ad libitum* for 74 days (Table 1). Feed intake was recorded daily. The basal diet was the same for all animals, formulated to have the same amount of nitrogen and energy (Table 1) according to the NRC (2000). The four experimental diets were as follows: CONT – basal diet; MIX2 – basal diet and 2 g/animal/d of extracts from baccharis, tamarind, cashew nut shell liquid (CNSL) and clove; MIX4 – basal diet and 4 g/animal/d of extracts from baccharis, tamarind, CNSL and clove; MIX6 – basal diet and 6 g/animal/d of extracts from baccharis, tamarind, CNSL and clove. These concentrations represent typical amounts of compounds from plant extracts and EO supplied to ruminants' diets (Ornaghi et al., 2020, 2017; Rivaroli et al., 2020; Souza et al., 2019). The mixture (MIX) contained 400 g/kg of baccharis (*Baccharis dracunculifolia*) leaves and stems, 400 g/kg of tamarind *(Tamarindus indica L.)* seeds, 100 g/kg of CNSL (*Anacardium occidentale* L.), and 100 g/kg of clove leaf (*Syzygium aromaticum*) essential oil per animal/day. Concentrate with the mixture was prepared every 15 days, adjusting the inclusion according to the intake of dry matter/day per animal, to maintain a constant dosage per animal/day and preserve antioxidant activities.

The baccharis leaves and stems were obtained from Maringá, Paraná, Brazil south, latitude 23º27'S and longitude 51º59' W, during the summer season. The tamarind seeds were obtained from Nova Redenção, Bahia, Brazil northeast, latitude 12º49'S and longitude 41º03'W, during the winter season. The cashew nut shell liquid (CNSL) was purchased from Safeeds® (Cascavel

city, Paraná state, Brazil south) and clove oil was obtained from FERQUIMA® (Vargem Grande Paulista, São Paulo, Brazil) and stored at 4 ºC.

2.2. Antioxidant activity

The antioxidant activity of the mixture was evaluated using the assays 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability power (FRAP), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and total phenolic compounds (TPC) were calculated.

The DPPH radical scavenging activity was performed according to Li et al. (2009), with modifications (Vital et al., 2016). The mixture (150 μL) was combined with 2850 μL of methanolic solution containing DPPH (60 μM), and reacted for 30 min. The absorbance at 515 nm was measured against pure methanol. Antioxidant activity was calculated as:

DPPH radical scavenging activity (%) = (1 – (sample t = 0/sample t) * 100;

where: sample t = 0 is the absorbance of the sample at time zero, and sample t is the absorbance of the sample at 30 min.

The FRAP assay was evaluated by spectrophotometry (595 nm) using tripyridyltriazine (TPTZ) and ferric chloride ($FeCl_3$) as describe by Benzie and Strain (1996).

Total phenolic compounds were determined according to the method described by Singleton and Rossi (1965), with some modifications. The extract (125 μL) was mixed with an equivalent volume of Folin-Ciocalteu reagent (diluted 1:1 in deionized water) and 2.25 mL sodium carbonate (28 g/L). The mixture was incubated in the dark for 30 min, and then the absorbance was measured at 725 nm using a spectrophotometer (Evolution™ 300, Thermo Fisher Scientific, UK). A standard absorbance curve was prepared using 0–300 mg/L gallic acid, and

results were obtained by interpolation using this curve and expressed as mg gallic acid equivalent (GAE)/g (Saraiva et al., 2019).

The ABTS assay was performed according to the protocol of Re et al. (1999). The ABTS activated radical was diluted with ethanol until an absorbance of 0.70 ± 0.02 was achieved, and 1960 µL of the resulting solution mixed 40 µL of with extract. The absorbance at 734 nm was measured after 6 min and the radical scavenging activity (%) was calculated using the equation proposed by (Saraiva et al., 2019).

2.3. Phytochemical profile

The identification of secondary compounds was assessed using the crude extracts (27.9 g) suspended in methanol/water (1:1, 50 mL, v/v), and successively partitioned with n-hexane and ethyl acetate. Then, ethyl acetate fraction was submitted to Nuclear Magnetic Resonance (NMR) analyses. ^{13}C NMR spectrum (Table 2) was recorded on a Bruker Avance III HD spectrometer (Bruker®, USA) operating at 75.5 MHz, using DMSO-d6 (Sigma-Aldrich) as solvent. The major compounds were identified based on the chemical shifts δ unit (ppm) data and comparison with data reported in the literature, as eugenol by HMDB0005809, cardol and cardanol (Ferreira et al., 2012; Maia et al., 2013) (Table 2).

2.4. Chemical analyses

Feed samples were collected weekly to determine the DM, and samples from four consecutive weeks were pooled for chemical analysis. Chemical analyses were performed in duplicates, adopting a variation coefficient ≤ 5% for repetition. The DM content of the ingredients was determined through oven-drying at 65 °C for 24 h and then drying at 135 °C

for 5 h (method 930.15); (AOAC, 2005). The organic matter (OM) content was calculated as the difference between the DM and ash content, being ash determined by combustion at 550 °C for 5 h (AOAC, 2005). The N content of the samples was determined by the Kjeldahl method (Method 976.05) (AOAC, 2005). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods described by Van Soest et al. (1991), with heat stable α-amylase and sodium sulphite used in the NDF procedure and expressed with residual ash. The content of non-fiber carbohydrates (NFC) was obtained using the equation proposed by Detmann and Valadares Filho (2010):

$$NFC = 100 - Ash - EE - NDFap - (CP - CPu + U);$$

where CP is the crude protein; CPu is the crude protein from urea; EE is the ether extract; NDFap, is the neutral detergent fiber corrected for ash and protein; and U is urea. All terms are expressed as % of DM.

The values of total digestible nutrients observed were estimated using the equation proposed by Sniffen et al. (1992):

$$TDN = CP_d + (EE_d \times 2.25) + NDF_d + NFC_d;$$

where: CP_d is the digestible crude protein; EE_d is the digestible ether extract; NDF_d is the digestible neutral detergent fiber; and NFC_d is the digestible non-fiber carbohydrates.

2.5. Animal performance and carcass characteristics

To determine animal performance, the bulls were weighed at the beginning of the experiment (after fasting of solids for 16 h) and at 28 days intervals throughout the study. The average daily gain (ADG) was calculated as the total body weight (BW) gain divided by the length of the experimental period (74 days). Feed conversion was calculated as the ratio between dry matter intake (DMI) and ADG.

On day 74 of the animal trial, the animals were weighed after 16 h of fasting of solids and transported to a commercial slaughterhouse (Colorado, PR, Brazil). The stocking density of the truck was 0.8 ± 0.2 bulls/m^2, and the transport distance was less than 40 km. The bulls were slaughtered following the usual practices of the Brazilian beef industry. The bulls were stunned using a captive-bolt pistol. Then, they were bled through exsanguination by cutting the throat vessels and had the head, hide, viscera, tail, legs, diaphragm and KPH (kidney, pelvic, heart fat) removed. Afterwards, the carcasses were divided medially from the sternum and spine, resulting in two similar halves, which were weighed to calculate the hot carcass weight. Then, the half-carcasses were washed, identified and stored in a chilling chamber at 4 °C, where they remained for a 24 h period. The cold carcass dressing (CCD) percentage was calculated as the cold carcass weight (CCW) divided by the final body weight (FBW) 16 h before slaughter, according to the following equation: CCD = (CCW/FBW) × 100.

2.6. Estimation of fecal production

Fecal production and digestibility were estimated following five weeks after start the experimental period. During the five days of the collection period, feces of each animal were sampled (> 250 g wet weight) daily off a clean floor shortly after defecation at alternate times (8:00; 10:00; 12:00; 14:00 and 16:00 hours) to ensure the collection of samples representative of the daily intestinal flow of the animals. Feces were stored at -20 °C until analysis.

2.7. Concentration of volatile fatty acids

Ruminal fluid was sampled 30 days after start the experimental period, 4 h ± 30 min after the morning feeding. Samples of ruminal fluid were collected via oral stomach tube (11 mm

diameter) and manual vacuum aspirator (TE-058, Tecnal, Piracicaba, SP, Brazil), filtered through a double cheese cloth, and conditioned at -20 °C. A total of 200 mL ruminal fluid was sampled from different anatomical regions of the rumen, being the first 100 mL of ruminal fluid discarded to minimize salivary contamination. The next 100 mL of ruminal fluid was filtered through four layers of cheesecloth and collected into a 500 mL plastic beaker.

Acetate, propionate, isobutyrate, butyrate, isovalerate and valerate fatty acids were evaluated by gas chromatography (GC-2010 Plus chromatograph, Shimadzu, Barueri, Brazil) equipped with a AOC-20i auto-sampler, Stabilwax-DA™ capillary column (30 m, 0.25 mm ID, 0.25 μm df; Restek©) and a flame ionization detector according to Del Valle et al. (2018).

2.8. Digestibility trial

The *in situ* digestibility of DM and their components (CP, NDF, and EE) were assessed by the internal indicator indigestible neutral detergent fiber (iNDF), following the methodology proposed by Casali et al. (2008), after incubation (feed and feces) for a period of 288 hours. Three ½ Holstein-Zebu crossbred bulls (400 ± 15 kg; mean ± SD) with ruminal cannulas were used for the determination of iNDF by the *in situ* method. During the trial, cattle were fed a diet consisting of 50% concentrate (corn gluten, yeast, limestone, mineral salt and urea) and 50% corn silage (% DM) for *ad libitum* intake (5%–10% refusal) at 08:00 h. After the incubation period, the bags containing the residual material were washed in running water until clear, and then subjected to the neutral detergent wash process under temperature (105 °C, 1 h) and pressure (Detmann et al., 2012). Fecal flow (FF) was determined using the following equation:

FF = indicator consumed/concentration indicator in feces.

The digestibility coefficient (DC) was calculated by the following equation:

DC = (nutrient intake – nutrient excreted)/nutrient intake).

2.9. Feeding behavior

Bulls were subjected to two 24-h observation periods, at 5-minute intervals, totaling 288 observations for each animal, performed by three previously trained evaluators throughout the feedlot to evaluate feeding behavior. Observations were performed without interrupting the animal's routine. Water and feed intake were measured as the time the animals spent drinking at the water cooler and eating at the feeder. Rumination was measured as the time the animals presented ruminal bolus and were chewing, and idleness was measured as the time the animals were idle (Silva et al., 2006). Water and feed intake, rumination and idle periods were obtained by the sum of the 288 observations (min/day).

2.10. Statistical analysis

Data were analyzed using analysis of variance and regression, using SAS 9.2. The experimental diet effect was evaluated using orthogonal contrast, which was used to assess the effects of the control diet versus diets with MIX, linear and quadratic response ($P \leq 0.05$).

3. Results

3.1. Phytochemical profile and antioxidant activity

The identification of compounds was conducted by Nuclear Magnetic Resonance (^{13}C NMR) and compared with literature data. Eugenol, cardol and cardanol, which are characteristic of vegetable oils from cashew nut shell and clove, were identified (Table 2). The TPC content of the MIX was 456.13 mg GAE/g. The DPPH, ABTS and FRAP assays revealed the antioxidant

properties of MIX, by demonstrating the free radical scavenging ability to be 27.21%, 7.47% and 10.23 mg GAE/g, respectively.

3.2. Animal performance, feed efficiency and intake

The inclusion of mixture of extracts from baccharis leaves and stems, tamarind seed, CNSL, and clove essential oil in the diet of young bulls finished in feedlot fed a high-grain diet did not alter (P > 0.05) the FBW, ADG, CCW and CCD (Table 3). Likewise, feed efficiency and intake were similar ($P > 0.05$) for all the diets (Table 3). The DMI and the intake of other nutrients (CP, OM, EE, NDF and TDN) were also similar ($P > 0.05$) among diets following the addition of the MIX compared to the CONT diet (Table 3).

3.3. In situ digestibility trial

The DM, NDF and OM digestibilities were higher ($P \leq 0.05$) when 2 g/animal/d of the mixture was included in the diet (Table 4). The *in situ* digestibilities were similar between the control diet and when 4 and 6 g/animal/d were added to the diets ($P > 0.05$). The CP digestibility was similar for all the diets (Table 4). The DM, NDF and OM digestibilities had a negative quadratic effect.

3.4. Concentration of volatile fatty acids

The DM, NDF and OM digestibilities were higher ($P \leq 0.05$) when 2 g/animal/d of the mixture was included in the diet (Table 4). The *in situ* digestibilities were similar between the control diet and when 4 and 6 g/animal/d were added to the diets ($P > 0.05$). The CP digestibility

was similar for all the diets (Table 4). The DM, NDF and OM digestibilities had a negative quadratic effect.

3.5. Feeding behavior

The time spent drinking water, rumination, feeding and idle was similar ($P > 0.05$) following the inclusion of the mixture in the diets (Table 6).

4. Discussion

4.1. Phytochemical profile and antioxidant activity

Novel additives to promote livestock production and health are demanded. Some of the isolated compounds present in the mixture, such as eugenol, cardol and cardanol have strong antibacterial, antiprotozoal and antifungal properties (Briozzo et al., 1989; Himejima and Kubo, 1991; Prakash et al., 2018). Natural extracts can be effective in inhibiting ruminal hyper ammonia-producing bacteria, thus improving the efficiency of nitrogen use (Wallace et al., 2002). Several studies support that natural additives have positive effects on the digestion and performance of beef cattle, including impact on meat quality (Fugita et al., 2018; Monteschio et al., 2017; Ornaghi et al., 2020; Rivaroli et al., 2016).

The antioxidant activity of mixture (TPC = 456.13 mg GAE/g) was a result of the main phytochemicals identified (Andrade et al., 2011; Gülçin et al., 2012). These could act as hydrogen donors, inhibiting the accumulation of free radicals (Ng et al., 2000) and, consequently, reducing the effects of the oxidative process. Results of antioxidant activity of the mixture (DPPH, ABTS and FRAP) is in accordance with the findings of Trevisan et al.

(2006) and Gülçin et al. (2012), who studied the antioxidant power of cashew nut shell liquid (with high amounts of cardol and cardanol) and clove oil (eugenol), respectively.

Gülçin et al. (2012) evlauted the antioxidant action of the constituents and derivatives of 45 μg/mL CNSL (cardol and cardanol) compared with 2,6-bis (1,1-dimethylethyl)-4-methylphenol; BHT) as reference antioxidant agent, and observed that the plant extract was more efficient to eliminate free radicals according to DPPH (CNSL = 83.6%; BHT = 67.8%) and the ABTS (CNSL = 98.7%; BHT = 97.8%) assays. The mixture of the plants extracts used to compose the MIX had great antioxidant activity, which could potentially be used as a natural antioxidant in feeds to replace synthetic compounds widely used in industry, such as BHT.

4.2. Animal performance, feed intake and feed efficiency

Natural alternatives to the use of antibiotics in livestock are highly needed. Plant extracts, vegetable and essential oils have the potential to modulate rumen fermentation, and to improve animal performance and feed efficiency (Fugita et al., 2018; Monteschio et al., 2017; Rivaroli et al., 2016; Souza et al., 2019). These compounds are potential substitutes for ionophores and antibiotics, without the potential antimicrobial resistance promotion. However, in the present study, animal performance and feed efficiency were not affected by the addition of plant material and vegetable oils to the diets. When used separately as additives in ruminant diets in some studies, clove and cashew oils have increased weight gain and improved feed efficiency (Ornaghi et al., 2017; Valero et al., 2016). In this study, there were no adverse effects with the addition of a mixture of baccharis leaves and stems, tamarind seed, CNSL, and clove oils on that DMI, carcass yield, animal performance and feed efficiency. Therefore, effects on performance and intake may vary with essential oil, the dose, type of diet and animal growth stage. Benchaar et al. (2006) evaluated the effects of dietary addition of monensin (33 mg/kg

DM) and mixture of essential oils (thymol, eugenol, vanillin and limonene) in different doses (2, 3 and 4 g/d), in the diet on in beef cattle (Angus Hereford, initial BW = 244 ± 4 kg) in feedlot. The authors observed that DMI and feed efficiency were not affected by addition of essential oils. Purevjav et al. (2013) supplemented Angus and Angus crossbred steers with CNSL and castor oil and found no effect on DMI. On the other hand, there is no data in the literature on baccharis extract as a dietary additive for animals. *Baccharis dracunculifolia*, which is composed by germacrene B, naringenin, kaempferol, artepillin C, α-pinene, hydroxycinnamic acid, apigenin, kaempferide, limonene, phenylethanol and β-caryophyllene (Bonin et al., 2020), is the main raw material used by bees (*Apis mellifera*) to produce green propolis, a waxy matrix composed by abundant bioactive compounds (Rodrigues et al., 2020). There is evidence that propolis fed to ruminants has positive effect of performance (Ítavo et al., 2011; Zawadzki et al., 2011). There was also performance improvement when propolis was combined to essential oils from cashew and castor in the diet of feedlot cattle (Valero et al., 2014). However, despite of the positive effects of propolis, there is a supply limitation. Thus, we prepared a low-dose natural extract using raw baccharis as there is evidence that natural ingestion of high doses (2g/kg) of *Baccharis megapotamica* was responsible for the death of goat in Brazil due to gastrointestinal disorders (Panziera et al., 2015). Finally, there is no data on the addition of tamarind seed on ruminant performance and feed efficiency of beef cattle. However, tamarind seed husk improved the efficiency of microbial protein synthesis *in vitro* (Bhatta et al., 2001). On the other hand, the addition of dry residues from tamarind pulp extract had no effect on animal performance or feed efficiency for sheep (Geron et al., 2015; Souza et al., 2018).

The intake CP, NDF, OM and EE were similar when the mixture was included in the diets. The intake of nutrients was similar when beef cattle were supplemented with providing clove and cinnamon essential oils doses (3.5 and 7.0 g/day per animal) (Ornaghi et al., 2017).

Similarly, the inclusion of 0.00, 100, 200 and 300 g/kg tamarind residues in a ruminant diet did not alter the intake of DM and other nutrients (Souza et al., 2018). Therefore, the effects of plant extracts and essential oils on DMI may vary depending on the dose, type of diet and diet interactions or adaptation of rumen microbial populations (Vakili et al., 2013; Yang et al., 2010).

The inclusion of mixture of baccharis leaves and stems, tamarind seed, CNSL, and clove oils to the diet did not alter feed efficiency (P = 0.145). This was also observed in some studies carried out with the inclusion of different essential and vegetable oils, as well as the addition of tamarind extract residues, to a cattle diet (Fugita et al., 2018; Ornaghi et al., 2017; Souza et al., 2019).

4.3. In situ digestibility trial

Dry matter, OM and NDF digestibilities were improved by 14.3%, 12.9% and 22.3%, respectively, when the mixture of baccharis, tamarind, CNSL, and clove oils (2 g/animal/d) were added to the diet. The maximum point estimated by the regression equation would be 2.5 g/animal/d. With that level of supply, DM, OM and NDF digestibilities would be improved. However, greater levels of additives did not influence the digestibility of these fractions. Regarding the biological properties of essential oils and plant extracts, it is known that they are complex mixtures of numerous molecules, which can result in several biological effects and, generally, the extent of their effects depends on the used concentration (Bakkali and Idaomar, 2008). Ornaghi et al. (2017) observed similar *in vitro* digestibilities of DM, NDF and OM when the mixture of essential oils containing eugenol, carophylene, eugenyl acetate, cinnamaldehyde and α-pinene were included in the diet. The addition of up to 2 g/animal/d of a mixture of natural compound oils increased the total production of volatile fatty acids in continuous culture,

although no increase in OM digestibility was observed (Castillejos et al., 2007). Busquet et al. (2006) studied various components of plant extracts and plant secondary metabolites and found that high doses of these products could even reduce feed digestion. However, some studies showed that the addition of natural compounds to the diet influenced the ratio of volatile fatty acids in the rumen and improved feed efficiency (Busquet et al., 2006). Wang et al. (2017) observed that the digestibility of DM, CP, and EE was 570 g/kg, 870 g/kg, and 860 g/kg, respectively, when tamarind residue was included in sheep diets.

4.4. Concentration of volatile fatty acids

The inclusion of monensin in feedlots cattle diets modulated ruminal profile of volatile fatty acids, mainly increasing the concentration of propionate and decreasing the acetate/propionate ratio (Yang and Russell, 1993). Although the monensin was not used in this experiment, similar effects were observed following the inclusion of mixture. The supplementation of ruminant diets with natural compounds increases the concentration of volatile fatty acids in the rumen, which could indicate an increase in feed efficiency, according to a limited number of studies (Benchaar et al., 2008; Benchaar and Greathead, 2011).

Increased ruminal concentrations of propionate provides increased supply of gluconeogenic precursors and reduces energy loss due to lower methane production (Benchaar et al., 2008; Benchaar and Greathead, 2011; Khorrami et al., 2015). Thus, we expected improved animal performance, which was not observed. Nonetheless, the inclusion of mixture in the diet could demonstrate positive effects as a potential substitute for another feed additives frequently used in beef cattle diets such as the ionophores. The positive effects observed in the concentration of propionate and acetate/propionate ratio must be the objective of nutritionists who work with beef cattle production.

4.5. Feeding behavior

There are few studies evaluating the addition of natural compounds and ingestive behavior of cattle (Ornaghi et al., 2017; Silva et al., 2014; Souza et al., 2019). The addition of natural compounds mixed into the diet did not produce a significant effect on ingestion behavior. Water ingestion can be affected by the ingredients of the diets, the temperature and the physiological state of the animal (Roguet et al., 1998). Rumination time can be affected by the physical form and content of fiber in the diet (Silva et al., 2016). However, rumination time was a very low percentage (233/1440 min, 16% per day) of the observation time because the diet had a 70: 30 concentrate forage ratio. This high-grain ratio affects the production of saliva and consequently influences rumination. Idle time can affect animal performance. Thus, an animal in an idle process has reduced energy expenditure; in this work, the observed idle time was high (1034 min/1440, 72% per day). Ornaghi et al. (2017) fed bulls in a feedlot with essential oils (clove or cinnamon, 3.5 or 7.0 g/animal/d), and did not observe differences in the idle time, in comparison to those fed a control diet. Similarly, Segabinazzi et al. (2011), fed cull cows with essential oils and plant extracts (thyme, garlic, and rosemary essential oils and canola and quillaja vegetable oils) with sodium monensin, did not observe differences in idle time, in comparison to cows fed the control diet.

5. Conclusions

Supplementation with of baccharis, tamarind, cashew nut shell liquid and clove oil showed no negative effects on food intake and nutrient digestibility. However, supplementation with 2, 4 and 6 g/animal/d increased the concentration of propionate, decreased the ratio

acetate/propionate and improved the digestibility the dry matter, neutral detergent fiber, and organic matter were. Our results suggest that baccharis, tamarind, cashew nut shell liquid and clove essential oil can be considered an appropriate feed additive and can be a promising candidate to replace antimicrobial feed additives for use in beef cattle production systems.

Author statement

The authors declare that they have approved the submission.

Declaration of Competing Interest

None.

Acknowledgements

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Brasília, Brazil) via a scholarship, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Grant no. 400375/2014-1) (Brasília, Brazil) and the Company Safeeds Nutrição Animal (Cascavel, Paraná, Brazil, safeeds@safeeds.com.br). The authors gratefully acknowledge the company for financing and providing the products used in this research, which made it possible to develop this work. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the Department of Animal Science, Universidade Estadual de Maringá, Maringá, Paraná, Brazil.

References

Abdallah, M., Ali, M., 2018. Antibacterial activity of leaves and fruits extract of Tamarindus indica against clinical isolates of Escherichia coli and Shigella at potiskum yobe state, Nigeria. J. Anal. Pharm. Res. 7, 606–609. https://doi.org/10.15406/japlr.2018.07.00290

Ali, R., Rahim, A., Islam, A., 2017. Synthesis and antimicrobial activity of 7-hydroxy-3', 4'-methylenedioxy-and 7-benzyloxy-3', 4'-methylenedioxy flavanones. Journal of Scientific Research 9, 297-306. https://doi.org/10.3329/jsr.v9i3.31229

Andrade, T.D.J.A.D.S., Araújo, B.Q., Citó, A.M.D.G.L., Da Silva, J., Saffi, J., Richter, M.F., Ferraz, A.D.B.F., 2011. Antioxidant properties and chemical composition of technical Cashew Nut Shell Liquid (tCNSL). *Food Chem.*, 126, 1044–1048. https://doi.org/10.1016/j.foodchem.2010.11.122

AOAC., 2005. Association Official Analytical Chemist, 18ª ed. ed. Gaitherburg, Washington DC.

Araújo, R.A. de, Rodrigues, R.C., Costa, C. dos S., Santos, F.N. de S., Cutrim Júnior, J.A.A., Jesus, A.P.R. de, Shigaki, F., Araújo, J. dos S., 2017. Grazing behavior and spatial distribution of feces of Young bulls in silvopastoral systems and Marandu monoculture in the Pre-Amazon region. *Acta Sci. Anim. Sci.*, 39, 83–90.

Bakkali, F., Idaomar, M., 2008. Biological effects of essential oils – A review 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. *Anim. Feed Sci. Technol.*, 145, 209–228. https://doi.org/10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Duynisveld, J.L., Charmley, E., 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. *Can. J. Anim. Sci.*, 86, 91–96.

Benchaar, C., Greathead, H., 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Anim. Feed Sci. Technol.*, 166–167, 338–355. https://doi.org/10.1016/j.anifeedsci.2011.04.024

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.*, 239, 70–76.

Bhatta, R., Krishnamoorthy, U., Mohammed, F., 2001. Effect of tamarind *(Tamarindus indica)* seed husk tannins on in vitro rumen fermentation. *Anim. Feed Sci. Technol.*, 90, 143–152.

Bonin, E., Carvalho, V.M., Avila, V.D., Santos, N.C.A. dos, Benassi-Zanqueta, É., Lancheros, C.A.C., Previdelli, I.T.S., Ueda-Nakamura, T., Abreu Filho, B.A. de, Prado, I.N., 2020. *Baccharis dracunculifolia*: Chemical constituents, cytotoxicity and antimicrobial activity. *LWT - Food Sci. Technol.*, 120, 1–10. https://doi.org/10.1016/j.lwt.2019.108920

Briozzo, J., Núncez, L., Chirife, J., Herszage, L., D'aquino, M., 1989. Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. *J. Appl. Bacteriol.*, 66, 69–75.

Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C., 2006. Plant Extracts Affect In Vitro Rumen Microbial Fermentation. J. Dairy Sci. 89, 761–771. https://doi.org/10.3168/jds.S0022-0302(06)72137-3

Campos, F.R., Bressan, J., Jasinski, V.C.G., Zuccolotto, T., Silva, L.E., Cerqueira, L.B., 2016. Baccharis (*Asteraceae*): Chemical constituents and biological activities. Chemistry & Biodiversity 13, 1-17.

Caro, A.A., Davis, A., Fobare, S., Horan, N., Ryan, C., Schwab, C., 2019. Antioxidant and pro-oxidant mechanisms of (+) catechin in microsomal CYP2E1-dependent oxidative stress. Toxicology in Vitro 54, 1-9.

Casali, A.O., Detmann, E., Valadares Filho, S. de C., Pereira, J.C., Henriques, L.T., Freitas, S.G. de, Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. *Rev. Bras. Zootec.*, 37, 335–342.

Castillejos, L., Calsamiglia, S., Ferret, A., Losa, R., 2007. Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Anim. Feed Sci. Technol.*, 132, 186–201.

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

Del Valle, T.A., Zenatt, T.F., Antonio, G., Campana, M., Gandra, J.R., Zilio, E.M.C., Mattos, L.F.A. de, Morais, J.G.P. de, 2018. Effect of chitosan on the preservation quality of sugarcane silage. *Grass Forage Sci.*, 73, 630–638. https://doi.org/10.1111/gfs.12356

Detmann, E., Souza, M., Valadares Filho, S., Queiroz, A., Berchielli, T., Saliba, E., Cabral, L.S., Pina, D.S., Ladeira, M., Azevedo, J., 2012. Métodos para análise de alimentos—INCT—Ciência Animal (p. 214). *Visconde do Rio Branco, Brazil Suprema*,.

Detmann, E., Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. *Arq. Bras. Med. Veterinária e Zootec.*, 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Devi, K.P., Nisha, S.A., Sakthivel, R., Pandian, S.K., 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *J. Ethnopharmacol.*, 130, 107–115. https://doi.org/10.1016/j.jep.2010.04.025

Ferreira, J.C., Teixeira, A., Esteves, P.M., 2012. A polyether glycol derived from cashew nutshell as a kinetic inhibitor for methane hydrate formation. *J. Braz. Chem. Soc.*, 23, 11–13. https://doi.org/10.1590/s0103-50532012000100003

Fugita, C.A., do Prado, R.M., Valero, M.V., Bonafé, E.G., Carvalho, C.B., Guerrero, A., Sañudo, C., Prado, I.N., do Prado, I.N., 2018. Effect of the inclusion of natural additives on animal performance and meat quality of crossbred bulls (Angus× Nellore) finished in feedlot. *Anim. Prod. Sci.*, 58, 2076–2083. https://doi.org/10.1071/AN16242

Geron, L.J.V., Costa, F.G., Aguiar, S.C., Garcia, J., Ribeiro, M.G., Zeoula, L.M., Silva, A.P., Pereira, S.R., Silveira, R.M., 2015. *In vitro* and *in vivo* nutrient digestibility in sheep of rations with and without residue from the extraction of tamarind pulp. *Semin. Ciências Agrárias*, 36, 4507–4517.

Guerrero, A., Rivaroli, D.C., Sañudo, C., Campo, M.M., Valero, M. V., Jorge, A.M., Prado, I.N., 2018. Consumer acceptability of beef from two sexes supplemented with essential oil mix. *Anim. Prod. Sci.*, 58. https://doi.org/10.1071/AN15306

Gülçin, İ., Elmastaş, M., Aboul-Enein, H.Y., 2012. Antioxidant activity of clove oil–A powerful antioxidant source. *Arab. J. Chem.*, 5, 489–499.

Himejima, M., Kubo, I., 1991. Antibacterial agents from the cashew *Anacardium occidentale* (Anacardiaceae) nut shell oil. *J. Agric. Food Chem.*, 39, 418–421.

Ítavo, C., Morais, M.G., Costa, C., Ítavo, L.C. V, Franco, G.L., Da Silva, J.A., Reis, F.A., 2011. Addition of propolis or monensin in the diet: Behavior and productivity of lambs in feedlot. *Anim. Feed Sci. Technol.*, 165, 161–166.

Kempinski, E.M.B.C., Vital, A.C.P., Oliveira Monteschio, J., Alexandre, S., Nascimento, K.F., Madrona, G.S., Mikcha, J.M.G., Prado, I.N., 2017. Development and quality evaluation of infant food with oregano essential oil for children diagnosed with cerebral palsy. *LWT - Food Sci. Technol.*, 84, 579–585.

Khorrami, B., Vakili, A.R., Mesgaran, M.D., Klevenhusen, F., 2015. Thyme and cinnamon essential oils: Potential alternatives for monensin as a rumen modifier in beef production systems. *Anim. Feed Sci. Technol.*, 200, 8–16. https://doi.org/10.1016/j.anifeedsci.2014.11.009

Li, W., Hydamaka, A.W., Lowry, L., Beta, T., 2009. Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. *Cent. Eur. J. Biol.*, 4, 499–506.

Maia, F., Ribeiro, V., Almeida, M., Lomonaco, D., Mafezoli, J., Mazzetto, S., 2013. Study of Antioxidant Activity of a Phenyl Phosphorylated Compound Derived from Hydrogenated Cardol by Thermogravimetric Analysis. *Br. J. Appl. Sci. Technol.*, 3, 546–556. https://doi.org/10.9734/bjast/2014/3046

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

Ng, T.B., Liu, F., Wang, Z.T., 2000. Antioxidative activity of natural products from plants. *Life Sci.*, 66, 709–723.

NRC, 2000. Nutrient requirements of beef cattle: update 2000. National Academies Press.

Olagaray, K.E., Bradford, B.J., 2019. Plant flavonoids to improve productivity of ruminants – A review. *Anim. Feed Sci. Technol.*, 251, 21–36. https://doi.org/10.1016/j.anifeedsci.2019.02.004

Ornaghi, M.G., Guerrero, A., Vital, A.C.P., Souza, K.A., Passetti, R.A.C., Mottin, C., Castilho, R.A., Sañudo, C., Prado, I.N., 2020. Improvements in the quality of meat from beef cattle fed natural additives. *Meat Sci.*, 163, 108059. https://doi.org/10.1016/J.MEATSCI.2020.108059

Ornaghi, M.G., Passetti, R.A.C., Torrecilhas, J.A., Mottin, C., Vital, A.C.P., Guerrero, A., Sañudo, C., del Mar Campo, M., Prado, I.N., 2017. Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Anim. Feed Sci. Technol.*, 234, 274–283. https://doi.org/10.1016/j.anifeedsci.2017.10.008

Panziera, W., Gonçalves, M.A., Lorenzett, M.P., Damboriarena, P., Argenta, F.F., Laisse, C.J.M., Pavarini, S.P., Driemeier, D., 2015. Intoxicação natural por *Baccharis megapotamica* var. *weirii* em caprinos. *Pesqui. Veterinária Bras.*, 35, 360–364.

Prado, I.N., Cruz, O.T.B., Valero, M.V., Zawadzki, F., Eiras, C.E., Rivaroli, D.C., Prado, R.M., Visentainer, J.V., 2015. Effects of glycerin and essential oils (*Anacardium occidentale* and *Ricinus communis*) on the meat quality of crossbred bulls finished in a feedlot. Animal Production Science 55, 2105-2114.

Prado, I.N., Cruz, O.T.B., Valero, M. V., Zawadzki, F., Eiras, C.E., Rivaroli, D.C., Prado, R.M., Visentainer, J. V., 2016. Effects of glycerin and essential oils (*Anacardium occidentale* and *Ricinus communis*) on the meat quality of crossbred bulls finished in a feedlot. *Anim. Prod. Sci.*, 56. https://doi.org/10.1071/AN14661

Prakash, A., Vadivel, V., Banu, S.F., Nithyanand, P., Lalitha, C., Brindha, P., 2018. Evaluation of antioxidant and antimicrobial properties of solvent extracts of agro-food by-products (cashew nut shell, coconut shell and groundnut hull). *Agric. Nat. Resour.*, 52, 451–459.

Purevjav, T., Hoffman, M.P., Ishdorj, A., Conover, A.J., Jedlicka, M.E., Prusa, K., Torrent, J., Pusillo, G.M., 2013. Effects of functional oils and monensin on cattle finishing programs. *Prof. Anim. Sci.*, 29, 426–434.

Razali, N., Junit, S.M., Ariffin, A., Siti, N., Ramli, F., Aziz, A.A., 2015. Polyphenols from the extract and fraction of *T. indica* seeds protected HepG2 cells against oxidative stress. *BMC Complement. Altern. Med.*, 15:438, 1–16. https://doi.org/10.1186/s12906-015-0963-2

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free*

Radic. Biol. Med., 26, 1231–1237.

Rivaroli, D.C., Campo, M.M., Sañudo, C., Guerrero, A., Jorge, A.M., Vital, A.C.P., Valero, M.V., Prado, R.M., Prado, I.N., 2020. Effect of an essential oils blend on meat characteristics of crossbred heifers finished on a high-grain diet in a feedlot. Anim. Prod. Sci. 60, 595–602. https://doi.org/https://doi.org/10.1071/AN18620.

Rivaroli, D.C., Guerrero, A., Valero, M.V., Zawadzki, F., Eiras, C.E., Campo, M. del M., Sañudo, C., Mendes, A.J., Prado, I.N., 2016. Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Sci.*, 121, 278–284. https://doi.org/10.1016/j.meatsci.2016.06.017

Rodrigues, D.M., Souza, M.C., Arruda, C., Pereira, R.A.S., Bastos, J.K., 2020. The Role of *Baccharis dracunculifolia* and its Chemical Profile on Green Propolis Production by *Apis mellifera*. *J. Chem. Ecol.*, 46, 150–162. https://doi.org/10.1007/s10886-019-01141-w

Roguet, C., Dumont, B., Prache, S., 1998. Selection and use of feeding sites and feeding stations by herbivores: a review, in: Annales de Zootechnie. EDP Sciences, pp. 225–244.

Saraiva, B.R., Vital, A.C.P., Anjo, F.A., Ribas, J.C.R., Matumoto Pintro, P.T., 2019. Effect of *yerba mate* (*Ilex paraguariensis* A. St.-Hil.) addition on the functional and technological characteristics of fresh cheese. *J. Food Sci. Technol.*, 56, 1256–1265. https://doi.org/10.1007/s13197-019-03589-w

Segabinazzi, L.R., Viégas, J., Freitas, L. da S., Brondani, I.L., Argenta, F.M., Binotto, J., 2011. Behavior patterns of cows with Charolais or Nellore breed predominance fed diets with plant extract or monensin sodium. *Rev. Bras. Zootec.*, 40, 2954–2962.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *J. Funct. Foods*, 18, 820–897.

Silva, T.H. da, Takiya, C.S., Vendramini, T.H.A., Jesus, E.F., Zanferari, F., Rennó, F.P., 2016. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows. *Anim. Feed Sci. Technol.*, 221, 35–43.

Silva, L.G., Torrecilhas, J.A., Passetti, R.A.C., Ornaghi, M.G., Eiras, C.E., Rivaroli, D.C., Valero, M.V., Prado, I.N., 2014. Glycerin and cashew and castor oils in the diets for bulls in finished in feed lot: Ingestive behavior. *Semin. Ciências Agrárias*, 35, 2723–2737.

Silva, R.R., Silva, F.F., Prado, I.N., Carvalho, G.G.P., Franco, I.L., Almeida, V.S., Cardoso, C.P., Ribeiro, M.H.S., 2006. Comportamento ingestivo de bovinos. Aspectos metodológicos. *Arch. Zootec.*, 55, 293–296.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16, 144–158.

Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G., Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.*,. https://doi.org/10.2527/1992.70113562x

Souza, C.M., Oliveira, R.L., Voltolini, T. V., Menezes, D.R., dos Santos, N.J.A., Barbosa, A.M., Silva, T.M., Pereira, E.S., Bezerra, L.R., Santos, N.J.A., Barbosa, A.M., Silva, T.M., Pereira, E.S., Bezerra, L.R., 2018. Lambs fed cassava silage with added tamarind residue: silage quality, intake, digestibility, nitrogen balance, growth performance and carcass quality. *Anim. Feed Sci. Technol.*, 235, 50–59. https://doi.org/10.1016/j.anifeedsci.2017.11.007

Souza, K.A., Monteschio, J. de O., Mottin, C., Ramos, T.R., Pinto, L.A. de M., Eiras, C.E., Guerrero, A., Prado, I.N., 2019. Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedl. *Livest. Sci.*, 220, 190–195. https://doi.org/10.1016/j.livsci.2018.12.026

Trevisan, M.T.S., Pfundstein, B., Haubner, R., Würtele, G., Spiegelhalder, B., Bartsch, H., Owen, R.W., 2006. Characterization of alkyl phenols in cashew *(Anacardium occidentale)* products and assay of their antioxidant capacity. *Food Chem. Toxicol.*, 44, 188–197.

Vakili, A. R., Khorrami, B., Mesgaran, M. D., & Parand, E.,2013. The effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in holstein calves consuming high concentrate diet. Asian-Australasian J. Anim. Sci., 26(7), 935–944. https://doi.org/10.5713/ajas.2012.12636

Valero, M.V., Prado, R.M., Zawadzki, F., Eiras, C.E., Madrona, G.S., Prado, I.N., 2014. Propolis and essential oils additives in the diets improved animal performance. *Acta Sci. - Anim. Sci.*, 36, 419–426. https://doi.org/10.4025/actascianimsci.v36i4.23856

Valero, M.V., Torrecilhas, J.A., Zawadzki, F., Bonafé, E.G., Madrona, G.S., Prado, R.M.,

Passetti, R.A.C., Rivaroli, D.C., Visentainer, J.V., Prado, I.N., 2014. Propolis or cashew and castor oils effects on composition of longissimus muscle of crossbred bulls finished in feedlot. *Chil. J. Agric. Res.*, 74. https://doi.org/10.4067/S0718-58392014000400011

Valero, M. V, Farias, M.S., Zawadzki, F., Prado, R.M., Fugita, C.A., Rivaroli, D.C., Ornaghi, M.G., Prado, I.N., 2016. Feeding propolis or essential oils (cashew and castor) to bulls: performance, digestibility, and blood cell counts. *Rev. Colomb. Ciencias Pecu.*, 29, 33–42.

Van Soest, P.J. van, Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74, 3583–3597.

Veiga, R.S., Mendonça, S., Mendes, P.B., Paulino, N., Mimica, M.J., Lagareiro Netto, A.A., Lira, I.S., López, B.G.C., Negrão, V., Marcucci, M.C., 2017. Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and *Baccharis dracunculifolia*. Journal of Applied Microbiology 122, 911-920.

Vital, A.C.P., Guerrero, A., Kempinski, E.M.B.C., Oliveira Monteschio, J., Sary, C., Ramos, T.R., del Mar Campo, M., Prado, I.N., 2018a. Consumer profile and acceptability of cooked beef steaks with edible and active coating containing oregano and rosemary essential oils. *Meat Sci.*, 143, 153–158.

Vital, A.C.P., Guerrero, A., Monteschio, J.D.O., Valero, M.V., Carvalho, C.B., Abreu Filho, B.A., Madrona, G.S., Prado, I.N., 2016. Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PLoS One*, 11. https://doi.org/10.1371/journal.pone.0160535

Vital, A.C.P., Guerrero, A., Ornaghi, M.G., Kempinski, E.M.B.C., Sary, C., Oliveira Monteschio, J., Matumoto-Pintro, P.T., Ribeiro, R.P., Prado, I.N., 2018b. Quality and sensory acceptability of fish fillet (*Oreochromis niloticus*) with alginate-based coating containing essential oils. *J. Food Sci. Technol.*, 55, 4945–4955.

Wallace, R.J., McEwan, N.R., McIntosh, F.M., Teferedegne, B., Newbold, C.J., 2002. Natural products as manipulators of rumen fermentation. *Asian-Australasian J. Anim. Sci.*, 15, 1458–1468.

Wang, L., Nakanishi, T., Sato, Y., Oishi, K., Hirooka, H., Takahashi, K., Kumagai, H., 2017. Effect of feeding tamarind kernel powder extract residue on digestibility, nitrogen availability and ruminal fermentation in wethers. *Asian-Australasian J. Anim. Sci.*, 30, 379. https://doi.org/10.5713/ajas.16.0137

Yang, C.M., Russell, J.B., 1993. The effect of monensin supplementation on ruminal ammonia accumulation in vivo and the numbers of amino acid-fermenting bacteria. *J. Anim. Sci.*, 71, 3470–3476.

Yang, W.Z., Ametaj, B.N., Benchaar, C., Beauchemin, K.A., 2010. Dose response to cinnamaldehyde supplementation in growing beef heifers: Ruminal and intestinal digestion. J. Anim. Sci. 88, 680–688. https://doi.org/10.2527/jas.2008-1652

Zawadzki, F., Prado, I.N., Marques, J.A., Zeoula, L.M., Rotta, P.P., Sestari, B.B., Valero, M. V., Rivaroli, D.C., 2011. Sodium monensin or propolis extract in the diets of feedlot-finished bulls: Effects on animal performance and carcass characteristics. *J. Anim. Feed Sci.*, 20, 16–25. https://doi.org/10.22358/jafs/66153/2011

Table 1. Ingredients and chemical composition of the basal diet (g/kg of DM)

Ingredients (g/kg)	Diet
Corn silage	308.4
Corn grain	636.1
Corn gluten	42.4
Limestone	3.5
Mineral and vitamin supplement*	3.5
Urea	5.7
Yeast (*Saccharomyces cerevisiae*)	0.4
Chemical composition (g/kg)	
Dry matter	710.3
Crude protein	122.8
Organic matter	930.9
Ash	69.1
Ether extract	33.6
Non-fiber carbohydrates.	610.4
Neutral detergent fiber	255.4
Acid detergent fiber	74.4
Total digestible nutrients	791.4
Calcium	5.2
Phosphorus	3.6

*Mineral salt composition (kg): calcium, 50 g; magnesium, 57 g; sodium, 81 g; sulphur, 3.75 g; cobalt, 20 mg; copper, 500 mg; iodine, 25 mg; manganese, 1.500 mg; selenium, 10 mg; zinc, 2.000 mg; vitamin A, 400.000 UI; vitamin D3, 50.000 UI; vitamin E, 750 UI; ether extract, 168 g; urea, 200 g.

Table 2. Phytochemical profile of a mixture of containing baccharis, tamarind, cashew nut shell liquid, and clove oil

Class of compounds	δ_C [ppm]/^nJ [Hz][1]
Eugenol[2]	39.92; 55.85; 111.29; 114.46; 121.27; 131.94; 137.92; 144.03; 146.60
Cardol	14.24; 22.63; 31.66; 35.83; 100.58; 106.91; 128.12; 128.40; 129.68; 130.25; 145.36; 157.91
Cardanol	14.24; 22.63; 31.66; 35.83; 121.13; 128.40; 128.60; 129.58; 129.68; 130.11; 130.25; 157.91

[1]Chemical shifts were reported in δ unit (ppm) with ^{13}C-NMR spectrum (DMSO-d6, 75.5 MHz) of crude extract of MIX.
[2]Confirmation of the compounds by HMDB: [1]HMDB0005809.

Table 3. Animal performance and nutrient intake of crossbred bulls finished in feedlot and fed a high-grain diet containing a mixture of baccharis, tamarind, cashew nut shell liquid, and clove essential oil

Parameters	Experimental diets				SEM[5]	*P-value*		CONT *vs.* MIX
	CONT[1]	MIX2[2]	MIX4[3]	MIX6[4]		Linear	Quadratic	
Initial body weight, kg	420	420	418	412	4.51	0.83	0.75	0.74
Final body weight, kg	525	533	528	525	3.50	0.48	0.46	0.72
Average daily gain, kg/day	1.42	1.52	1.50	1.53	0.05	0.68	0.73	0.64
Feed efficiency, kg gain/DM intake	0.14	0.14	0.15	0.15	0.00	0.81	0.97	0.49
Feed conversion, DM intake/ kg gain	7.30	7.11	7.06	6.71	0.20	0.96	0.82	0.87
Cold carcass weight, kg	291	288	288	296	3.30	0.51	0.45	0.99
Cold carcass dressing (%)	55.4	54.2	53.9	56.3	0.01	0.42	0.29	0.54
	Daily intake of nutrients (kg/day)							
Dry matter	10.31	10.44	10.04	9.98	0.15	0.94	0.79	0.34
Crude protein	1.33	1.34	1.30	1.30	0.02	0.71	0.59	0.47
Neutral detergent fiber	2.09	2.11	2.06	2.06	0.02	0.87	0.71	0.21
Organic matter	9.62	9.75	9.37	9.31	0.14	0.88	0.73	0.34
Ether extract	0.35	0.35	0.34	0.33	0.01	0.91	1.00	0.68
Total digestible nutrients	8.82	8.8	8.87	8.28	0.15	0.51	0.38	0.70

[1]CONT - control (no mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil).
[2]MIX2 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (2 g/animal/d).
[3]MIX4 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (4 g/animal/d).
[4]MIX6 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (6 g/animal/d).
[5]SEM - standard error of the mean.

Table 4. *In situ* digestibility of crossbred bulls finished in feedlot and fed a high-grain diet containing a mixture of baccharis, tamarind, cashew nut shell liquid, and clove essential oil

Digestibility, g/kg	Experimental diets				SEM[5]	*P-value*		CONT *vs.* MIX
	CONT[1]	MIX2[2]	MIX4[3]	MIX6[4]		Linear	Quadratic	
Dry matter	608[b]	695[a]	636[b]	600[b]	0.84	0.28	< 0.001[6]	0.01
Crude protein	723	779	734	760	1.01	0.47	0.461	0.13
Neutral detergent fiber	448[b]	548[a]	477[b]	454[b]	0.94	0.53	< 0.001[7]	0.06
Organic matter	626[b]	707[a]	653[b]	621[b]	0.77	0.31	< 0.001[8]	0.01

[a,b,c] Means within rows with different superscripts differ (P < 0.05).

[1]CONT - control (no mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil).

[2]MIX2 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (2 g/animal/d).

[3]MIX4 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (4 g/animal/d).

[4]MIX6 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (6 g/animal/d).

[5]SEM - standard error of the mean.

[6]$\hat{Y}$=50.216 +14.569X- 3.0802 X^2 (r^2=0.47).

[7]$\hat{Y}$=34.246 + 14.791X – 3.0684 X^2 (r^2=0.35).

[8]$\hat{Y}$= 52.932 + 13.362X – 2.8155 X^2 (r^2=0.46).

Table 5. Concentration of volatile fatty acids of crossbred bulls finished in feedlot and fed a high-grain diet containing a mixture of baccharis, tamarind, cashew nut shell liquid, and clove essential oil

Volatile fatty acids (mmol/l)	Experimental diets				SEM[5]	*P-value*		CONT *vs.* MIX
	CONT[1]	MIX2[2]	MIX4[3]	MIX6[4]		Linear	Quadratic	
Acetate	21.85	23.1	23.59	24.74	1.43	0.48	0.99	0.58
Propionate	10.94[b]	17.1[ab]	16.82[ab]	22.03[a]	1.31	< 0.001	0.83	0.006
Acetate/propionate ratio	1.99[a]	1.35[ab]	1.40[ab]	1.12[b]	0.14	0.01	0.43	0.008
Isobutyrate	0.50	0.55	0.66	0.52	0.04	0.64	0.26	0.42
Butyrate	4.69	3.29	4.27	3.05	0.34	0.19	0.90	0.14
Isovalerate	0.78	0.99	1.38	1.09	0.13	0.27	0.33	0.23
Valerate	0.88	0.61	1.03	1.27	0.12	0.14	0.28	0.74

[a,b,c] Means within rows with different superscripts differ ($P < 0.05$).

[1]CONT - control (no mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil).

[2]MIX2 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (2 g/animal/d).

[3]MIX4 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (4 g/animal/d).

[4]MIX6 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (6 g/animal/d).

[5]SEM - standard error of the mean.

The use of the MIX did not influence concentrations of acetic, isobutyric, butyric, isovaleric and valeric acids (Table 5).

Table 6. Feeding behavior of crossbred bulls finished in feedlot and fed a high-grain diet containing a mixture of baccharis, tamarind, cashew nut shell liquid, and clove essential oil

Activity, min/day	Experimental diets				SEM[5]	*P-value*		CONT *vs.* MIX
	CONT[1]	MIX2[2]	MIX4[3]	MIX6[4]		Linear	Quadratic	
Water ingestion	26	16	18	18	2.33	0.47	0.38	0.59
Feed intake	149	169	155	139	5.42	0.98	0.18	0.27
Rumination	234	253	255	192	13.31	0.26	0.69	0.45
Idle	1031	1002	1012	1091	16.35	0.30	0.80	0.29

[1]CONT - control (no mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil).
[2]MIX2 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (2 g/animal/d).
[3]MIX4 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (4 g/animal/d).
[4]MIX6 - mixture of baccharis steam and leaves, tamarind seed, cashew nut shell liquid and clove essential oil (6 g/animal/d).
[5]SEM - standard error of the mean.