UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

COMPOSTOS NATURAIS SOBRE O DESEMPENHO, COMPORTAMENTO, RESPOSTA IMUNE E CARACTERÍSTICAS DE CARCAÇA DE BOVINOS TERMINADOS EM CONFINAMENTO

Autor: Kennyson Alves de Souza
Orientador: Prof. Dr. Ivanor Nunes do Prado

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TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADA em 26 de fevereiro de 2018.

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“*Procure ser um homem de valor, em vez de ser um homem de sucesso.*”

Albert Einstein

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BIOGRAFIA

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

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RESUMO

Atualmente persiste uma polêmica relacionada à utilização dos antibióticos, os quais têm o objetivo na nutrição de ruminantes de moduladores fermentativos. Mas o seu uso frequente na alimentação de bovinos de corte promoveu um possível surgimento de bactérias resistentes que, consequentemente, pode ser grande ameaça à saúde humana. Deste modo, no atual contexto a cadeia produtiva da carne bovina necessita adequar à produção para poder atender o mercado consumidor, com o uso de ingredientes de qualidade alimentar alternativos que não promovam perdas ao sistema. Por meio dos estudos realizados, os objetivos foram avaliar o efeito de ingredientes de origem natural sobre o desempenho animal, resposta fisiológica, bem-estar animal, resposta imune e características de carcaça de bovinos de corte. No primeiro estudo foram utilizadas 40 novilhas Nelores (peso corporal inicial 297,6 ± 31,2 kg) distribuídas aleatoriamente às dietas testadas, sendo CON - Sem óleo essencial; ROS - Óleo essencial de alecrim; BLE - Mistura protegida de eugenol, timol e vanilina; BCL - Mistura protegida + óleo essencial de cravo; e BRC - Mistura protegida + óleo essencial de alecrim + óleo essencial de cravo. Foram quantificados o desempenho animal e as características de carcaça, digestibilidade *in situ*, comportamento ingestivo e composição de tecido da carcaça. Os pesos corporais iniciais e finais não mostraram efeito. Entretanto, o ganho médio diário e o consumo de matéria seca foram maiores em novilhas alimentadas com dietas BLE, BCL e BCR do que novilhas alimentadas com dietas CON e ROS. A eficiência alimentar e a digestibilidade *in situ* foram melhores para as novilhas alimentadas com as três dietas misturadas e o pior para as novilhas alimentadas com a dieta ROS. Os pesos de carcaça e suas características, bem como as percentagens de músculo, gordura ou osso da carcaça não mostraram alteração. Para o comportamento ingestivo, os dados sobre ruminação e ócio tendem a ser alterados pela dieta. No segundo estudo foram utilizados 40 novilhos Nelores designados aleatoriamente para quatro dietas experimentais, sendo TEST - sem ingredientes de suplemento; BAC05 - Folhas de *Baccharis dracunculifolia in natura* (5 g/animal/dia); BAC10 - Folhas de *Baccharis dracunculifolia in natura* (10 g/animal/dia); e BAC15 – Folhas de *Baccharis dracunculifolia in natura* (15 g/animal/dia). Foram quantificados o desempenho animal, o comportamento ingestivo e os parâmetros sanguíneos. O uso das folhas da *Baccharis* não afetou o peso corporal final, ganho médio diário, ingestão de matéria seca, eficiência alimentar e comportamento ingestivo. Para as concentrações plasmáticas de ureia, creatina, aspartato aminotransferase, gamma glutamil transferase e creatina quinase, nenhum efeito foi detectado entre os tratamentos testados. No terceiro estudo foram utilizados 105 bezerros Angus x Hereford (63 novilhos + 42 novilhas). Os animais foram designados aleatoriamente às baias (7 baias/tratamento) e distribuídos aos tratamentos, sendo PC - lasalocida (360 mg/animal/dia de Bovatec, Zoetis, Florham Park, NJ, EUA) + clorotetraciclina (350 mg/animal de Aureomicina em ciclos de inclusão de cinco dias e remoção de dois dias da dieta, Zoetis) do dia 0 a 32 e apenas monensina (360 mg/animal/dia de Rumensin, Elanco Animal Health, Greenfield, IN, EUA) do dia 33 ao 60; EG - edulcorante à base de sacarina de sódio (Sucram a 0,04 g/kg, Pancosma SA, Genebra, Suíça) + extratos de plantas contendo eugenol, cinamaldeído e capsicum (800 mg/animal dia de XTRACT Ruminants 7065; Pancosma SA) do dia 0 a 32, e XTRACT apenas (800 mg/animal/dia) do dia 33 a 60; CON - nenhum ingrediente suplementar do dia 0 a 60. Diariamente os bezerros foram avaliados quanto aos sinais da doença respiratória bovina durante todo período experimental, bem como analises sanguíneas foram quantificados. O ganho médio diário de bezerros foi maior em PC vs. EG e tendeu a ser maior em PC vs. CON. A eficiência alimentar também tendeu a ser maior no PC vs. CON, embora o efeito principal do tratamento para esta resposta não tenha sido significativo. Os títulos séricos médios contra o *vírus sincitial respiratório bovino* foram maiores em EG vs. PC e CON apresentando uma tendência. Os resultados desses estudos sugerem que os ingredientes de origem natural podem ser utilizados como substituintes aos antibióticos utilizados na alimentação de animais confinados.

Palavras-chave: Dieta alto grão, Extrato natural, Imunidade, Modulador fermentativo, Nutrição, Qualidade de carcaça

ABSTRACT

Actually, there is a controversy related to the use of antibiotics, which have the objective in the nutrition of ruminants of fermentative modulators. But its frequent use in feed of beef cattle has promoted a possible emergence of resistant bacteria, which can be a great threat to human health. Thus, in the current context, the beef production chain needs to adapt to production in order to attend the consumer market, with the use of alternative feed quality ingredients that do not promote losses to the system. The objective of these studies was to evaluate the effect of natural ingredients on animal performance, physiological response, animal welfare, immune response and carcass characteristic of beef cattle. In the first study 40 Nellore heifers (initial body weight 297.6 ± 31.2 kg) were randomly distributed to the diets tested, CON - no essential oil; ROS - essential oil of rosemary; BLE - protected blend of eugenol, thymol and vanillin; BCL - protected blend + clove essential oil; and BRC - protected blend + rosemary essential oil + clove essential oil. Animal performance and carcass characteristics, *in situ* digestibility, ingestive behavior and carcass tissue composition were quantified. Initial and final body weights showed no effect. However, average daily gain and dry matter intake were higher in heifers fed BLE, BCL and BCR diets than heifers fed with CON and ROS diets. Feed efficiency and *in situ* digestibility were better for heifers fed with the three mixed diets and worse for heifers fed the ROS diet. The carcass weights and their characteristics, as well as the percentages of muscle, fat, and bone showed no change. For ingestive behavior, data of rumination and idleness tend to be altered by diet. In the second study, 40 Nellore steers randomly assigned to four experimental diets were used, TEST – no supplement ingredients; BAC05 – Leaves of *Baccharis dracunculifolia in nature* (5 g/animal/day); BAC10 – Leaves of *Baccharis dracunculifolia in nature* (10 g/animal/day); and BAC15 – Leaves of *Baccharis* *dracunculifolia in nature* (15 g/animal/day). Animal performance, ingestive behavior and blood parameters were quantified. The use of *Baccharis* leaves did not affect final body weight, average daily gain, dry matter intake, feed efficiency and ingestive behavior. For plasma concentrations of urea, creatine, aspartate aminotransferase, gamma glutamyl transferase and creatine kinase, no effect was detected among the treatments tested. In the third study, 105 Angus x Hereford calves (63 steers + 42 heifers) were used. The animals were randomly assigned to the pens (7 pens/treatment) and distributed to the treatments, PC - lasalocid (360 mg/calf daily of Bovatec; Zoetis, Florham Park, NJ, USA) + chlortetracycline (350 mg/calf of Aureomycin in cycles of 5-day inclusion and 2-day removal from diet; Zoetis) from day 0 to 32 , and monensin only (360 mg/calf daily of Rumensin; Elanco Animal Health, Greenfield, IN, USA) from day 33 to 60; EG - sodium saccharin-based sweetener (Sucram at 0.04 g/kg, Pancosma SA, Geneva, Switzerland) + plant extracts containing eugenol, cinnamaldehyde, and capsicum (800 mg/calf daily of XTRACT Ruminants 7065; Pancosma SA) from day 0 to 32, and XTRACT only (800 mg/calf daily) from day 33 to 60; CON - no supplemental ingredients from day 0 to 60. Calves were evaluated for signs of bovine respiratory disease daily throughout the experimental period, as well as blood tests were quantified. Calf average daily gain was greater in PC vs. EG and tended to be greater in PC vs. CON. Feed efficiency also tended to be greater in PC vs. CON, although main treatment effect for this response was not significant. Mean serum titers against *bovine respiratory syncytial virus* were greater in EG vs. PC and CON showing a tendency. The results of these studies suggest that ingredients of natural origin may be used as substituent for the antibiotics used in feed of feedlot animal.

Key words: Carcass quality, Fermentative modulator, High-grain diet, Immunity, Natural extract, Nutrition

I – INTRODUÇÃO

A eficiência na produção de proteína animal seja ela na área da nutrição ou na área do bem-estar animal é considerado um grande desafio e tem sido alvo de inúmeros estudos ao decorrer dos anos. Isso indica a necessidade de potencializar a produção por meio do incremento de toda a cadeia produtiva da bovinocultura de corte. Trabalhos estão sendo realizados, com o objetivo da elaboração de novos aditivos, os quais são fundamentados pela procura e desenvolvimento de novas tecnologias à base de produtos naturais (Jayasena et al., 2013) em decorrência das exigências impostas pelos mercados compradores de carne bovina. Esta exigência, que vem se consolidando nos últimos anos com a utilização rotineira de antibióticos e promotores de crescimento na alimentação animal, tem preocupado a saúde pública.

Da mesma maneira, o código de regulamentação federal do FDA (21CFR182.20) (2013) estabelece que os óleos essenciais, as oleorresinas (sem solventes) e os extratos naturais (incluindo destilados) são geralmente reconhecidos como seguros (GRAS - Generally Recognized as Safe) para o uso. Neste contexto, várias pesquisas estão sendo destinadas à descoberta de novos ingredientes de origem natural, com o objetivo de serem utilizados como potenciais substituintes dos antibióticos utilizados na nutrição animal. Entretanto, esses ingredientes necessitam apresentar capacidade semelhante aos produtos sintéticos, atuando como moduladores fermentativos, e ainda, ter boa aceitabilidade pelos consumidores da carne bovina.

A utilização desses extratos vegetais é reportada por vários autores como uma interessante alternativa em substituição aos antibióticos (Benchaar et al., 2008; Yang et al., 2010) por ser considerado aditivo alimentar seguro. Em paralelo, o uso de um sistema de acabamento de animais em confinamento com dieta alto concentrada está sendo disseminada rapidamente em razão do maior ganho por animal/dia e pela maior qualidade das carcaças (Miguel et al., 2013). Entretanto, dietas com 70% de concentrado ou mais necessitam de moduladores da fermentação ruminal em função da alta degradabilidade dos carboidratos usados; neste sentido, os aditivos de origem natural passam a ser grandes aliados nas pesquisas.

Dieta alto concentrada na nutrição de ruminantes

O uso de dietas alto-concentrado fornecidas *ad libitum* é uma prática muito comum nas indústrias de gado de corte dos Estados Unidos. Segundo Preston (1998) essa prática caracteriza-se por um rápido ganho de peso, alta eficiência de conversão alimentar e consequente diminuição no tempo de terminação para abate, menor custo de mão-de-obra, menor necessidade de armazenamento de alimentos e geralmente maior uniformidade do rebanho.

Mas para determinar a eficiência do sistema dois fatores estão envolvidos, sendo o preço do milho grão no momento da aquisição e o preço pago pela arroba. O conhecimento do custo da dieta na fase de terminação em confinamento é de fundamental importância, lembrando que é a porção mais onerosa de um sistema de engorda de bovinos. Assim, levando em consideração os custos de produção, obtém-se uma maior competitividade no setor.

Entretanto, o uso de dietas com um elevado teor de concentrado pode promover distúrbios metabólicos, como acidose, timpanismo, laminite, diarréia, perca de peso e aparecimento de lesões no fígado. De acordo com Mendes et al. (2010), no uso de dietas com alta proporção de ingredientes concentrados, é mais seguro e recomendado a utilização de um teor mínimo de fibra capaz de estimular a ruminação e permitir um ambiente ruminal adequado para não prejudicar o desempenho animal.

Neste contexto o grão de milho apresenta anatomia e composição química interessante para tal prática. A composição química expressada à base da matéria seca é 72% de amido, 9,5% proteínas, 9% fibras e 4% de óleo. Sendo considerada uma importante fonte de fibra, especialmente do tipo insolúveis (hemicelulose, celulose e lignina), que correspondem à fração fibra em detergente neutro. Já em relação à estrutura física do grão de milho, o mesmo é formado por quatro principais estruturas físicas, sendo o endosperma, gérmen, pericarpo (casca) e ponta (NRC, 2001).

Sobrinho et al. (1996) trabalhando com grãos inteiros para cordeiros em confinamento, observaram diminuição do ritmo de fermentação ruminal e aumento do tempo de ruminação e de ingestão, elevando a secreção de saliva e o pH do rúmen. Os mesmos autores verificaram também que o fornecimento de grãos inteiros não causou prejuízos à digestibilidade nem à conversão alimentar.

Autores como Woody et al. (1983) estudaram o efeito de diferentes níveis de grãos nas dietas de bovinos em fase de terminação e relataram que animais alimentados com dietas com 90% de grãos ganharam peso 7% mais rápido e tiveram redução de 16% no requerimento alimentar por unidade de ganho em relação a animais alimentados com 70% de grãos.

Doença respiratória bovina

A incidência da doença respiratória bovina é extremamente elevada durante os primeiros 30 dias dos animais em confinamento, com sintomas clínicos observados em até 75% de morbidade e 50% a 70% de mortalidade (média dos Estados Unidos), apesar dos esforços associados com a minimização do estresse e ganhos com a vacinação para as doenças respiratórias bovinas (Kirkpatrick et al., 2008). O complexo da doença respiratória bovina é a doença mais dispendiosa encontrada nos bovinos confinados nos Estados Unidos da America (EUA), e custa a indústria nacional da carne aproximadamente 1 bilhão de dólares anualmente. Estas perdas econômicas incluem, além de mortalidade dos animais, custos associados com o desempenho animal reduzido e a compra de antibióticos (Loerch & Fluharty, 1999). No Brasil os dados para a doença respiratória bovina ainda são inexpressiva, mas acredita - se que na atualidade esta doença é um dos principais desafios referente à saúde animal, permanecendo à frente de problemas como acidose e laminite (Millen et al., 2009).

Entre os fatores ambientais e de manejo os bovinos estão expostos a uma série de desafios de estresse e de saúde, os quais afetam diretamente ao bem-estar e a produtividade animal em todo o período de alimentação (Duff & Galyean, 2007). Estes desafios incluem o transporte rodoviário, mistura entre animais diferentes e a exposição a novas dietas e ambientes (Arthington et al., 2008). Essa exposição a novas dietas promovem um consumo inadequado pelos animais durante os primeiros dias de confinamento, o que contribui ainda mais com prejuízos a imunidade e a resistência ao surgimento de possíveis doenças (Araujo et al., 2010).

A identificação dos sinais clínicos da doença respiratória bovina segue o sistema DART (Zoetis, Florhan Park, Nj), onde se atribuí pontuações de escore variando de 0 a 4 com base nos sinais clínicos e a gravidade dos sinais observados. Sendo o escore 0 nenhum sintoma detectado, 1 sintoma leve, 2 sintoma moderado, 3 sintoma grave e 4 moribundo. Todos os animais quando detectados com algum sintoma clínico e apresentando temperatura retal acima de 40 ^{o}C administra - se o antimicrobiano. Este sistema leva em consideração critérios de avaliação como depressão, apetite, sistema respiratório e temperatura.

Com relação aos sinais clínicos de depressão observa - se nos animais a atitude, movimentação da cabeça, postura, olhos (vidrados ou afundados) e capacidade de levantar; sinais clínicos de apetite - interesse em se alimentar, disposição em comer, quantidade ingerida, ritmo de consumo, preenchimento ruminal e perca de peso; sinais clínicos do sistema respiratório - caráter respiratório e o esforço, sons de respiração auditiva e extensão da cabeça e pescoço; sinais clínicos da temperatura - quantificado, após de ser considerado um candidato para tratamento.

Além da vacinação contra os agentes infecciosos da doença, se utiliza estratégias nos confinamentos, como é o caso do uso de rações comerciais para mitigar a incidência da doença respiratória bovina e outros tipos de enfermidades com a inclusão de ionóforo e clortetraciclina na dieta (Duff & Galyean, 2007).

Ingredientes de origem natural utilizado na alimentação animal - Introdução a *Baccharis dracunculifolia*

A *Baccharis dracunculifolia* é um gênero de planta com mais de 500 espécies localizadas em todo continente sul americano, principalmente encontradas nas regiões do Brasil, Argentina, Colômbia, Chile e México. Essa espécie de planta é habitualmente conhecida como “Alecrim do campo e/ou Vassoura” tendo sua aplicação amplamente utilizada na medicina popular na prevenção de disfunções como anemia, inflamação, diabetes, distúrbios hepáticos e próstata (Menezes, 2005; Verdi et al., 2005). Por meio de estudos *in vitro* demonstrou a participação de algumas classes que constituem a *Baccharis*, destacando - se os flavonóides (isosakuranetina, aromadendrin-4'-éter metílico) terpenos (bacarina), ácidos fenólicos (artepelina C, ácido cafeico, ácido *p*-

cumárico, ácido ferúlico) (Bohlmann et al., 1981; Banskota et al., 1998; Akao et al., 2003; Silva Filho et al., 2004; Mendez, 2005; Loots et al., 2006).

A *Baccharis dracunculifolia* é explorada como substrato no Brasil com o objetivo da produção da própolis verde, assim chamada devido a sua coloração (Marcucci et al., 1998; Park et al., 2002). A qual é um elemento natural resinosa, coletada por abelhas das flores das plantas, que apresenta uma atividade biológica, antimicrobiana e antioxidante (Da Silva Leitão et al., 2004; Simões et al., 2004; Jorge et al., 2008; Pontin et al., 2008). Autores estudando a composição da planta *Baccharis* e da própolis verde (Labbe et al., 1986; Marcucci et al., 1998; Kumazawa et al., 2003) encontraram várias substâncias que estão presente nas duas fontes, como derivados do ácido *p*-cumárico, prenilados e flavonóides.

Óleo essencial e sua capacidade de atuação na flora bacteriana de ruminantes

Definição

Os óleos essenciais são misturas complexas de metabolitos secundários lipófilos voláteis. Tradicionalmente extraído das plantas, mas o método utilizado varia conforme a localização do óleo essencial na planta e com o tipo de uso. Os tipos de extração utilizados são por enfloração, arraste com vapor d'água, solvente, prensagem e dióxido de carbono supercrítico. Embora a destilação a vapor seja o procedimento mais empregado na extração dos óleos essenciais, a utilização de dióxido de carbono líquido à baixa temperatura e alta pressão preserva de forma mais eficiente às características organolépticas dos compostos existentes no óleo. Essas diferenças influenciam na composição dos óleos essenciais e nas propriedades antimicrobianas, porém, é um processo de custo elevado comparados aos demais processos (Burt, 2004).

A composição dos óleos essenciais pode variar dentre a mesma espécie de planta, a partir do método de extração, época da colheita, fertilidade do solo e locais geográficos. Vokou et al. (1993) observaram que as concentrações dos compostos principais do óleo essencial de orégano (*Origanum vulgare* ssp. *Hirtum*) variaram entre as áreas geográficas da Grécia, da qual a planta foi colhida.

Embora se acreditasse que os metabólitos secundários e, portanto, os óleos essenciais, não tinham função na planta, agora são aceitos que eles fornecem a planta proteção contra estressores abióticos e bióticos, bem como sendo atrativos a organismos que polinizam e dispersam sementes (Wink & Schimmer, 1999).

Mecanismos de ação (propriedade antimicrobiana)

Os óleos essenciais atuam de várias formas sobre os microorganismos do rúmen, mas de uma forma geral a sua atuação é sobre a membrana celular dos mesmos, desnaturando e coagulando as proteínas, afetando o transporte de elétrons, o gradiente de íons, a translocação de proteínas, a fosforilação e outras reações enzimo-dependentes (Dorman & Deans, 2000). Essa capacidade de atuação sobre a membrana celular, bem como a membrana da mitocôndria estão relacionadas com a característica hidrofóbica dos óleos quando se encontram na forma indissociada (Calsamiglia et al., 2007) causando assim, o rompimento da membrana e conseqentemente o extravasamento de íons e de material citoplasmático (Figura 1).

Dentre as bactérias ruminais encontra-se as bactérias gram-positivas e as gram-negativas. As bactérias gram-positivas são mais suscetíveis aos efeitos dos óleos essências quando comparado com as bactérias gram-negativas, isso ocorre devido a uma camada extra, além da membrana celular o que promove uma proteção e uma permeabilidade o que limita o acesso dos compostos hidrofóbicos (Burt, 2004; Tajkarimi et al., 2010).

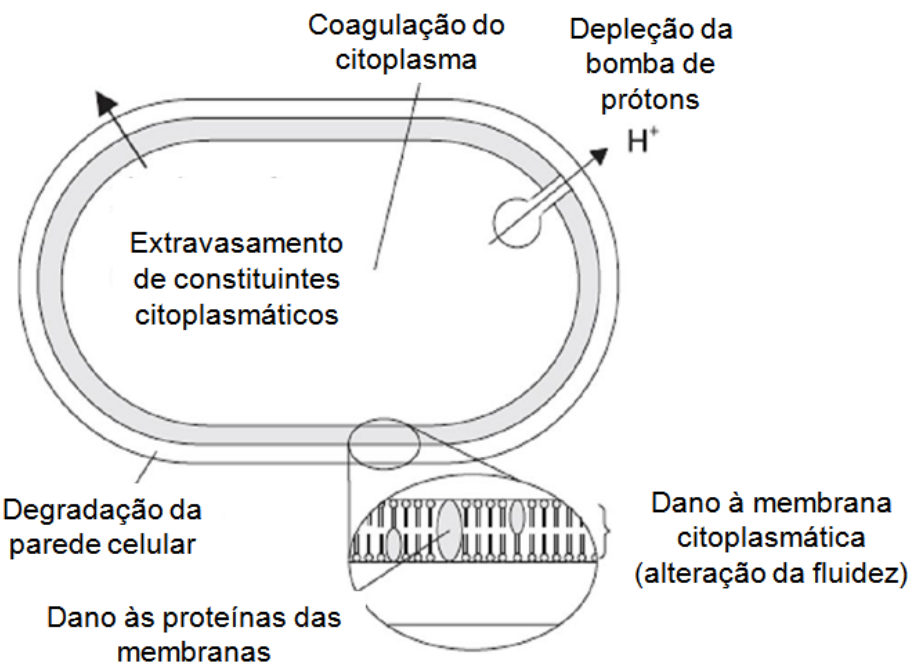

Figura 1. Possíveis mecanismos de atuação dos óleos essências sobre as bactérias ruminais. Adaptado de Burt (2004).

Os extratos naturais apresentando esta capacidade de seleção bacteriana podem atuar inibindo a atividade de bactérias nocivas a produção de ruminantes, como é o caso da “Hyper-ammonia producing - HAP” as quais são responsáveis pela desaminação dos aminoácidos. Processo este que é responsável por liberar um agrupamento amina na forma de amônia dos aminoácidos (Patra & Saxena, 2010). O crescimento de algumas espécies de bactérias HAP (i,g., Clostridium sticklandii e Peptostreptococcus anaerobius) são facilmente inibidas pela ação antimicrobiana dos óleos essências, mas McInotch et al. (2003) pôde observar que existe um outro grupo de bactérias menos sensíveis a ação dos óleos (e,g., Clostridium aminoplhilus).

Vale ressaltar que existem outros fatores que podem atuar neste tipo de seleção bacteriana, como é o caso de baixos níveis de proteína ofertada aos animais via suplemento, bem como, o tipo de fornecimento dos óleos essências a população bacteriana. Por meio da diferenciação dos mecanismos de atuação dos óleos propriamente ditos e dos seus compostos (princípios ativos) isoladamente (Prata et al., 2011).

Existem relatos que os óleos essências de uma forma geral também podem atuar como seletores de protozoários, em trabalhos realizados por Cardozo et al. (2006) observaram que a adição de uma mistura de cinamaldeído e eugenol na dietas de novilhas diminui a população dos protozoários da espécie holotrichas e entodiniomorphs. Apesar da importância dos protozoários no meio ruminal como recicladores intra-ruminal da proteína bacteriana e responsáveis em minimizar a ocorrência de acidoses, a sua parcial defaunação promove um aumento no escape de proteína bacteriana, menor concentração de amônia e consequentemente uma melhora no desempenho animal (Eugène et al., 2004).

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II - OBJETIVOS GERAIS

- Avaliar os efeitos dos óleos essenciais e suas misturas no desempenho animal, ingestão de matéria seca, características de carcaça, digestibilidade *in situ* e comportamento ingestivo de novilhas terminadas em confinamento recebendo uma dieta alto concentrada.

- Investigar os efeitos da adição de folhas de *Baccharis dracunculifolia in natura* sobre o desempenho animal, ingestão de matéria seca, atividades de comportamento ingestivo e parâmetros sanguíneos de novilhos terminados em confinamento com dieta alto concentrada.

- Comparar desempenho, saúde e respostas fisiológicas de bezerros recém-desmamados suplementado com antibióticos alimentares, ingredientes alternativos ou sem tais suplementos durante um período inicial de confinamento de 60 dias.

III – 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 of Nellore heifers finished in feedlot

Journal: Livestock Science

ABSTRACT

This study was carried out to evaluate the influence of essential oils and their blends on animal performance, feed intake, *in situ* digestibility, ingestive behavior activities, and carcass characteristics of heifers finished in feedlot and fed with a high-grain system. Forty Nellore heifers (initial body weight 297.6 ± 31.2 kg) were used in the experiment and distributed randomly among individual pens. The diets tested were CON – Without essential oil; ROS – Rosemary essential oil; BLE – Protected blend of eugenol, thymol, and vanillin; BCL – Protected blend + clove essential oil; and BRC – Protected blend + rosemary essential oil + clove essential oil. The initial and final body weights did not show effect. However, average daily gain was greater ($p < 0.001$) in heifers fed with BLE, BCL, and BRC diets than in heifers fed with CON and ROS diets. Dry matter intake (kg/d) was greater ($p < 0.05$) in heifers fed with BLE, BCL, and BRC diets than in heifers fed with CON and ROS diets. Feed efficiency was better ($p < 0.0001$) in heifers fed the three blended diets and the worst in heifers fed the ROS diet. Carcass weights and characteristics did not show effect. I*n situ* digestibility of dry matter and neutral detergent fiber were greater ($p < 0.0001$) in heifers fed the three blended diets and lower in heifers fed the ROS diet. The addition of essential oils to the diets of heifers did not alter the muscle, fat, or bone percentages in the carcass. For ingestive behavior activities, data on rumination and idleness tended to be altered by diet. The addition of 4 g/animal/d of a blend of essential oils to the diets of Nellore heifers improve average daily gain, dry matter intake, feed efficiency, *in situ* digestibility, and ingestive behavior activities.

Keywords: Beef cattle; Carcass quality; Fermentative modulator; *In situ* digestibility; Natural extract

Highlights:

- Use of essential oil improved the animal performance of feedlot heifers.
- No differences were observed in the carcass tissue composition of heifers.
- Digestibility of NDF was lower in heifers fed rosemary essential oil.
- Inclusion of essential oils improved the ingestive behavior activities of heifers.

1. Introduction

In Brazil, the traditional production systems of cattle are extensive and pasture based, and the Zebu breeds (*Bos taurus indicus*), such as Nellore and European crossbreds (*Bos taurus taurus* × *B. taurus indicus*), are frequently used (Rotta et al., 2009). In recent years, due to the increase in domestic and export beef demand, large annual growth has occurred in the meat market and consequently, the use of more intensive production systems with the inclusion of a high percentage of concentrate are also being utilized to reach market demand (Prado et al., 2008).

The addition of antibiotics to livestock production systems has been common, especially when animals are reared intensively, in order to prevent diseases and metabolic disorders and to improve feed efficiency. However, due to the emergence of bacteria resistant to antibiotics and the possible risks to human health from possible residues in the final products (Russell and Houlihan, 2003), the use of antibiotics has been forbidden in some regions. Thus, those who are responsible for the production chain are seeking alternative solutions, including the use of essential oils as a potential alternative/substitute for antibiotics to improve the performance of cattle (Cruz et al., 2014).

The essential oils are liquid aromatic extracts due to the volatile nature of the components extracted from plant material, such as flowers, buds, seeds, leaves, twigs, barks, wood, fruit, and roots. They may be obtained by fermentation, extraction, or most commonly, by steam distillation (Burt, 2004). Chemically, essential oils are variable mixtures of terpenoids that primarily include monoterpenes (C^{10}) and sesquiterpenes (C^{15}), although diterpenes (C^{20}) may also be present. They also include a variety of low-molecular-weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, or lactones. Besides to count with compounds such as coumarins and homologues of phenylpropanoids. These products consist of various concentrations and chemical variations acting as antimicrobial and antioxidant agents, benefiting the immune and digestive systems of animals, which is reflected in animal performance indices (Jayasena and Jo, 2013).

Interest in the use of essential oils as a potential substituent of antibiotics in cattle feed has been generated from the results of *in vitro* studies (Meyer et al., 2009) showing that essential oils have antimicrobial activity against the microflora present in the gastrointestinal tract. There is still a large portion of open research in the area, because the results of the use of essential oils are dependent on their compounds, the doses used, and the synergistic effects among them.

This study was carried out to evaluate the effects of essential oils and their blends on animal performance, feed intake, carcass characteristics, *in situ* digestibility, and ingestive behavior activities of heifers finished in feedlot with high-grain diets.

2. Materials and Methods

This experiment was approved by the Committee for Ethics in the use of Animals (CEUA) of the Universidade Estadual de Maringá, following protocol 3624120116.

2.1. Locale, animals, housing, and experimental treatments

The experiment was carried out at Sector Rosa & Pedro at the experimental farm of Universidade Estadual de Maringá, Paraná, Brazil. Forty Nellore purebred heifers with a mean initial body weight (BW) of 297.6 ± 31.2 kg were used in this study. Heifers were distributed randomly in individual pens, with dimensions of 10 m^2 for each animal, partially covered and equipped with automatic drinkers and masonry feeders. The period of adaptation to the feedlot and concentrate diet was 7 days; afterwards, the experimental period was extended to 73 days until animals reached a mean BW of 356.6 ± 32.6 kg. During the experimental period, Nellore heifers were weighed monthly in order to record weight gain and productivity variables.

Nellore heifers were randomly assigned to one of five studied diets with eight heifers per diet group. The diets tested were CON – Without essential oil; ROS – Rosemary essential oil (4 g/animal/d); BLE – Protected blend of eugenol, thymol, and vanillin (4 g/animal/d); BCL – Protected blend – eugenol, thymol, and vanillin (2 g/animal/d) +

clove essential oil (2 g/animal/d); and BRC – Protected blend – eugenol, thymol, and vanillin (1.33 g/animal·d) + rosemary essential oil (1.33 g/animal/d) + clove essential oil (1.33 g/animal/d).

The rosemary and clove essential oils had a liquid texture and were obtained from FERQUIMA® (Vargem Grande Paulista, São Paulo, Brazil). The essential oil blend was powder (eugenol, thymol, and vanillin) and was obtained from Safeeds® (Cascavel, Paraná, Brazil). These plant extracts were chosen from the best results of the analysis of detection antioxidant power (Biondo et al., 2016), while the dosage was determined after performed research (Benchaar et al., 2006a, 2006b; Busquet et al., 2006). The preparation of the diet with the essentials oils were made every 15 days, but in order to calculate and adjust the dose by period depending on the intake of dry matter (DM)/d per animal were made monthly after weighing the animals. Preparation of diets was made with a pre-mix of essential oils in the soybean meal and then led to the feed mixer together with other ingredients. The diets were reviewed by the oxygen radical absorbance capacity (ORAC) method as reported by Zulueta et al. (2009), as the antioxidant power of essential oils in the diet remains for up to 30 days of exposure.

The five basal diets, consisting of corn silage; soybean meal that was dosed the amount to guarantee the supply of 4 g/animal/d, and provided twice a day; and the corn grain, was provided *ad libitum*. All diets were isonitrogenous, isoenergetics, and formulated to meet the requirements for a gain of 1.0 kg/d (NRC, 2000) with adequate concentrations of nutrients for the growth and finishing of animals (Table 1).

2.2. Chemical analyses

The chemical compositions of ingredients and experimental diets were presented as g/kg of DM (Table 2). DM was determined after oven drying at 65 °C for 24 h and milling through a 1-mm screen following method ID 934.01 (AOAC, 2005). Ash content was measured by combustion at 550 °C for 16 h according to method ID 942.05 (AOAC, 2005). Nitrogen concentration was estimated by the Kjeldahl method (ID 988.05) (AOAC, 2005). Following the determination of nitrogen concentration, crude protein was calculated by multiplying the nitrogen content by a factor of 6.25. Ether extract content was determined by method ID 920.39 (AOAC, 2005). The neutral detergent fiber (NDF) content was measured according to the recommendations of Mertens (2002) using α-amylase and was expressed inclusive of residual ash. The acid detergent fiber was measured by using method ID 973.18 (AOAC, 2005) and was expressed inclusive of residual ash. Total carbohydrates were estimated by the procedure of Sniffen et al. (1992) as follows. Non-fibrous carbohydrates were determined as the difference between total carbohydrates and NDF. Metabolizable energy of feed stuffs was estimated according to NRC (2000) recommendations.

2.3. Feed intake, growth performance, and carcass characteristics

Diets were offered at 08:00 and 16:00 h every day. Feed intake was estimated as the difference between the feed supplied and refusals in the trough. Feed efficiency was calculated as the ratio between average daily gain and DM intake. To determine growth performance, animals were weighed at the beginning of the experiment and then every month (after fasting for 16 h), throughout the experiment. The average daily gain was calculated as the total BW gain divided by the length of the experimental period (73 days).

When the Nellore heifers reached a mean final body weight of 356.6 ± 32.6 kg, they were slaughtered in a commercial slaughterhouse 130 km from the Iguatemi Experimental Farm. Animal transport was carried out in the late afternoon to minimize stress. Upon arrival the at the slaughterhouse, animals were kept in resting pens and were subsequently stunned using a penetrating captive bolt pistol as per Brazilian federal inspection regulations according to the Brazilian RIISPOA – Regulation of Industrial and Sanitary Inspection of Animal Products.

After slaughter, the carcasses were identified, weighed, and chilled for 24 h at 4 °C. The cold carcass weight was determined after chilling. The carcass dressing percentage (hot and cold) was calculated by applying the following equation:

$$CDP = CW \times 100/FBW \qquad (1)$$

where: *CDP*, *CW*, and *FBW* are Carcass dressing percentage; Carcass weight; and Final body weight, respectively.

The carcass dripping loss was performed by measuring the difference between the weight obtained before and after refrigeration for 24 h (± 4 ^{o}C).

2.4. Carcass tissue composition

Carcass tissue composition was determined/estimated by dissection of the 6th rib according to the methodology of Robelin and Geay (1975). Muscle, fat (subcutaneous and inter-muscular), bone, and other tissues (tendons and fascia) were separated.

2.5. *In situ digestibility*

The determination of total digestibility from the indicator indigestible neutral detergent fiber (iNDF) was carried out according to the methodology described by Zeoula et al. (2002). Samples of feed, feces, and leavings were incubated in the rumen of animals cannulated using F57 filter bags for 288 h (Ankom Technology, NY, USA) with dimensions of 5.0 x 5.0 cm and a porosity of 50 mm. A 1.0-g sample was incubated for concentrated food and 0.5 g for silage, feces, and leavings. Following removal of the bags from the rumen, they were washed by hand under running water until the resulting wash water became clear and subsequently placed to dry in a forced ventilation oven at 60 °C for 48 h and finally, boiled in a neutral detergent solution (TE-149, Tecnal, SP, Brazil) to obtain the iNDF.

Fecal flow was determined using the following equation:

$$FF = IC/CIF \tag{2}$$

where: *FF*, *IC*, and *CIF* are Fecal flow; Indicator consumed; and Concentration indicator in feces, respectively.

The digestibility coefficient was calculated by the following equation:

$$DC = (NI - NE)/NI \tag{3}$$

where: *DC*, *NI*, and *NE* are Digestibility coefficient; Nutrient intake; and Nutrient excreted, respectively.

2.6. *Ingestive behavior activities*

Data on feeding behavior were obtained between the 6th and 7th weeks of feedlot. The record of time spent on different activities was obtained by visual observation of the animals every 5 min, carried out by a trained team over 24 uninterrupted hours (Silva et al., 2006). Data were collected to estimate the duration of periods spent feeding, drinking, ruminating, and idle. The total time spent on each activity was determined by the sum of repetitions.

The efficiencies of feeding and rumination of DM and NDF were determined by adapting the methodology proposed by Bürger et al. (2000), according to the equations described below:

$FE_{DM} = DMI/FD$

$FE_{NDF} = NDFI/FD$

$RE_{DM} = DMI/RUD$

$RE_{NDF} = NDFI/RUD$ (4)

where: FE_{DM}, DMI, FD, FE_{NDF}, $NDFI$, RE_{DM}, RUD, RE_{NDF} are Feeding efficiency of dry matter (kg DM/h); Dry matter intake (kg DM/d); Feeding duration (h/d); Feeding efficiency of neutral detergent fiber (kg NDF/h); Neutral detergent fiber intake (kg NDF/d); Rumination efficiency of dry matter (kg DM/h); Rumination duration (h/d); and Rumination efficiency of Neutral detergent fiber (kg NDF/h), respectively.

3. Statistical Analyses

Data were analyzed by using the ANOVA procedure of SAS (SAS, 2004) to perform a randomized complete experiment with five diets and eight replications. The model included the fixed effects of essential oil diets according the following equation:

$Yij = \mu + Ti + eij$ (5)

where: Yij, μ, Ti, eij are Dependent variables; Mean value common to all observations; Fixed effect of essential oils diets; and The error term, respectively.

For each studied variable, the mean and standard error of the mean (SEM) were calculated and differences between means were evaluated using Duncan's Multiple Range Test ($p \leq 0.05$).

4. Results

Final body weight (FBW) was not affected ($p > 0.05$) by essential oil addition to the diets (Table 3). The average daily gain (ADG) was significantly greater ($p < 0.001$) for heifers fed three diets with essential oil blends than for heifers fed CON and ROS diets (Table 3). The blend of essential and protected oils in the diets increased ($p < 0.0001$) dry matter intake (DMI) (Table 3). The feed efficiency rate was better ($p < 0.0001$) in heifers fed three essential oil blends (BLE, BCL, and BRC) (Table 3). Feed efficiency presented intermediate values in heifers fed the CON diet and were not significantly different from those in the BLE group. Heifers fed the ROS diet had the lowest feed efficiency ($p < 0.0001$).

Hot and cold carcass weights were not modified ($p > 0.05$) by the addition of essential oils in the diets of heifers. Similarly, the hot carcass and cold dressing were similar ($p > 0.05$) among heifers fed the five diets provided (Table 3). Dripping losses (after 24 h of chilling) were not influenced ($p > 0.05$; Table 3) by the addition of essential oils or their blends to the diets. No differences were observed ($p > 0.05$) in the muscle, fat, and bone percentages on the 6th ribs of heifers (Table 4).

The addition of essential oils and their blends to the diet resulted in differences ($p < 0.05$) in *in situ* digestibility of DM and neutral detergent fiber (NDF) (Table 5). The *in situ* digestibility of DM was lower in heifers fed ROS diet relative to the other diets. This diet also presented the lowest values for NDF digestibility, with BCL being the group with the greatest value. In this study, it may be noted that when rosemary essential oil was used, the total digestibility of DM and NDF showed the lowest results when compared with other treatments. The best results for total digestibility were for the animals fed the diet containing clove essential oil.

For ingestive behavior activities, data on rumination and idleness tended to be altered by diet ($p < 0.10$; Table 6). The values of feeding and drinking were not affected ($p > 0.05$, Table 6) by the use of vegetable extracts.

5. Discussion

The observed value of FBW was in accordance with the Nellore standard, as well as with the requirements of Brazilian slaughterhouses, which advocate a final body weight for heifers from 320 to 380 kg (Ferraz and Felício, 2010). The rosemary essential oil in the diet showed the lowest gain, whereas when it was supplied with other oils, it presented a higher ADG than the CON group. Heifers finished in feedlot must have weight gain from 0.8 to 1.2

kg/animal/d. The low body weight gains of heifers fed CON and ROS diets were due the lower feed intake of those experimental groups.

The DMI of heifers from ROS diet was the lowest. However, heifers fed the BRC diet that contains rosemary essential oil in the composition was better than CON and ROS. As any other living being, plants also develop defense mechanisms, and in this case, the defense is against herbivorism, making use of substances to this situation (Gershenzon and Croteau, 1991). These substances can be found in essential oils as volatile compounds, for example, camphor, limonene, α-pinene, β-carophylenne, p-cymene, α-humulene, and others (Burt, 2004). Working with isolated camphor and carophylenne compounds, Estell et al. (1998) observed a reduction of 14% and 16% in DMI of sheep, respectively. The essential oil from rosemary is rich in volatile compounds of 1.8 cineole, α-pinene, β-carophylenne, camphene, camphor, and borneol (Smeti et al., 2013), which could affect DMI when not applied with other essential oils.

The lowest feed efficiency seen in heifers fed the ROS diet could have been due the plants' palatability. The best value for feed efficiency in heifers was seen for diets with blends of essential oils, maybe because of a possible synergism that occurred between the oils, which was probably due to an ruminal environment appropriate (pH 5.5), promoted by highly concentrated diets (Cardozo et al., 2005).

The hot and cold carcass weights values observed were in accordance with those of standard Nellore heifers, as well as with the requirements of Brazilian slaughterhouses, which advocate a final body weight for heifers from 180 to 200 kg (Ferraz and Felício, 2010). The average hot and cold carcass dressings were superior, when compared that the carcass dressing are approximately of 52% can be considered normal for Nellore heifers slaughtered at 24 months, as observed in other studies of heifers slaughtered at a similar weight (Marques et al., 2010; Farias et al., 2012). In general, the dripping losses are between 1.5% and 2.0% after 24 h of chilling which is in agreement with the present study. Thus, the losses observed in this experiment are consistent with losses considered normal (Andreotti et al., 2015). Studies carried out with crossbred heifers in feedlot have reported muscle, fat, and bone percentages from 56% to 62%, 20% to 25%, and 16% to 19%, respectively (Andreotti et al., 2015). Thus, the muscle, fat, and bone percentages obtained in this study can be considered normal for these animal categories.

According to Oh et al. (1968) a hypothesis exists that the low palatability of some natural extracts to ruminants, as is the case of rosemary essential oil, could be due not only to sensorial effects but also to effect on microbial flora, thus affecting directly the total digestibility of DM. Nagy and Tengerdy (1968) proved this fact in their studies evaluating the sensitivity of ruminal microorganisms using the essential oil of *Artemisia tridentate,* which has as its main compound 1.8-cineole, also found in essential oil of rosemary, because some evidence indicated that high intake of this compound resulted in digestive problems in ruminants. In general, high doses of this oil, when added to *in vitro* cultures of rumen bacteria, reduced total viable bacteria counts. A possible explanation for the low levels of NDF total digestibility is related to the higher starch content of the diets (Table 1), which can decrease the digestibility of the fiber as a result of lower ruminal pH, ruminal passage rate, and changes in rumen microbial populations (Allen and Mertens, 1988). However the use of clove essential oil is beneficial because it has as main compound eugenol, which is considered a phenolic compound, and it has demonstrated a high antimicrobial activity due to the presence of a phenolic hydroxyl group in its structure (Burt, 2004).

The reason of values for ingestive behavior activities could be related to the fact that all animals received a basal diet in which there was no differentiation of the diet ingredients, as fiber content and particle size are the main factors involved in this situation (Mendes et al., 2007). However, essential oils are able to reduce protein degradation, causing a reduction in adherence and colonization of bacteria with proteolytic activity toward substrates (Benchaar et al., 2008); consequently, rumination rates increase in order to reduce particulate ingredients found in the rumen.

The inclusion of essential oils in the diet was highly beneficial, even resulting in an increased rumination rate, while there was a decrease in the idleness rate. These observed frequencies of ingestive behavior are consistent with the values of DM feeding efficiency and NDF rumination efficiency (Table 6), thus demonstrating that essential oils can act as fermentative modulators, which positively influences animal production (Marques et al., 2008). These values of feeding and drinking are very important because such extracts have a rather sharp odor and taste and can be used as stimulators of consumption. According to Yang et al. (2010), very high doses of essential oils administered in the diet, from absent to present, influence animal consumption differently from when using a low dose.

6. Conclusions

The present results suggest that the use of a blend of 4 g/animal/d of natural additives in the diets of Nellore heifers improves animal production. Rosemary essential oil in independent doses supplied as single oil shows no improvement in animal production, but when administered with other essential oils, the response is positive. The blend of clove essential oil (2 g/animal/d) and protected oils [eugenol, thymol, and vanillin (2 g/animal/d)] proved to be promising, promoting the best results.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1. Ingredients of experimental diets (g/kg of DM)

Ingredients	CON[a]	ROS[b]	BLE[c]	BCL[d]	BRC[e]
Corn silage	250	250	250	250	250
Corn grain	647	647	647	647	647
Soybean meal	100	100	100	100	100
Yeast	0.40	0.40	0.40	0.40	0.40
Phosphorus	0.70	0.70	0.70	0.70	0.70
Mineral salt[f]	1.45	1.45	1.45	1.45	1.45
Rosemary essential oil	-	0.04	-	-	-
Protected blend – eugenol + thymol + vanilinn	-	-	0.04	-	-
Protected blend + clove	-	-	-	0.04	-
Protected blend + clove + rosemary	-	-	-	-	0.04

[a] CON – Without essential oil.

[b] ROS – Rosemary essential oil (4 g/animal/d).

[c] BLE –Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

[d] BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

[e] BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

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

Table 2. Chemical composition of ingredients of diets (g/kg of DM)

Ingredients	DM[a]	OM[b]	Ash	CP[c]	EE[d]	NDF[e]	ADF[f]	TC[g]	NFC[h]	ME[i]
Corn silage	306	969	30.9	71.1	27.1	424	224	870	446	23.4
Corn grain	853	984	16.4	96.1	47.1	175	45.8	840	665	30.0
Soybean meal	850	933	67.0	489	19.0	159	87.8	425	266	31.3
Yeast	920	954	46.1	331	21.0	26.0	9.22	572	546	-
Phosphorus	995	38.0	962	-	-	-	-	-	-	-
Mineral salt	986	55.0	945	-	-	-	-	-	-	-
Diet	716	973	27.1	129	39.1	235	94.4	804	568	28.4

[a] Dry matter.

[b] Organic matter.

[c] Crude protein.

[d] Ether extract.

[e] Neutral detergent fiber.

[f] Acid detergent fiber.

[g] Total carbohydrates.

[h] Non-fiber carbohydrates.

[i] Metabolizable energy (Mcal/ kg).

Table 3. Effect of diets with and without inclusion of essential oils on animal performance, feed conversion/efficiency and carcass characteristics of Nellore heifers finished in feedlot

Parameters	CON[a]	ROS[b]	BLE[c]	BCL[d]	BRC[e]	SEM[f]	p-value
Initial body weight (kg)	292	311	289	290	306	4.93	0.5030
Final body weight (kg)	343	346	356	361	377	5.16	0.2852
Average daily gain (kg/d)	0.70[b]	0.47[c]	0.91[ab]	0.97[a]	0.97[a]	0.04	0.0002
Dry matter intake (kg)	5.49[bc]	5.07[c]	6.25[a]	5.90[ab]	6.21[a]	0.13	0.0001
Dry matter intake (% Initial body weight)	1.74[b]	1.55[c]	1.95[a]	1.82[ab]	1.82[ab]	0.04	0.0014
Feed efficiency[g]	0.13[b]	0.09[c]	0.15[ab]	0.16[a]	0.16[a]	0.02	0.0001
Hot carcass weight (kg)	186	190	191	193	203	2.84	0.3732
Cold carcass weight (kg)	183	186	186	188	198	2.86	0.5443
Hot dressing carcass (%)	54.0	54.7	53.7	53.4	54.0	0.19	0.3172
Cold dressing carcass (%)	53.1	53.6	52.4	52.2	52.5	0.24	0.4109
Carcass dripping losses (%)	1.62	1.58	1.50	1.58	1.86	0.05	0.1917

[a] CON – Without essential oil.

[b] ROS – Rosemary essential oil (4 g/animal/d).

[c] BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

[d] BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

[e] BRC – Protected blend protected – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

[f] SEM: Standard error of mean.

[g] kg average daily gain/kg dry matter feed intake.

[abc] Values with different letters in the same row are different by Duncan test.

Table 4. Effect of diets with and without inclusion of essential oils on carcass characteristics of Nellore heifers finished in feedlot

	Diets						
Tissues, %	CON[a]	ROS[b]	BLE[c]	BCL[d]	BRC[e]	SEM[f]	p-value
Muscle	55.7	55.1	55.2	58.2	55.4	1.20	0.8066
Fat	24.8	24.8	25.8	21.8	24.6	1.26	0.5254
Bone	17.6	17.7	16.5	17.5	17.6	0.66	0.9708
Other	1.85	2.39	2.44	2.42	2.42	0.24	0.3921

[a] CON – Without essential oil.

[b] ROS – Rosemary essential oil (4 g/animal/d).

[c] BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

[d] BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

[e] BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

[f] SEM: Standard error of mean.

Table 5. Effect of diets with and without inclusion of essential oils on *in situ* digestibility (g/kg of DM)

	CON[a]	ROS[b]	BLE[c]	BCL[d]	BRC[e]	SEM[f]	p-value
Dry matter	0.65^{a}	0.62^{b}	0.64^{a}	0.66^{a}	0.65^{a}	0.35	0.0064
Crude protein	0.71	0.68	0.70	0.71	0.71	0.31	0.9723
Neutral detergent fiber	0.47^{b}	0.45^{c}	0.47^{b}	0.48^{a}	0.47^{b}	1.41	0.0001

[a] CON – Without essential oil.

[b] ROS – Rosemary essential oil (4 g/animal/d).

[c] BLE – Protected blend of eugenol + thymol+vanillin (4 g/animal/d).

[d] BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

[e] BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

[f] SEM: Standard error of mean.

[abc] Values with different letters in the same row statistically different by Duncan test.

Table 6. Effect of diets with and without inclusion of essential oils on ingestive behaviour activities of Nellore heifers finished in feedlot

Activities	CON[a]	ROS[b]	BLE[c]	BCL[d]	BRC[e]	SEM[f]	p-value
Drinking (No. visits)	4.00	3.06	2.75	4.43	2.25	0.35	0.2882
Feeding (No. visits)	17.3	22.2	19.1	24.3	22.4	1.24	0.4105
Rumination time (hours)	198^{b}	215^{b}	231^{ab}	216^{b}	274^{a}	8.89	0.0663
Idleness time (hours)	1135^{b}	1098^{ab}	1099^{ab}	1080^{ab}	1042^{a}	11.0	0.0999
FE_{DM} (kg DM/h)[g]	6.22	4.94	5.63	4.87	5.19	0.36	0.7685
FE_{NDF} (kg DM/h)[h]	1.57	1.25	1.42	1.23	1.31	0.09	0.7661
RE_{DM} (kg DM/h)[i]	2.40	2.05	2.51	2.50	1.94	0.10	0.2125
RE_{NDF} (kg DM/h)[j]	0.61	0.52	0.63	0.63	0.49	0.03	0.2083

[a] CON – Without essential oil.

[b] ROS – Rosemary essential oil (4 g/animal/d).

[c] BLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

[d] BCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

[e] BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d), clove essential oil (1.33 g/animal/d).

[f] SEM: Standard error of mean.

[g] FE_{DM}: Dry matter feeding efficiency.

[h] FE_{NDF}: Neutral detergent fiber feeding efficiency.

[i] RE_{DM}: Dry matter rumination efficiency.

[j] RE_{NDF}: Neutral detergent fiber rumination efficiency.

[ab] Values with different letters in the same row statistically different by Duncan test.

IV – Leaves of *Baccharis dracunculifolia* added in the diet of steers finished in feedlot, effect on performance and immune response

Short Title: Natural ingredients to finished feedlot steers

Journal: Animal Production Science

ABSTRACT

This study was carried out to investigate the effects that have the addition on the diet of Leaves of *Baccharis dracunculifolia in nature* on animal performance, feed intake, ingestive behavior activities, and blood parameters of steers finished in feedlot with high-grain diets. Nellore purebred steers (forty) were distributed in individual pens, equipped with automatic drinkers and masonry feeders. The steers were randomly assigned to one of four studied diets, therefore the TEST – no supplement ingredients; BAC05 – Leaves of *B. dracunculifolia in nature* (5 g animal^{-1} day^{-1}); BAC10 – Leaves of *B. dracunculifolia in nature* (10 g animal^{-1} day^{-1}); and BAC15 – Leaves of *B. dracunculifolia in nature* (15 g animal^{-1} day^{-1}). The Leaves of *B. dracunculifolia in nature* had a powder texture and were obtained of a single property through the manual collection of the leaves of the plant, which were processed to be offered to the animals. The use of plant *in nature* did not affect ($P \geq 0.47$) final body weight, average daily gain, dry matter intake, or feed efficiency. Neither on ingestive behavior activities ($P \geq 0.23$) and plasma concentrations of urea, creatine, aspartate aminotransferase, gamma glutamyl transferase, and creatine kinase ($P \geq 0.12$) no effects were detected between diets. The inclusion of plant *in nature* in steer's diet did not negatively impact performance and health. However, further field studies with beef cattle are needed for greater clarification of its effects and dosages.

Keywords: Beef cattle; High-grain diet; Ingestive behavior; Plasma metabolites

Summary

Leaves of *Baccharis dracunculifolia in natura* used as potential substitute for the antibiotics offered in the steers feed feedlot with the objective of fermentative modulators. The performance, ingestive behavior, and blood parameters were not affected by the inclusion of up to 15 grams of the *Baccharis*, proving that this product can be used in the beef cattle feed. But more studies are needed in the area to define the dosages and the true functioning of this plant in animal feed.

Introduction

The efficiency in the beef cattle production is considered a great challenge and has been target of innumerable research and discussions over the years, indicating the need to maximize production through the developed of the entire meat production chain. The finishing phase in feedlot of the animals, which was been studied in this work is one of the phases more important on the production cycle. This is an onerous phase due to the high costs of a quality feed, thus allowing the animals to express their full genetic potential. Thus, the use of new alternatives to increase the productivity of the Brazilian cattle herd has been studied more frequently, being the class of growth promoters in the highlighted (Ornaghi *et al.* 2017).

In the last decades, antibiotics were commonly administered in the diet of animals with the function of modulating the bacterial flora, but their use is undergoing some restrictions in European Union (OJEU 2003) and USA (US Food and Drug Administration 2015). Therefore alternative products that promote a satisfactory animals' performance without compromising the quality of the final product (meat) offered to beef consumers are being investigated.

In this situation many studies are being carried out in this area, searching for a natural substitute that meets such requirements. The use of plant extracts is an alternative to replace antibiotics (Benchaar *et al.* 2008; Yang *et al.* 2010; Cruz *et al.* 2014), besides acting as antimicrobials and antioxidants, benefiting the immune and digestive system of animals (Jayasena and Jo 2013; Ornaghi *et al.* 2017).

This includes the plant of *Baccharis dracunculifolia*, being a native plant from Brazil, commonly known as "Alecrim do campo". This extract is composed of aliphatic hydrocarbons, cyclic hydrocarbons, terpenes (baccharin), isopropenol, flavonoids (isosakuranetin, aromadendrin-4′-methyl ether) and phenolic acid (artepelin C, caffeic acid, *p*-coumaric acid, ferulic acid) (Kumazawa *et al.* 2003; Campos *et al.* 2016), however having the artepelin C as the principal compound (Veiga *et al.* 2017), besides presenting potential as antioxidants (Burdock 1998), classifying themselves as biological, antimicrobial, antioxidant and anti-inflammatory agents (Tiveron *et al.* 2016).

This study was carried out to evaluate the effects of the addition/inclusion of plant leaves of *Baccharis dracunculifolia in nature* on animal performance, feed intake, ingestive behavior activities, and blood parameters of steers finished in feedlot with high-grain diets.

Materials and Methods

Animals and experimental diet

Forty Nellore purebred steers with a mean initial body weight of 412.9 ± 22.0 kg were used in this study. Steers were distributed randomly in individual pens, with dimensions of 10 m^2 for each animal, partially covered and equipped with automatic drinkers and masonry feeders. The period of adaptation to the feedlot and concentrate diet was 14 days; afterwards, the experimental period was extended to 56 days until animals reached a mean FBW of 499.9 ± 25.6 kg. During the experimental period, Nellore steers were weighed monthly in order to record weight gain and productivity variables.

Steers were randomly assigned to one of four studied diets with ten steers per diet group. The diets tested were TEST – no supplement ingredients; BAC05 – Leaves of *B. dracunculifolia in nature* (5 g $animal^{-1}$ day^{-1}); BAC10 – Leaves of *B. dracunculifolia in nature* (10 g $animal^{-1}$ day^{-1}); and BAC15 – Leaves of *B. dracunculifolia in nature* (15 g $animal^{-1}$ day^{-1}). The plant included in the diet was made every 15 days, in order to calculate and adjust the dose by period depending on the intake of dry matter (DM)/d per animal. Preparation of diets was made with a pre-mix of plant *in nature* in the soybean meal and ground corn then led to the feed mixer together with other ingredients. The Leaves of *B. dracunculifolia in nature* had a powder texture and were obtained of a single property through the manual collection of the leaves of the plant, which were processed in a knife mill to be offered to the animals.

The four basal diets, consisting of pre-dried Tifton 85 hay, corn grain, and the leaves of *B. dracunculifolia* in nature was mixed with soybean meal, ground corn, yeast, mineral salt, and top-dressed daily into the morning feeding of respective treatments pens (1.60 kg of mixture/steers daily). Soybean meal was also top-dressed into the morning feeding of TEST pens (1.60 kg/steers daily), without the addition of the experimental ingredients. All diets were isonitrogenous, isoenergetics, and formulated to meet the requirements for a gain of 1.7 kg/d (NRC 2000) with adequate concentrations of nutrients for the growth and finishing of animals (Table 1).

Chemical analyses

The chemical compositions of ingredients and experimental diets were presented as g/kg of DM (Table 2). DM was estimated after oven drying at 65 °C for 24 h and milling through a 1-mm screen following method ID 934.01 (AOAC 2005). Ash content was measured by combustion at 550 °C for 16 h according to method ID 942.05 (AOAC 2005). Nitrogen concentration was determined by the Kjeldahl method (ID 988.05) (AOAC 2005). Following the determination of nitrogen concentration, crude protein was calculated by multiplying the nitrogen content by a factor of 6.25. Ether extract content was determined by method ID 920.39 (AOAC 2005). The neutral detergent fiber (NDF) content was measured according to the recommendations of Mertens (2002) using α-amylase and was expressed inclusive of residual ash. The acid detergent fiber was measured by using method ID 973.18 (AOAC 2005) and was expressed inclusive of residual ash. The non-nitrogen extract was obtained by equation according to (AOAC 2005). The digestible energy was calculated according to the recommended equations (NRC 2000). Total digestible nutrients (TDN) content of diets was obtained by the methodology descript by Kearl (1982), using the equation for pre-dried:

Hay = – 17.2649 + 1.2120 (% CP) + 0.8352 (% ENN) + 2.4637 (% EE) + 0.4475 (% CF).

Energetic foods = 40.2625 + 0.1969 (% CP) + 0.4228 (% ENN) + 1.1903 (% EE) + 0.1379 (% CF).

Protein foods = 40.3227 + 0.5398 (% CP) + 0.4448 (% ENN) + 1.4218 (% EE) – 0.7007 (% CF).

Animal performance

Diets were offered at 0800 and 1600 h every day. Feed intake was estimated as the difference between the feed supplied and refusals in the trough. Feed efficiency was calculated as the ratio between average daily gain and DM intake. To determine growth performance, animals were weighed at the beginning of the experiment and then every month (after fasting for 16 h), throughout the experiment. The average daily gain was calculated as the total BW gain divided by the length of the experimental period (56 days).

When the Nellore steers reached a mean final body weight of 499.9 ± 25.6 kg, they were slaughtered in a commercial slaughterhouse 153 km from the Iguatemi Experimental Farm. Animal transport was carried out in the late afternoon to minimize stress. Upon arrival the at the slaughterhouse, animals were kept in resting pens and were subsequently stunned using a penetrating captive bolt pistol as per Brazilian federal inspection regulations according to the Brazilian RIISPOA – Regulation of Industrial and Sanitary Inspection of Animal Products.

Ingestive behavior activities

Data relative to ingestive behavior of steers were obtained between the 7^{th} and 8^{th} weeks of feedlot. The record of the quantitative data on the basic behavioral patterns was according to Silva *et al.* (2005), through visual observation of the animals every 5 min during 1 minute performed by a trained team during 12 uninterrupted hours. A spreadsheet was used to organize the records collected chronologically regarding the duration of feeding and drinking by number of action observation times. For ruminating and idle periods, the total time spent on each activity was determined by the sum of the repetitions.

Blood analyses

Blood samples were evaluated every 18 days for a total of three individual collection per animal in the vacutioner® tube, and maintained at temperature of 25 ^{0}C with the mean for facilitating the coagulation, and then were performed the serum separation by centrifugation (Centrifuge, Rotina 420-R, Tuttlingen, Germany), being used a speed of 3000 rpm/15 min. The evaluation of parameters like urea and creatine were performed according Vasconcelos *et al.*

(2007). The activities of muscle injury indicative enzymes aspartate aminotransferase (AST) and creatine kinase (CK) were measured in spectrophotometer (Spectrophotometer UV-Vis-Evolution 200, Massachusetts, United State of America) by means of commercial enzymatic dosage kits (Bioclin, Belo Horizonte, Brazil) according the manufacturer's instructions. The gamma glutamyl transferase (GGT) was performed using Roche assay reagents in the Roche 900 series automated clinical chemical analyzer (Roche Diagnostics, Indiana, United State of America).

Statistical Analyses

The experimental design was completely randomized with four treatments and ten replications. The results were statistically interpreted using regression equations performed in SAS (2004) (PROC REG):

$$Yijk = \beta 0 + \beta 1Xi + \beta 2\, Xi^2 + \alpha ijk + \varepsilon ijk$$

where: *Yijk*, *β0*, *Xijk*, *αijk*, and *εijk* are Dependent variables (plant levels); Regression coefficient; Independent variables; Regression deviations; and Residual error, respectively.

Results

The inclusion of up to 15 grams per animal/day of the leaves of *Baccharis dracunculifolia in nature* in the steers' diets finished in the feedlot did not affect ($P \geq 0.47$) final body weight, average daily gain, dry matter intake, and feed efficiency (Table 3). No treatment effects were detected ($P \geq 0.23$) for ingestive behavior activities during the 12 uninterrupted hours for activities drinking, feeding, rumination, idleness (Table 4). No treatment effects were detected ($P \geq 0.12$; Table 5) for plasma concentrations of urea, creatine, aspartate aminotransferase, gamma glutamyl transferase, and creatine kinase.

Discussion

The finished period, especially feedlot is a term in which beef cattle need a contribution against diseases, metabolic disorders, and ruminal digestion as fermentative modulators (Russell and Strobel 1989). The *B. dracunculifolia* has great importance in Brazilian botany (Bankova *et al.* 1999; Da Silva Filho *et al.* 2004; Campos *et al.* 2016), because of their antibacterial effect (Silva Filho *et al.* 2008; Veiga *et al.* 2017) this same effect is found both in the plant *in nature* and in the propolis and / or as it can be called "green propolis" that is produced by bees that use the nectar of the plant flowers.

The studies reported by other authors (Zawadzki *et al.* 2011; Valero *et al.* 2014) prove that the use of propolis in beef cattle diets can improve the average daily gain. This improvement is due to the efficiency of use of nutrients in the rumen, as the decrease of the losses coming from the methane gas (Callaway *et al.* 2003). However the results from the current experiment did not report an improvement on average daily gain variables with the addition/inclusion of the leaves of *B. dracunculifolia*.

According to laboratory works performed by Búfalo *et al.* (2009); Massignani *et al.* (2009); Parreira *et al.* (2010); Guimarães *et al.* (2012), using *B. dracunculifolia in nature* as substrate prove *in vitro* the antiviral, antiprotozoal, antioxidant, and antibacterial power; in particular the antibacterial effect is related to the greater sensitivity of the gram positive bacteria to the action mechanisms of this extract, corroborating with the results found by Zawadzki *et al.* (2011); Valero *et al.* (2014) on natural propolis.

Even without the differentiation between the treatments from animals that received or not the supplementation with the plant extracts, the average daily gain can be considered satisfactory for feedlot animals fed a high grain diet on Nellore breed (Maggioni *et al.* 2009; Françozo *et al.* 2013). The final body weight, dry matter intake, and feed efficiency had similar results between treatment animals throughout the experimental period (Table 3). The plant *in nature* presents high levels of flavonoids and phenol (Kumazawa *et al.* 2003; Paula *et al.* 2017), consequently, these concentrations in steers’ diet negatively influenced ruminal dynamics, justifying the lack of effect detection for feed efficiency.

Ingestive behavior activities (feeding, drinking, ruminating, and idle; Table 4) were similar among the treatments that received the vegetal extract or not, this resemblance is possibly explained due to the similarity between the feedlot pens, as well as the basal diet offered to the animals. Corroborating with these results another works carried out under similar conditions and with inclusion of natural additives (essential oils) as those from our research group (Ornaghi *et al.* 2017) who did not detect either effect for ingestive behavior on young bulls receiving a high concentrated diet.

Results for feeding and ruminating are in according with Missio *et al.* (2010); Eiras *et al.* (2013); Ornaghi *et al.* (2017) who also evaluated ingetsive behavior on beef cattle feedlot supplemented with a high concentrated diet. The low levels for these activities are understood due to the greater energetic support that this type of diet provides, thus the animals reach their nutritional requirements and cease their consumption. According Van Soest *et al.* (1994), a diet with a higher percentage of forage increase the time used for rumination, that is, high concentrate diet due to the size of its particles may have reduced the rumination capacity of the present study steers. Another factor that may have compromised the rumination rate are the high levels of phenolic substances found in *B. dracunculifolia* (Park *et al.* 2002; Tiveron *et al.* 2016), which adversely affected the use of feed by ruminal bacteria.

The observation of the beef cattle behavior from feedlot present a great importance, to guarantee the maximum production of the animals without taking unnecessary management, avoiding more intense periods used by the animals in the use of feed intake. In addition, the feed offer to animals at shorter intervals of time is aimed at improving the nutrients absorption (Ítavo *et al.* 2011).

Stresses being by transport, dehydration, or nutrient-poor diets have an effect on metabolism, through changes in plasma concentrations of urea, total protein, and creatine kinase (Tarrant *et al.* 1992; Earley and O'Riordan 2006; Buckham Sporer *et al.* 2008). Corroborating with this affirmation Bershauer *et al.* (1983) report that with increasing feed intake there is a decrease in blood urea concentrations. In the present study the results for urea, creatinine, and creatine kinase were above what is classified as a reference for cattle (Table 5). Therefore, these results indicate that the steers did not suffer any type of metabolic alteration during the experimental period, but in the period before the experiment.

The plasma blood concentration of steers fed *B. dracunculifolia* for aspartate aminotransferase and gamma glutamyl transferase were higher according the results found by Gandra *et al.* (2012). The possible explanation for this small difference is the forage: concentrate ratio of the diet, since high concentrate diets can induce liver injury (Mori *et al.* 2007). Therefore, with the inclusion of the plant extract, no clinical alterations were observed due to infectious, neurological or metabolic diseases, which could negatively influence the performance and health of the steers.

Conclusion

The inclusion up to 15 g animal^{-1} day^{-1} of leaves of *Baccharis dracunculifolia in nature* do not affect animal performance, ingestive animal behavior, and blood plasma parameters on finished steers in feedlot. The results from this study suggest that the use of this plant in the diet of steers does not cause any feed injury

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Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Ethics statement

This experiment was approved by the committee for ethics in the use of animals (CEUA) of the Universidade Estadual de Maringá, following protocol 3624120116.

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Table 1 Ingredient composition of total mixed ration offered during the experiment[1]

Item	**TEST**	**BAC05**	**BAC10**	**BAC15**
Ingredients (g/ kg of DM)				
Pre-dried hay	150	150	150	150
Corn grain	710	710	710	710
Soybean meal	51.0	51.0	51.0	51.0
Ground corn	85.0	85.0	85.0	85.0
Yeast	0.40	0.40	0.40	0.40
Mineral mix[2]	3.20	3.20	3.20	3.20
Baccharis dracunculifolia	-	0.05	0.10	0.15

[1]TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g animal^{-1} day^{-1}); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal^{-1} day^{-1}); and BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal^{-1} day^{-1}).

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

850 **Table 2** Nutrient profile of total mixed ration offered during the experiment

Item	DM	Ash	CP	EE	NDF	ADF	NNE	TDN	DE
Ingredients (g/ kg of DM)									
Pre-dried hay	337	73.1	155	18.1	828	358	461	581	25.6
Corn grain	853	16.4	96.1	47.1	175	45.8	845	635	2.80
Ground corn	875	15.7	90.7	39.8	136	43.6	838	857	37.8
Soybean meal	850	67.0	489	19.0	159	87.8	450	822	36.2
Yeast	920	46.1	331	21.0	26.0	9.22	-	-	-
Mineral salt	986	945	-	-	-	-	-	-	-
Baccharis dracunculifolia	539	61.3	130	22.3	533	356	602	655	28.9
Diet	776	32.1	138	39.8	269	95.9	750	651	10.8

Table 3 Performance parameters of steers supplemented or not with plant *in nature* during the feedlot finishing period[1]

						P-value	
Parameters	**TEST**	**BAC05**	**BAC10**	**BAC15**	**SEM**	**L**	**Q**
Initial body weight (kg)	415	415	413	409	3.89	0.61	0.85
Final body weight (kg)	499	499	506	496	4.56	0.97	0.86
Average daily gain (kg/d)	1.50	1.50	1.66	1.55	0.05	0.53	0.72
Dry matter intake (kg)	9.13	9.29	9.16	9.09	0.13	0.84	0.89
Feed efficiency[2]	0.17	0.16	0.18	0.17	0.01	0.47	0.75

[1]TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g animal^{-1} day^{-1}); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal^{-1} day^{-1}); and BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal^{-1} day^{-1}).

[2]kg average daily gain/kg dry matter feed intake.

Table 4 Ingestive behavior activities parameters of steers supplemented or not with plant *in nature* during the feedlot finishing period[1]

						P-value	
Activities	**TEST**	**BAC05**	**BAC10**	**BAC15**	**SEM**	**L**	**Q**
Drinking (No. visits)	3	3	4	3	0.36	0.85	0.95
Feeding (No. visits)	21	22	22	24	1.02	0.23	0.48
Rumination time (hours)	100	109	70.0	112	8.02	0.97	0.61
Idleness time (hours)	500	486	520	471	9.27	0.51	0.52

[1]TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g animal^{-1} day^{-1}); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal^{-1} day^{-1}); and BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal^{-1} day^{-1}).

Table 5 Concentrations of plasma urea (mg/dL), creatine (mg/dL), aspartate aminotransferase (U/L), gamma glutamyl transferase (U/L), creatine kinase (U/L) in steers supplemented or not with plant *in nature* during the feedlot finishing period[1]

						***P*-value**	
Parameters	**TEST**	**BAC05**	**BAC10**	**BAC15**	**SEM**	**L**	**Q**
Urea	38.5	40.0	37.0	38.0	1.26	0.47	0.62
Creatine	2.79	2.99	2.77	3.29	0.09	0.13	0.23
Aspartate aminotransferase	186	197	174	181	6.11	0.49	0.79
Gamma glutamyl transferase	44.5	46.2	43.7	47.8	1.94	0.12	0.22
Creatine kinase	334	384	429	430	24.8	0.14	0.31

[1]TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g animal^{-1} day^{-1}); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal^{-1} day^{-1}); and BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal^{-1} day^{-1}).

V – Performance, health, and physiological responses of newly-weaned feedlot cattle supplemented with feed-grade antibiotics or alternative feed ingredients

Short Title: Alternative feed ingredients to feedlot cattle

Journal: Animal Journal

ABSTRACT: With increased regulations regarding the use of feed-grade antimicrobials in livestock systems, alternative strategies to enhance growth and immunity of feedlot cattle are warranted. Hence, this experiment compared performance, health, and physiological responses of cattle supplemented with feed-grade antibiotics or alternative feed ingredients during the initial 60 days in the feedlot. Angus × Hereford calves (63 steers + 42 heifers) originating from 2 cow-calf ranches were weaned on day -3, obtained from an auction yard on day -2 and road-transported (800 km; 12-h) to the feedlot. Upon arrival on day -1, shrunk BW was recorded. On day 0, calves were ranked by sex, source, and shrunk BW, and allocated to 1 of 21 pens. Pens were assigned to receive (7 pens/treatment) a free-choice total mixed ration containing: 1) lasalocid (360 mg/calf daily of Bovatec; Zoetis, Florham Park, NJ, USA) + chlortetracycline (350 mg/calf of Aureomycin at cycles of 5-day inclusion and 2-day removal from diet; Zoetis) from day 0 to 32, and monensin only (360 mg/calf daily of Rumensin; Elanco Animal Health, Greenfield, IN, USA) from day 33 to 60 (**PC**), 2) sodium saccharin-based sweetener (Sucram at 0.04 g/kg of diet DM; Pancosma SA; Geneva, Switzerland) + plant extracts containing eugenol, cinnamaldehyde, and capsicum (800 mg/calf daily of

XTRACT Ruminants 7065; Pancosma SA) from day 0 to 32, and XTRACT only (800 mg/calf daily) from day33 to 60 (**EG**), or 3) no supplemental ingredients (**CON**; day 0 to 60). Calves were assessed for bovine respiratory disease (**BRD**) signs and DMI was recorded from each pen daily. Calves were vaccinated against BRD pathogens on day 0 and 22. Shrunk BW was recorded on day 61, and blood samples collected on days 0, 6, 11, 22, 33, 43, and 60. Calf ADG was greater (P = 0.04) in PC vs. EG and tended (P = 0.09) to be greater in PC vs. CON. Feed efficiency also tended (P = 0.09) to be greater in PC vs. CON, although main treatment effect for this response was not significant (P = 0.23). Mean serum titers against *bovine respiratory syncytial virus* were greater in EG vs. PC (P = 0.04) and CON (tendency; P = 0.08). Collectively, inclusion of alternative feed ingredients prevented the decrease in feed efficiency when chlortetracycline and ionophores were not added to the initial feedlot diet, and improved antibody response to vaccination against *bovine respiratory syncytial virus*in newly-weaned cattle.

Key Words: beef cattle; growth; immunity; nutrition

Implications

Supplementing newly-weaned cattle with feed-grade antibiotics (chlortetracycline and ionophores) during the initial 60 days in the feedlot tended to improve average daily gain and feed efficiency compared with non-supplemented cattle. Replacing these feed-grade antibiotics with plant extracts (eugenol, cinnamaldehyde, and capsicum) and sodium saccharin-based sweetener prevented the decrease in feed efficiency when feed-grade antibiotics were not added to the diet. Cattle supplemented with these alternative ingredients also had improved humoral response to vaccination against *bovine respiratory syncytial virus*. Hence, plant extracts and sodium saccharin-based sweetener may replace feed-grade antibiotics without substantially impairing feed efficiency of newly-weaned feedlot cattle.

Introduction

Feedlot receiving is one of the most critical phases within the beef production cycle, comprising the initial 8 weeks in the feedlot when cattle are exposed to several stress and health challenges that impact their welfare and productivity (Duff and Galyean, 2007). These include weaning, road transport, commingling with different animals, and exposure to novel diets and environments (Cooke, 2017). Feed intake is often inadequate during the receiving period because of these stressors, which further impairs cattle growth and immunocompetence (Lippolis *et al.*, 2017). Accordingly, incidence of bovine respiratory disease (**BRD**) is elevated during feedlot receiving, despite vaccination against BRD pathogens and efforts to minimize stress (Snowder *et al.*, 2006).

Prophylactic medication with feed-grade antimicrobials, including ionophores and chlortetracycline, is often effective in mitigating incidence of BRD and other health syndromes during feedlot receiving (Duff and Galyean, 2007; Wilson *et al.*, 2017). With increased regulations regarding the use of feed-grade antimicrobials in livestock systems (US Food and Drug Administration, 2015), alternative dietary strategies that enhance immunity of receiving cattle are warranted. These include the use of sodium saccharin-based sweetener in feedlot receiving diets to increase cattle DMI (Ponce *et al.*, 2014). Another strategy is supplementing plant extracts containing organic compounds known to enhance rumen function and immunity in cattle, such as cinnamaldehyde, eugenol, and capsicum oleoresin (Yang *et al.*, 2010a; Yang *et al.*, 2010b; Ayrle *et al.*, 2016). Based on this information, we hypothesized that plant extracts and sodium saccharin-based sweetener are alternatives to feed-grade antimicrobials in enhancing cattle immunocompetence and productivity during feedlot receiving. Hence, this experiment compared performance, health, and physiological responses of newly-weaned cattle supplemented with feed-grade antibiotics, the aforementioned alternative ingredients, or without such supplements during a 60-day feedlot receiving period.

Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns, OR, USA) from April to June, 2017. During the experiment, environmental temperature ranged from 35 to -5°C, with an average of 12°C and 54% humidity, and 16 mm of total precipitation as rain. For all management procedures that required cattle to be restrained, a Silencer Chute (Moly Manufacturing, Lorraine, KS, USA) mounted on Avery Weigh-Tronix load cells (Fairmount, MN, USA; readability 0.45 kg) was utilized.

Animals and treatments

One hundred and five Angus x Hereford calves (63 steers and 42 heifers) were purchased from a commercial auction yard (Producers Livestock Marketing Association; Vale, OR, USA) and utilized in this experiment (day-2 to 61). Calves originated from 2 cow-calf operations (eastern Oregon and western Idaho, USA) and weaned on day -3, loaded into a double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC., Purcell, OK, USA) at the auction yard (day -2; 1800 h),and transported for 800 km. During transport, the driver stopped once after 6 h of driving to rest for 60 min, whereas total transport time was 12 h. Calves remained in the truck throughout the 12-h transportation period. Minimum, maximum, and average environmental temperatures during transport were -3, 8, and 3°C, respectively, whereas average humidity was 70% and no precipitation was observed. Transportation length and distance were selected to simulate the stress of along-haul that beef cattle originated from western or southeastern U. S. cow-calf operations experience when transferred to feedlots in the midwestern U. S. (Cooke *et al.*, 2013).

On day -1, calves were unloaded (0600 h) at the Eastern Oregon Agricultural Research Center, immediately weighed (initial shrunk BW = 197 ± 3 kg), and maintained in a single paddock (160 × 100 m) with *ad libitum* access to alfalfa-grass hay, water, and a commercial mineral mix (described in Table 1) for 24 h. On day 0, calves were ranked according to sex, source and shrunk BW, and allocated to 1 of 21 drylot pens (7×15 m; 3 steers and 2 heifers per pen) in a manner that pens had equivalent initial shrunk BW and calves from both sources to stimulate the stress of comingling (Step *et al.*, 2008). Pens were assigned to receive 1 of 3 treatments: 1) lasalocid (360 mg/calf daily of Bovatec; Zoetis, Florham Park, NJ, USA) + chlortetracycline (350 mg/calf of Aureomycin at cycles of 5-day inclusion and 2-day removal from diet; Zoetis) from day 0 to 32, and monensin only (360 mg/calf daily of Rumensin; Elanco Animal Health, Greenfield, IN, USA) from day 33 to 60 (**PC**; n = 7), 2) sodium saccharin-based sweetener (Sucram at 0.04 g/kg of diet DM; Pancosma SA; Geneva, Switzerland) + plant extracts containing eugenol, cinnamaldehyde, and capsicum (800 mg/calf daily of XTRACT Ruminants 7065; Pancosma SA) from day 0 to 32, and XTRACT only (800 mg/calf daily) from day 33 to 60 (**EG**; n = 7), or 3) no supplemental ingredients (**CON**; n = 7). The inclusion and administration rate of the PC and EG ingredients were according to manufacturer's recommendations for growing cattle. Ionophores and chlortetracycline were chosen based on traditional U.S. feedlot practices (Samuelson *et al.*, 2016). Chlortetracycline was supplemented from day 0 to 32 when elevated BRD incidence was expected (Snowder *et al.*, 2006; Lippolis *et al.*, 2017), whereas lasalocid was used during this period because it is approved for use in combination with chlortetracycline (US Food and Drug Administration, 2017). In turn, monensin was supplemented from day 33 to 60 when chlortetracycline supplementation ended, given that monensin is the primary ionophore used by U.S. commercial feedlots (Samuelson *et al.*, 2016). Sucram was supplemented from day 0 to 32 to stimulate DMI upon feedlot arrival (McMeniman *et al.*, 2006), whereas XTRACT was supplemented throughout the receiving period as an immunostimulant and dietary alternative to ionophores (Yang *et al.*, 2010a; Yang *et al.*, 2010b).

From day 0 to 60, calves had free-choice access to water and total mixed ration (**TMR**; Table 1), which was offered twice daily (0800 and 1300 h). Sucram was mixed daily in 4 L of water, whereas 2 L were mixed with the morning and 2 L with the afternoon TMR allocation of each EG pen (day 0 to 32). The PC and CON pens received the same amount of water without the addition of Sucram (day 0 to 32). Lasalocid, chlortetracycline, monensin, and XTRACT were mixed with soybean meal and top-dressed daily into the morning TMR feeding of respective PC or EG pens during the supplementation period (0.25 kg of mixture/calf daily). Moreover, chlortetracycline was supplemented to PC calves on day 0 to 4, day 7 to 11, day 14 to 18, day 21 to 25, and day 28 to 32. Soybean meal was also top-dressed into the morning TMR feeding of CON pens (0.25 kg/calf daily) from day 0 to 61, without the addition of the experimental ingredients. Based on daily visual observations, all pens consumed the top-dress within 5 min after feeding.

On day 0, calves were vaccinated against *Clostridium* and *Mannheimia haemolytica* (One Shot Ultra 7; Zoetis), *bovine respiratory syncytial virus* (**BRSV**), *bovine herpesvirus-1* (**BHV-1**), *bovine viral diarrhea virus* (**BVD**) *1* and 2, and *parainfluenza-3 virus* (**PI3;** Bovi-Shield Gold 5; Zoetis), and were administered an anthelmintic (Dectomax; Zoetis). On day22, calves were re-vaccinated against *Clostridium* (Ultrabac 8; Zoetis), BRSV, BHV-1, BVD 1 and 2, and PI3 (Bovi-Shield Gold 5; Zoetis), following the manufacturer's recommendation for revaccination against these pathogens (Zoetis).

Sampling

Samples of TMR ingredients were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA). All samples were analyzed by wet chemistry procedures for concentrations of CP, ADF, and NDF as described by Lippolis *et al*. (2017). Calculations for net energy for maintenance and gain were calculated with the equations proposed by the NRC (2000). Nutrient profile of TMR is described in Table 1.

Full BW was recorded on days0, 5, 11, 22, 33, 43, and 60 of the experiment at 0700 h, prior to the first TMR feeding of the day. Shrunk BW was recorded on day 61, after 16 h of water and feed withdrawal. Shrunk BW values from days -1 and 61 were used to calculate calf ADG during the experiment. Intake of TMR (DM basis) was evaluated daily from day 0 to 60 from each pen by collecting and weighing offered and non-consumed TMR. All samples were dried for 96 h at 50°C in forced-air ovens for DM calculation. Total TMR intake of each pen was divided by the number of calves within each pen, and expressed as kg per calf/day. Total BW gain and TMR intake of each pen were used for feed efficiency calculation. Calves were observed daily for BRD signs according to the DART system (Zoetis), and received antimicrobial treatment as in Lippolis *et al.* (2017).

Blood samples were collected from all calves, concurrently with full BW evaluation into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing no additive or containing freeze-dried sodium heparin for serum and plasma collection, respectively. During each sampling day, approximately 16 mL of blood was collected from each calf, being 8 mL in each collection tube. After collection, all blood samples were placed immediately on ice, centrifuged (2 500 × g for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection.

Laboratorial analyses.

Plasma samples collected from day 0 to 33 were analyzed for cortisol (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) and haptoglobin concentrations (Cooke and Arthington, 2013), given that adrenocortical and acute-phase protein responses return to baseline levels in receiving cattle within 4 wk after feedlot entry (Cooke, 2017). Plasma samples collected on days 0, 33 and 60 were analyzed for IGF-I concentrations (Immulite 1000; Siemens Medical Solutions Diagnostics) to metabolically assess calf nutritional status throughout the experimental period (Lippolis et al., 2017). The intra- and inter-assay CV for haptoglobin were, respectively, 2.4 and 10.8%. Plasma IGF-I and cortisol were analyzed within single assays, and the intra-assay CV were, respectively, 7.4 and 1.9%.

Serum samples collected from 2 calves/pen not observed with BRD signs during the experiment were selected for analysis of antibody titers against BRD pathogens, to ensure that this response was associated with vaccine efficacy rather than pathogenic infection (Callan, 2001). More specifically, samples collected on days 0, 5, 11, 22, 33, and 43 were analyzed for antibody titers against BRSV,BHV-1, BVD-1, and PI3 using virus neutralization tests, and for antibodies against *M. haemolytica* via a quantitative agglutination test (Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX, USA). It is not certain if selected calves were indeed healthy or just asymptomatic to

BRD, although none of them exhibited BRD signs and clinical symptoms throughout the experimental period as mentioned previously.

Statistical analysis.

Pen was considered the experimental unit for all analyses. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.) with a binomial distribution and logit link function. All data were analyzed using Satterthwaite approximation to determine the denominator df for tests of fixed effects, with pen (treatment) and calf (pen) as random variables, but for DMI and feed efficiency that used pen (treatment) as random variable. Model statements for initial and final BW, ADG, feed efficiency, and morbidity-related results contained the effects of treatment and calf sex as independent covariate. Model statements for DMI, cumulative BRD incidence, full BW change, and blood variables contained the effects of treatment, day, the resultant interaction, and calf sex as independent covariate. Blood variables were analyzed using results from day 0 as independent covariate, whereas calf source was also included as independent covariate for antibody titers against BRD pathogens. The specified term for all repeated statements was day, with pen (treatment) as subject for DMI and calf (pen) as subject for all other analyses. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. All results are reported as covariately-adjusted least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Repeated measures are reported according to main treatment effect if the treatment × day interaction was $P > 0.10$.

Results

A tendency for a treatment effect was detected ($P = 0.10$) for ADG, which was greater ($P = 0.04$) in PC vs. EG calves, and tended ($P = 0.09$) to be greater in PC vs. CON calves (Table 2). However, no main treatment effects were detected ($P \geq 0.55$) for final shrunk BW (day 61; Table 2) or full BW during the 60-day receiving period (Figure 1). No treatment effects were detected for DMI ($P = 0.52$; Table 2 and Figure 2) and feed efficiency ($P = 0.23$; Table 2). Despite the lack of main treatment effect for feed efficiency, it should be noted that this response tended to be greater ($P = 0.09$) in PC vs. CON calves, and did not differ ($P \geq 0.38$) in EG vs. PC and CON calves.

No treatment differences were detected ($P = 0.94$) for BRD incidence (Table 3), which were only observed during the initial 15 days of feedlot receiving (Figure 2; day effect, $P < 0.01$). No treatment differences were detected ($P \geq 0.39$; Table 3) for other morbidity reasons (physical injury), number of antimicrobial treatments required upon BRD diagnosis, and percentage of cattle that required ≥ 1 antimicrobial treatment upon BRD diagnosis. No incidence of mortality was observed during the experiment.

No treatment effects were detected ($P \geq 0.56$) for plasma concentrations of cortisol, haptoglobin, and IGF-I (Table 4), whereas day effects were detected ($P \leq 0.01$) for these variables (Table 5). No treatment effects were detected ($P \geq 0.35$) for serum titers against *M. haemolytica*, PI3, BVD-1, and BHV-1 (Table 4). A tendency for a treatment effect was detected ($P = 0.09$) for serum titers against BRSV, which was greater (P = 0.04) in EG vs. PC and tended to be greater (P = 0.08) in EG vs. CON calves, and were similar (P = 0.80) between CON and PC calves. Moreover, day effects were also detected ($P \leq 0.01$) for all serum titers against BRD pathogens (Table 5).

Discussion

Calves utilized in this experiment were considered high-risk, given that their prior management and health history were not fully known (Wilson *et al.*, 2017). Moreover, cattle experienced the stress of weaning, auction, transportation, commingling, vaccination, and feedlot entry within a 72-h period, whereas the combination of these stressors impacts cattle immunocompetence and performance (Cooke, 2017). Hence, the experimental model adopted herein represented the stress and health challenges that commercial feeder cattle typically experience during feedlot receiving in the U.S. (Duff and Galyean, 2007).

Inclusion of chlortetracycline and ionophores in the receiving diet tended to improve cattle ADGas previously reported by others (Perry *et al.*, 1986; Duffield *et al.*, 2012), although such difference was not sufficient to impact final BW on day 61 (Table 2; Figure 1). This outcome should be primarily attributed to the tendency for improved feed efficiency in PC vs. CON cattle (Perry *et al.*, 1986; Birkelo, 2003), given that DMI, BRD incidence, as well as physiological responses were similar among all treatment groups (Table 2, 3, and 4). Accordingly, ionophores and chlortetracycline have been show to increase feed efficiency in cattle by, respectively, improving rumen fermentation efficiency (Russell and Strobel, 1989; Callaway *et al.*, 2003), and increasing nutrient supply due to reduced intestinal mass and energy loss as methane (Visek *et al.*, 1978;

Zinn, 1993; Baldwin *et al.*, 2000). It also should be noted that DMI in the present experiment was not depressed by ionophore inclusion (Table 2; Figure 1), either lasalocid or monensin according to the experimental schedule, despite previous research reporting such outcome in feedlot cattle (Zinn, 1987; Duff *et al.*, 1995; Duff and Galyean, 2007).

Cattle DMI and ADG were not improved by inclusion of sodium saccharin-based sweetener and plant extracts (Table 2; Figure 1). Contrary to our findings, Ponce *et al.* (2014) reported that supplementing the same sweetener utilized herein increased DMI by 17% during feedlot receiving, although this effect was observed when sweetener was included at 200 g/ton of diet DM. McMeniman *et al.* (2006) also included sodium saccharin-based sweetener into a feedlot receiving diet at 200 g/ton of diet DM, and reported a trend in increased DMI from day 29 to 56 after feedlot arrival. Yet, both authors also reported that ADG and feed efficiency were not impacted by inclusion of sodium saccharin-based sweetener. Perhaps DMI was not improved in EG cattle herein because the sweetener was included at a different dose than Ponce *et al.* (2014) and McMeniman *et al.* (2006), and was only fed from day 0 to 32 when receiving DMI is inconsistent and often inadequate (Duff and Galyean, 2007). Others have also reported that supplementing plant extracts also failed to improve ADG in ruminants consuming concentrate-based diets (Yang *et al.*, 2010a; Geraci *et al.*, 2012). It should be noted that the EG treatment prevented the decrease in feed efficiency when chlortetracycline and ionophores were not included into the diet, based on similar feed efficiency between EG vs. PC and tendency for greater feed efficiency in PC vs. CON calves (Table 2). Plant-derived organic compounds such as eugenol, cinnamaldehyde, and capsicum appear to enhance rumen fermentation in cattle consuming high-concentrate diets (Cardozo *et al.*, 2005; Cardozo *et al.*, 2006), and may be used as alternatives to ionophores such as monensin (Fandiño *et al.*, 2008). Accordingly, Geraci *et al.* (2012) reported similar feed efficiency in feedlot cattle supplemented with monensin or a mixture of eugenol, cinnamaldehyde, and capsicum during an 84-day feeding period. In contrast, no research has compared the effects of plant extracts and/or sodium saccharin-based sweetener with chlortetracycline in feedlot receiving diets to further debate the performance results reported herein.

Morbidity and BRD-related responses were similar among treatments (Table 3; Figure 2), which does not support our experimental hypothesis and the use of chlortetracycline supplementation to reduce morbidity during feedlot receiving (Duff *et al.*, 2000; Edwards, 2010; Samuelson, 2016). Others have also reported that dietary inclusion of sodium saccharin-based sweetener failed to mitigate BRD incidence in receiving cattle (McMeniman *et al.*, 2006; Ponce *et al.*, 2014), whereas research investigating the effects of plant extracts on BRD is lacking. It should be noted, however, that BRD incidence observed herein were not as elevated compared with previous research from our group (Lippolis *et al.*, 2017), as well as research conducted at commercial receiving yards reporting up to 43% of BRD incidence (Snowder *et al.*, 2006). In fact, Lippolis *et al.* (2017) commingled cattle obtained from 7 cow-calf operations, which is typical of U.S. commercial feed yards, and reported BRD incidence at 66% during an 80-day receiving period. In this experiment, cattle were obtained from 2 different sources due to market availability, which likely reduced commingling-elicited stress (Step *et al.*, 2008) and resulted less BRD incidence compared with previous research (Snowder *et al.*, 2006; Lippolis *et al.*, 2017). Hence, the reduced prevalence of BRD in the present experiment may have hindered proper assessment of feedlot receiving morbidity, and contributed to the lack of treatment effects on BRD-related responses.

Supplementing sodium saccharin-based sweetener and plant extracts improved acquired humoral immunity against BRSV during the 60-day receiving period (Table 4). Serum antibody titers against all other BRD pathogens were not impacted by treatments but increased during the experiment (Table 5), denoting that cattle effectively acquired humoral immunity against these pathogens upon vaccination (Richeson *et al.*, 2008). It should be noted that calves were not revaccinated against *M. haemolytica* based on recommendations by the manufacturer (Zoetis), explaining why concentrations of serum titers against this pathogen did not increase beyond day 22. The exact mechanisms by which the EG treatment improved efficacy to BRSV vaccination (Callan, 2001) warrants investigation, and could be attributed to potential immunomodulatory effects of plant extracts (Ayrle *et al.*, 2016). Yet, such outcome was also not sufficient to alter BRD incidence in the present experiment, which corroborates with the lack of treatment differences on acquired humoral immunity against *M. haemolytica*, parainfluenza-3 virus, BHV-1, BVD-1, and PI3.

Similar concentrations of plasma cortisol, haptoglobin, and IGF-I among PC, CON, and EG cattle (Table 4) indicate that none of the experimental treatments modulated the physiological and acute-phase responses typically associated with feedlot receiving (Cooke, 2017). In turn, the specific impact of ingredients evaluated herein on these plasma variables are either variable or mostly undetermined. As examples, monensin has either increased or failed to change circulating IGF-I concentrations in growing beef cattle (Vendramini *et al.*, 2015; Vendramini *et al.*, 2016). Supplementing cinnamaldehyde to feedlot steers did not impact serum haptoglobin concentrations, but reduced concentrations of the acute-phase protein serum amyloid A (Yang *et al.*, 2010a). Nonetheless, day effects reported for plasma variables (Table 5) corroborate that cattle were exposed to the stress and nutritional changes associated with feedlot entry. Plasma haptoglobin concentrations transiently increased across all treatments upon feedlot arrival, corroborating that calves experienced an acute-phase protein response elicited by weaning, transport, vaccination, and feedlot entry (Cooke, 2017). Plasma IGF-I concentrations increased across all treatments during feedlot receiving, mainly due to increased nutrient intake (Table 1) and growth (Table 2) during the experimental period (Lippolis *et al.*, 2017). Plasma cortisol concentration also increased across all treatments as the experiment progressed; the exact reason for this outcome is unknown, but may be associated with increasing concentrate inclusion in the TMR (Enemark, 2008). Hence, the tendencies for improved ADG and feed efficiency in PC calves were not associated with altered cortisol, IGF-I, and acute-phase responses elicited by transport and feedlot entry, although these responses influence nutrient utilization and growth in beef cattle (Cooke, 2017). Collectively, plasma variables evaluated herein failed to elucidate biological mechanisms by which chlortetracycline and ionophore supplementation benefited performance of receiving cattle; perhaps these occurred without substantial impacts on systemic inflammatory and metabolic responses.

Conclusions

Beef cattle supplemented with feed-grade antibiotics (lasalocid, chlortetracycline, and monensin) during feedlot receiving tended to have improved ADG and feed efficiency compared with non-supplemented cohorts. Replacing feed-grade antibiotics with plant extracts and sodium saccharin-based sweetener prevented the decrease in feed efficiency when feed-grade antibiotics were not included in the receiving diet. Moreover, cattle supplemented with these alternative feed ingredients had greater serum antibody response to BRSV, suggesting improved humoral response to immunization against this pathogen compared to all other treatments. Yet, supplementing feed-grade antibiotics or plant extracts and sodium saccharin-based sweetener did not reduce BRD incidence, which was not as prevalent as typically observed in commercial feedlot systems. Nonetheless, results from this experiment suggest that plant extracts and sodium saccharin-based sweetener may replace feed-grade antibiotics in feedlot receiving diets without substantially impairing feed efficiency.

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Declaration of interest

No conflict of interest to report.

Ethics statement

All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (#4937).

Software and data repository resources

No software, data, or models were deposited in official repositories.

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Table 1 *Ingredient composition and nutrient profile of total mixed ration offered during the experiment (day 0 to 60)*[1]

Item	**A**	**B**	**C**	**D**
Ingredient (% DM basis)				
Grass hay	74.5	58.2	37.0	33.7
Cracked corn	17.5	35.0	54.6	58.2
Soybean meal	7.2	6.0	7.7	7.4
Mineral mix[2]	0.80	0.80	0.70	0.70
Nutrient profile (DM basis)				
Net energy for maintenance (Mcal/kg)	1.38	1.55	1.76	1.80
Net energy for growth (Mcal/kg)	0.80	0.95	1.14	1.17
NDF, %	46.8	39.3	29.5	27.9
ADF, %	30.9	24.9	17.2	16.0
CP, %	13.7	13.1	13.6	13.5

[1]A = day 0 to 7; B = day 8 to 18; C = day19 to 32; and D = day 33 to 60. Calves had free-choice access to the total mixed ration and water throughout the experimental period.

[2]Cattleman's Choice (Performix Nutrition Systems, Nampa, ID, USA) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3 200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6 000 mg/kg of Zn, 136000 IU/kg of vitamin A, 13000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E.

***Table 2** Performance parameters from beef calves supplemented or not (**CON**; n = 7) with feed-grade antibiotics (**PC**; n = 7) or alternative feed ingredients (**EG**; n = 7) during a 60-day feedlot receiving*[1]

Item	**CON**	**PC**	**EG**	**SEM**	***P*-value**
Initial BW (day -1; kg)	199	198	195	7	0.90
Final BW (day 61; kg)	291	297	287	6	0.58
ADG (kg/day)	1.50[b]	1.59[a]	1.47[b]	0.04	0.10
DMI (kg/day)	8.36	8.45	8.05	0.19	0.52
Feed efficiency[3] (g/kg)	0.181	0.191	0.186	0.003	0.23

[1]PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; EG = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; CON = no supplemental ingredients. Within rows, values with different superscripts differ ($P \leq 0.05$).

***Table 3** Morbidity and mortality parameters in beef calves supplemented or not (**CON**; n = 7) with feed-grade antibiotics (**PC**; n = 7) or alternative feed ingredients (**EG**; n = 7) during a 60-day feedlot receiving*[1]

Item	**CON**	**PC**	**EF**	**SEM**	***P*-value**
Incidence of bovine respiratory disease signs (%)	25.7	28.6	22.9	11.8	0.94
Number of antimicrobial treatments required	1.22	1.20	1.00	0.12	0.39
Calves that required ≥ 1 antimicrobial treatment (%)	22.2	20.0	0.0	12.1	0.39
Other morbidity reasons[2] (%)	2.86	2.86	0.00	2.33	0.61
Mortality (%)	0.0	0.0	0.0	-	-

[1]PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; EG = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; CON = no supplemental ingredients.

[2]All non-BRD related morbidity were due to physical injury.

Table 4 *Physiological and humoral responses from beef calves supplemented or not (**CON**; n = 7) with feed-grade antibiotics (**PC**; n = 7) or alternative feed ingredients (**EG**; n = 7) during a 60-day feedlot receiving*[1]

Item	**CON**	**PC**	**EF**	**SEM**	***P*-value**
Physiological variables					
Plasma cortisol (ng/mL)	54.5	52.5	54.9	3.1	0.84
Plasma haptoglobin (mg/mL)	0.283	0.292	0.318	0.037	0.78
Plasma IGF-I (ng/mL)	223	235	232	8	0.56
Serum antibody variables (titer log 2)					
Mannheimia haemolytica	9.34	9.53	9.42	0.19	0.79
Parainfluenza-3 virus	6.60	5.89	6.05	0.35	0.35
Bovine respiratory syncytial virus	2.82^{y}	2.62^{b}	3.82^{ax}	0.42	0.09
Bovine viral diarrhea virus-1	2.62	2.87	3.42	0.43	0.39
Bovine herpesvirus-1	1.07	1.37	1.58	0.30	0.51

[1]PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; EG = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; CON = no supplemental ingredients. Within rows, values with different superscripts differ at *P* = 0.04 (a,b) or *P* = 0.08 (x,y).

Table 5 *Concentrations of plasma cortisol (ng/mL), haptoglobin (mg/mL), IGF-I (ng/mL), and serum titers against Mannheimia haemolytica (**MH**), parainfluenza-3 virus (**PI3**), bovine respiratory syncytial virus (**BRSV**), bovine viral diarrhea virus-1 (**BVD-1**), and bovine herpesvirus-1 (**BHV**) in beef cattle during an 60-day feedlot receiving* [1]

	Plasma variables			Serum antibody titers				
Day	**Cortisol**	**Haptoglobin**	**IGF-I**	**MH**	**PI3**	**BRSV**	**BVD**	**BHV**
0	41.5^{d}	0.151^{d}	88^{c}	6.05^{e}	4.86^{d}	0.63^{d}	0.76^{d}	0.19^{d}
5	40.4^{d}	0.383^{a}	-	7.34^{d}	5.31^{cd}	3.11^{b}	0.63^{d}	0.52^{cd}
11	54.0^{c}	0.278^{bc}	-	9.61^{c}	5.94^{b}	3.07^{b}	0.81^{d}	0.79^{bc}
22	57.6^{b}	0.312^{b}	-	10.63^{a}	5.88^{bc}	1.41^{c}	2.38^{c}	1.08^{b}
33	64.7^{a}	0.228^{c}	221^{b}	10.02^{b}	6.83^{a}	4.09^{a}	4.83^{b}	2.14^{a}
43	-	-	-	9.32^{c}	6.43^{ab}	3.68^{ab}	6.52^{a}	1.99^{a}
60	-	-	234^{a}	-	-	-	-	-
SEM	1.6	0.030	5	0.18	0.32	0.33	0.33	0.22
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1] Within columns, values with different superscripts differ ($P \leq 0.05$).

Figure captions

Figure 1. Body weight (Panel A) and DMI (total mixed ration; Panel B) during a 60-day feedlot receiving from beef cattle assigned to: **PC** = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; **EG** = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; **CON** = no supplemental ingredients. Values reported are least square means ± SEM. No treatment effect was detected ($P \geq 0.52$).

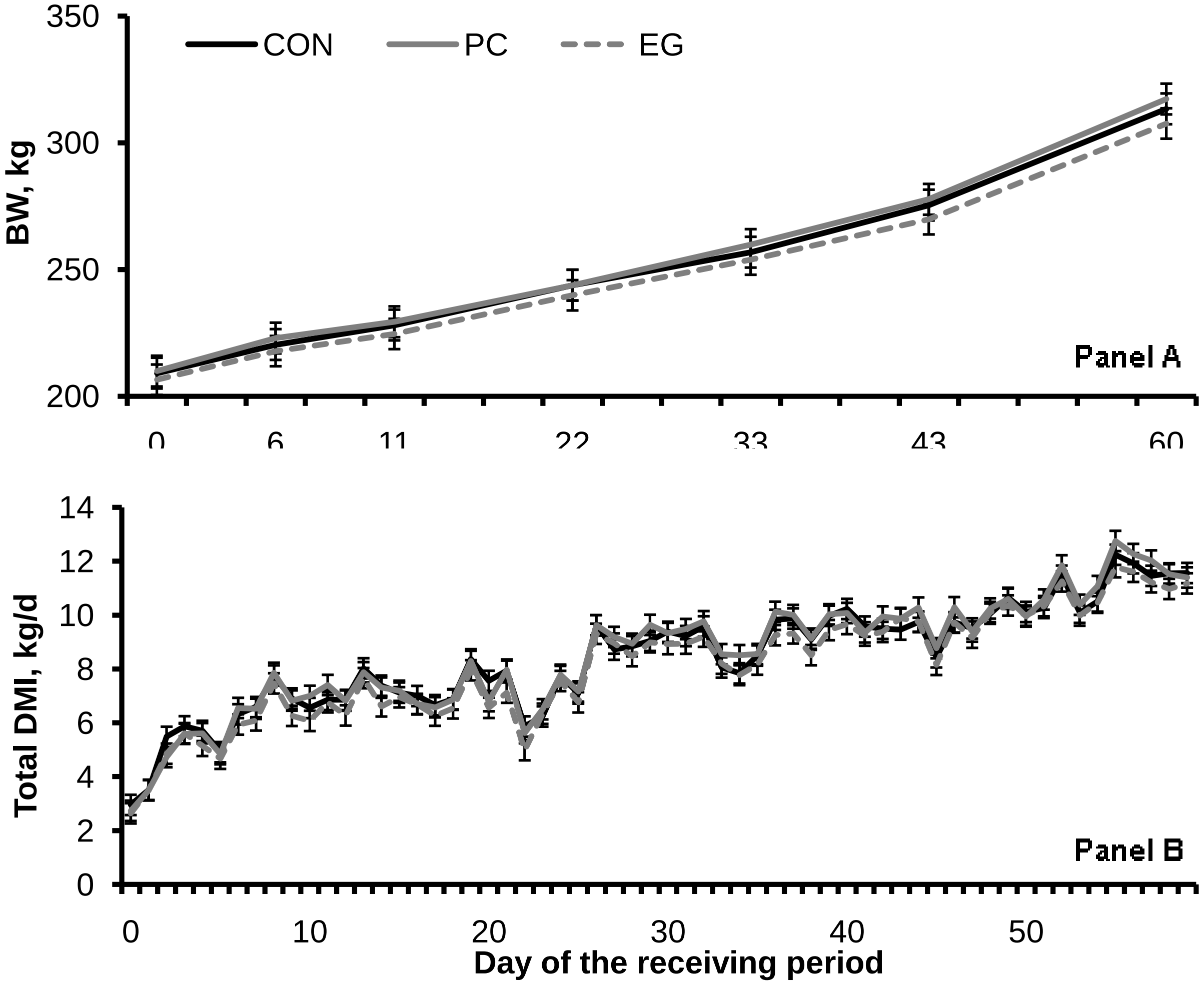

Figure 2. Cumulative incidence of bovine respiratory disease (**BRD**) signs during a 60-day feedlot receiving in beef cattle assigned to: **PC** = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; **EG** = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; **CON** = no supplemental ingredients. Values reported are least square means ± SEM. No treatment effect or treatment × day interaction were detected ($P \geq 0.94$).