

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

MARINGÁ
Estado do Paraná
Fevereiro – 2018

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

S729c Souza, Kennyson Alves de
Compostos naturais sobre o desempenho,
comportamento, resposta imune e características de
carcaça de bovinos terminados em confinamento /
Kennyson Alves de Souza. -- Maringá, 2018.
83 f. : il., color., figs., tabs.

Orientador(a): Prof. Dr. Ivanor Nunes do Prado.
Tese (doutorado) - Universidade Estadual de
Maringá, Centro de Ciências Agrárias, Programa de
Pós-Graduação em Zootecnia - Área de Concentração:
Produção Animal, 2018.

1. Dieta do grão. 2. Extrato natural. 3.
Imunidade. 4. Modulador fermentativo. 5. Nutrição.
6. Qualidade de carcaça. I. Prado, Ivanor Nunes do,
orient. II. Universidade Estadual de Maringá. Centro
de Ciências Agrárias. Programa de Pós-Graduação em
Zootecnia: Área de Concentração: Produção Animal.
III. Título.

CDD 21.ed. 636.213

AHS-CRB-9/1065



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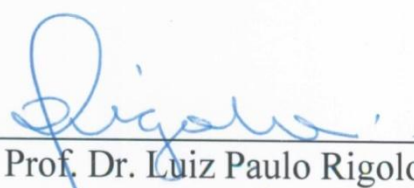
Autor: Kennyson Alves de Souza
Orientador: Prof. Dr. Ivanor Nunes do Prado

TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção
Animal

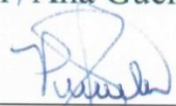
APROVADA em 26 de fevereiro de 2018.



Prof^ª Dr^ª Ana Guerrero Barrado



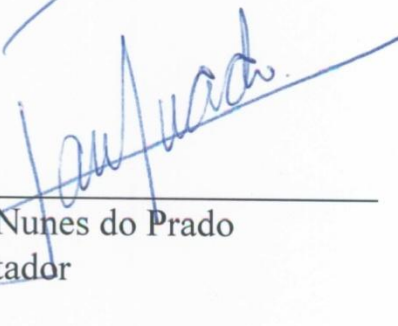
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“Procure ser um homem de valor, em vez de ser um homem de sucesso.”

Albert Einstein

AGRADECIMENTOS

Primeiramente agradeço a Deus por ter sido o guia em todos os momentos da minha vida, por que sem sua presença não conseguiria chegar aonde cheguei com o espírito tranquilo.

Aos meus pais, Antonio Souza de Oliveira e Maria de Fátima Alves Gonçalves, por serem meu alicerce, partilhando tanto dos meus momentos felizes como dos momentos difíceis desta caminhada.

À Universidade Estadual de Maringá, professores e funcionários, pelo suporte necessário em todos os momentos necessitados, bem como pelo agradável ambiente de trabalho.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos no Brasil e no Exterior na modalidade de Doutorado-sanduíche no Exterior (PDSE).

A Oregon State University e seus funcionários, por ter proporcionado minha estadia muito produtiva e confortável.

Ao professor Dr. Ivanor Nunes do Prado, pelo incentivo ao conhecimento profissional e pessoal, e pelos momentos de descontração dentro e fora da universidade.

Ao professor Dr. Reinaldo Fernandes Cooke e toda sua equipe, pelo apoio, ensinamentos e compreensão durante todo o período do meu intercâmbio.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), a empresa Safeeds Nutrição Animal (Paraná, Brazil) e a empresa Pancosma SA (Geneva, Switzerland), pelo financiamento dos projetos de pesquisa.

Ao José Carlos da Silva, por ter sido uma pessoa essencial na realização deste trabalho em nível de campo, pelos ensinamentos, conselhos, momentos de descontração e pela amizade construída durante a fase do doutorado.

Aos amigos conquistados durante esta fase do doutorado que me auxiliaram nos momentos mais oportunos e contribuíram em meu crescimento pessoal e profissional.

Aos colegas de trabalho por terem permitido o meu aprendizado por meio do nosso convívio diário.

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, conseqüentemente, 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 $297,6 \pm 31,2$ 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 novinhos 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 análises 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 sincicial 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

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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

39 concentrado ou mais necessitam de moduladores da fermentação ruminal em função da
40 alta degradabilidade dos carboidratos usados; neste sentido, os aditivos de origem
41 natural passam a ser grandes aliados nas pesquisas.

42

43 **Dieta alto concentrada na nutrição de ruminantes**

44

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

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

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

63 Neste contexto o grão de milho apresenta anatomia e composição química
64 interessante para tal prática. A composição química expressada à base da matéria seca é
65 72% de amido, 9,5% proteínas, 9% fibras e 4% de óleo. Sendo considerada uma
66 importante fonte de fibra, especialmente do tipo insolúveis (hemicelulose, celulose e
67 lignina), que correspondem à fração fibra em detergente neutro. Já em relação à
68 estrutura física do grão de milho, o mesmo é formado por quatro principais estruturas
69 físicas, sendo o endosperma, gérmen, pericarpo (casca) e ponta (NRC, 2001).

70 Sobrinho et al. (1996) trabalhando com grãos inteiros para cordeiros em
71 confinamento, observaram diminuição do ritmo de fermentação ruminal e aumento do

72 tempo de ruminção e de ingestão, elevando a secreção de saliva e o pH do rúmen. Os
73 mesmos autores verificaram também que o fornecimento de grãos inteiros não causou
74 prejuízos à digestibilidade nem à conversão alimentar.

75 Autores como Woody et al. (1983) estudaram o efeito de diferentes níveis de grãos
76 nas dietas de bovinos em fase de terminação e relataram que animais alimentados com
77 dietas com 90% de grãos ganharam peso 7% mais rápido e tiveram redução de 16% no
78 requerimento alimentar por unidade de ganho em relação a animais alimentados com
79 70% de grãos.

80

81 **Doença respiratória bovina**

82

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

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

104 A identificação dos sinais clínicos da doença respiratória bovina segue o sistema
105 DART (Zoetis, Florhan Park, Nj), onde se atribuí pontuações de escore variando de 0 a
106 4 com base nos sinais clínicos e a gravidade dos sinais observados. Sendo o escore 0
107 nenhum sintoma detectado, 1 sintoma leve, 2 sintoma moderado, 3 sintoma grave e 4
108 moribundo. Todos os animais quando detectados com algum sintoma clínico e
109 apresentando temperatura retal acima de 40 °C administra - se o antimicrobiano. Este
110 sistema leva em consideração critérios de avaliação como depressão, apetite, sistema
111 respiratório e temperatura.

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

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

123

124 **Ingredientes de origem natural utilizado na alimentação animal -** 125 **Introdução a *Baccharis dracunculifolia***

126

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

136 cumárico, ácido ferúlico) (Bohlmann et al., 1981; Banskota et al., 1998; Akao et al.,
137 2003; Silva Filho et al., 2004; Mendez, 2005; Loots et al., 2006).

138 *A Baccharis dracunculifolia* é explorada como substrato no Brasil com o objetivo
139 da produção da própolis verde, assim chamada devido a sua coloração (Marcucci et al.,
140 1998; Park et al., 2002). A qual é um elemento natural resinosa, coletada por abelhas
141 das flores das plantas, que apresenta uma atividade biológica, antimicrobiana e
142 antioxidante (Da Silva Leitão et al., 2004; Simões et al., 2004; Jorge et al., 2008; Pontin
143 et al., 2008). Autores estudando a composição da planta *Baccharis* e da própolis verde
144 (Labbe et al., 1986; Marcucci et al., 1998; Kumazawa et al., 2003) encontraram várias
145 substâncias que estão presente nas duas fontes, como derivados do ácido *p*-cumárico,
146 prenilados e flavonóides.

147

148 **Óleo essencial e sua capacidade de atuação na flora bacteriana de** 149 **ruminantes**

150

151 ***Definição***

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

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

167 Embora se acreditasse que os metabólitos secundários e, portanto, os óleos
168 essenciais, não tinham função na planta, agora são aceitos que eles fornecem a planta

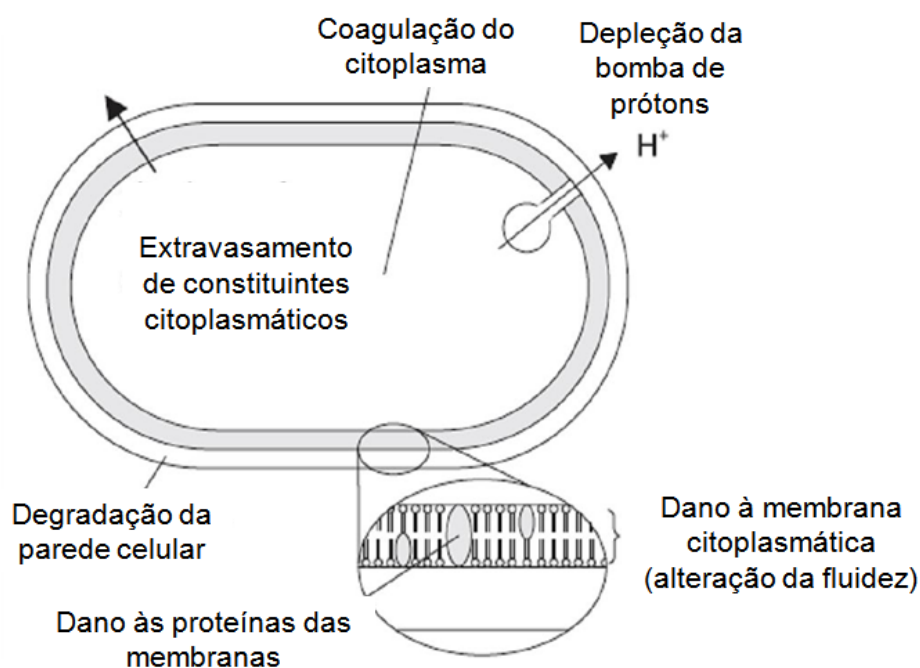
169 proteção contra estressores abióticos e bióticos, bem como sendo atrativos a organismos
170 que polinizam e dispersam sementes (Wink & Schimmer, 1999).

171

172 ***Mecanismos de ação (propriedade antimicrobiana)***

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

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



188 **Figura 1.** Possíveis mecanismos de atuação dos óleos essenciais sobre as bactérias ruminais. Adaptado de
189 Burt (2004).

190

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

200 Vale ressaltar que existem outros fatores que podem atuar neste tipo de seleção
201 bacteriana, como é o caso de baixos níveis de proteína ofertada aos animais via
202 suplemento, bem como, o tipo de fornecimento dos óleos essenciais a população
203 bacteriana. Por meio da diferenciação dos mecanismos de atuação dos óleos
204 propriamente ditos e dos seus compostos (princípios ativos) isoladamente (Prata et al.,
205 2011).

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

215

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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.

26 **Highlights:**

27

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

32

33 **1. Introduction**

34

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

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

46 The essential oils are liquid aromatic extracts due to the volatile nature of the components extracted from plant
47 material, such as flowers, buds, seeds, leaves, twigs, barks, wood, fruit, and roots. They may be obtained by
48 fermentation, extraction, or most commonly, by steam distillation (Burt, 2004). Chemically, essential oils are
49 variable mixtures of terpenoids that primarily include monoterpenes (C¹⁰) and sesquiterpenes (C¹⁵), although
50 diterpenes (C²⁰) may also be present. They also include a variety of low-molecular-weight aliphatic hydrocarbons,
51 acids, alcohols, aldehydes, acyclic esters, or lactones. Besides to count with compounds such as coumarins and
52 homologues of phenylpropanoids. These products consist of various concentrations and chemical variations acting as

53 antimicrobial and antioxidant agents, benefiting the immune and digestive systems of animals, which is reflected in
54 animal performance indices (Jayasena and Jo, 2013).

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

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

63

64 2. Materials and Methods

65

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

68

69 2.1. *Locale, animals, housing, and experimental treatments*

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

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

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

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

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

96

97 2.2. Chemical analyses

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

108 carbohydrates and NDF. Metabolizable energy of feed stuffs was estimated according to NRC (2000)
109 recommendations.

110

111 2.3. *Feed intake, growth performance, and carcass characteristics*

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

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

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

$$125$$
$$126 \text{ CDP} = \text{CW} \times 100/\text{FBW} \quad (1)$$

127

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

129 The carcass dripping loss was performed by measuring the difference between the weight obtained before and
130 after refrigeration for 24 h (± 4 °C).

131

132 2.4. *Carcass tissue composition*

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

136

137 *2.5. In situ digestibility*

138 The determination of total digestibility from the indicator indigestible neutral detergent fiber (iNDF) was carried
139 out according to the methodology described by Zeoula et al. (2002). Samples of feed, feces, and leavings were
140 incubated in the rumen of animals cannulated using F57 filter bags for 288 h (Ankom Technology, NY, USA) with
141 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
142 for silage, feces, and leavings. Following removal of the bags from the rumen, they were washed by hand under
143 running water until the resulting wash water became clear and subsequently placed to dry in a forced ventilation
144 oven at 60 °C for 48 h and finally, boiled in a neutral detergent solution (TE-149, Tecnal, SP, Brazil) to obtain the
145 iNDF.

146 Fecal flow was determined using the following equation:

147

$$148 \quad FF = IC/CIF \quad (2)$$

149

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

151

152 The digestibility coefficient was calculated by the following equation:

153

$$154 \quad DC = (NI - NE)/NI \quad (3)$$

155

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

157

158 *2.6. Ingestive behavior activities*

159 Data on feeding behavior were obtained between the 6th and 7th weeks of feedlot. The record of time spent on
160 different activities was obtained by visual observation of the animals every 5 min, carried out by a trained team over
161 24 uninterrupted hours (Silva et al., 2006). Data were collected to estimate the duration of periods spent feeding,
162 drinking, ruminating, and idle. The total time spent on each activity was determined by the sum of repetitions.

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

$$\begin{aligned}
 166 \quad FE_{DM} &= DMI/FD \\
 167 \quad FE_{NDF} &= NDFI/FD \\
 168 \quad RE_{DM} &= DMI/RUD \\
 169 \quad RE_{NDF} &= NDFI/RUD
 \end{aligned} \tag{4}$$

170
 171 where: FE_{DM} , DMI , FD , FE_{NDF} , $NDFI$, RE_{DM} , RUD , RE_{NDF} are Feeding efficiency of dry matter (kg DM/h); Dry
 172 matter intake (kg DM/d); Feeding duration (h/d); Feeding efficiency of neutral detergent fiber (kg NDF/h); Neutral
 173 detergent fiber intake (kg NDF/d); Rumination efficiency of dry matter (kg DM/h); Rumination duration (h/d); and
 174 Rumination efficiency of Neutral detergent fiber (kg NDF/h), respectively.

175 176 **3. Statistical Analyses**

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

$$181 \quad Y_{ij} = \mu + T_i + e_{ij} \tag{5}$$

182
 183 where: Y_{ij} , μ , T_i , e_{ij} are Dependent variables; Mean value common to all observations; Fixed effect of essential oils
 184 diets; and The error term, respectively.

185
 186 For each studied variable, the mean and standard error of the mean (SEM) were calculated and differences
 187 between means were evaluated using Duncan's Multiple Range Test ($p \leq 0.05$).

188 189 **4. Results**

190

191 Final body weight (FBW) was not affected ($p > 0.05$) by essential oil addition to the diets (Table 3). The average
192 daily gain (ADG) was significantly greater ($p < 0.001$) for heifers fed three diets with essential oil blends than for
193 heifers fed CON and ROS diets (Table 3). The blend of essential and protected oils in the diets increased ($p <$
194 0.0001) dry matter intake (DMI) (Table 3). The feed efficiency rate was better ($p < 0.0001$) in heifers fed three
195 essential oil blends (BLE, BCL, and BRC) (Table 3). Feed efficiency presented intermediate values in heifers fed the
196 CON diet and were not significantly different from those in the BLE group. Heifers fed the ROS diet had the lowest
197 feed efficiency ($p < 0.0001$).

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

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

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

211

212 5. Discussion

213

214 The observed value of FBW was in accordance with the Nellore standard, as well as with the requirements of
215 Brazilian slaughterhouses, which advocate a final body weight for heifers from 320 to 380 kg (Ferraz and Felício,
216 2010). The rosemary essential oil in the diet showed the lowest gain, whereas when it was supplied with other oils, it
217 presented a higher ADG than the CON group. Heifers finished in feedlot must have weight gain from 0.8 to 1.2

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

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

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

233 The hot and cold carcass weights values observed were in accordance with those of standard Nellore heifers, as
234 well as with the requirements of Brazilian slaughterhouses, which advocate a final body weight for heifers from 180
235 to 200 kg (Ferraz and Felício, 2010). The average hot and cold carcass dressings were superior, when compared that
236 the carcass dressing are approximately of 52% can be considered normal for Nellore heifers slaughtered at 24
237 months, as observed in other studies of heifers slaughtered at a similar weight (Marques et al., 2010; Farias et al.,
238 2012). In general, the dripping losses are between 1.5% and 2.0% after 24 h of chilling which is in agreement with
239 the present study. Thus, the losses observed in this experiment are consistent with losses considered normal
240 (Andreotti et al., 2015). Studies carried out with crossbred heifers in feedlot have reported muscle, fat, and bone
241 percentages from 56% to 62%, 20% to 25%, and 16% to 19%, respectively (Andreotti et al., 2015). Thus, the muscle,
242 fat, and bone percentages obtained in this study can be considered normal for these animal categories.

243 According to Oh et al. (1968) a hypothesis exists that the low palatability of some natural extracts to ruminants,
244 as is the case of rosemary essential oil, could be due not only to sensorial effects but also to effect on microbial flora,
245 thus affecting directly the total digestibility of DM. Nagy and Tengerdy (1968) proved this fact in their studies

246 evaluating the sensitivity of ruminal microorganisms using the essential oil of *Artemisia tridentate*, which has as its
247 main compound 1.8-cineole, also found in essential oil of rosemary, because some evidence indicated that high
248 intake of this compound resulted in digestive problems in ruminants. In general, high doses of this oil, when added to
249 *in vitro* cultures of rumen bacteria, reduced total viable bacteria counts. A possible explanation for the low levels of
250 NDF total digestibility is related to the higher starch content of the diets (Table 1), which can decrease the
251 digestibility of the fiber as a result of lower ruminal pH, ruminal passage rate, and changes in rumen microbial
252 populations (Allen and Mertens, 1988). However the use of clove essential oil is beneficial because it has as main
253 compound eugenol, which is considered a phenolic compound, and it has demonstrated a high antimicrobial activity
254 due to the presence of a phenolic hydroxyl group in its structure (Burt, 2004).

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

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

268

269 **6. Conclusions**

270

271 The present results suggest that the use of a blend of 4 g/animal/d of natural additives in the diets of Nellore
272 heifers improves animal production. Rosemary essential oil in independent doses supplied as single oil shows no
273 improvement in animal production, but when administered with other essential oils, the response is positive. The

274 blend of clove essential oil (2 g/animal/d) and protected oils [eugenol, thymol, and vanillin (2 g/animal/d)] proved to
275 be promising, promoting the best results.

276

277 **Conflict of interest**

278

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

281

282 **Acknowledgements**

283

284 This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Brasília,
285 Brazil) for the scholarship, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Brasília,
286 Brazil) and the Company Safeeds Nutrição Animal (Paraná, Brazil). The authors gratefully acknowledge the
287 company for financing and providing the products used in this research which it was possible to develop this work.
288 The mention of trade names or commercial products in this publication is solely for the purpose of providing specific
289 information and does not imply recommendations or endorsement by the Department of Animal Science,
290 Universidade Estadual de Maringá, Paraná, Brazil.

291

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377 **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 + vanillin	-	-	0.04	-	-
Protected blend + clove	-	-	-	0.04	-
Protected blend + clove + rosemary	-	-	-	-	0.04

378 ^aCON – Without essential oil.379 ^bROS – Rosemary essential oil (4 g/animal/d).380 ^cBLE –Protected blend of eugenol + thymol + vanillin (4 g/animal/d).381 ^dBCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).382 ^eBRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d),
383 clove essential oil (1.33 g/animal/d).384 ^fMineral salt composition (kg): calcium, 50 g; magnesium, 57 g; sodium, 81 g; sulfur, 3.75 g; cobalt, 20 mg; copper,
385 500 mg; iodine, 25 mg; manganese, 1.500 mg; selenium, 10 mg; zinc, 2.000 mg; vitamin A, 400.000 UI; vitamin D3,
386 50.000 UI; vitamin E, 750 UI; ether extract, 168 g; urea, 200 g.

387 **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

388 ^a Dry matter.389 ^b Organic matter.390 ^c Crude protein.391 ^d Ether extract.392 ^e Neutral detergent fiber.393 ^f Acid detergent fiber.394 ^g Total carbohydrates.395 ^h Non-fiber carbohydrates.396 ⁱ Metabolizable energy (Mcal/ kg).

397 **Table 3.** Effect of diets with and without inclusion of essential oils on animal performance, feed
 398 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

399 ^aCON – Without essential oil.

400 ^bROS – Rosemary essential oil (4 g/animal/d).

401 ^cBLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

402 ^dBCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

403 ^eBRC – Protected blend protected – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33
 404 g/animal/d), clove essential oil (1.33 g/animal/d).

405 ^fSEM: Standard error of mean.

406 ^gkg average daily gain/kg dry matter feed intake.

407 ^{abc} Values with different letters in the same row are different by Duncan test.

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

Tissues, %	Diets					SEM ^f	p-value
	CON ^a	ROS ^b	BLE ^c	BCL ^d	BRC ^e		
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

410 ^aCON – Without essential oil.

411 ^bROS – Rosemary essential oil (4 g/animal/d).

412 ^cBLE – Protected blend of eugenol + thymol + vanillin (4 g/animal/d).

413 ^dBCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).

414 ^eBRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d),
 415 clove essential oil (1.33 g/animal/d).

416 ^fSEM: Standard error of mean.

417 **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

418 ^aCON – Without essential oil.419 ^bROS – Rosemary essential oil (4 g/animal/d).420 ^cBLE – Protected blend of eugenol + thymol+vanillin (4 g/animal/d).421 ^dBCL – Protected blend – eugenol + thymol + vanillin (2 g/animal/d) + clove essential oil (2 g/animal/d).422 ^eBRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d),
423 clove essential oil (1.33 g/animal/d).424 ^fSEM: Standard error of mean.425 ^{abc} Values with different letters in the same row statistically different by Duncan test.

426 **Table 6.** Effect of diets with and without inclusion of essential oils on ingestive behaviour activities of Nellore
 427 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

428 ^a CON – Without essential oil.

429 ^b ROS – Rosemary essential oil (4 g/animal/d).

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

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

432 ^e BRC – Protected blend – eugenol + thymol + vanillin (1.33 g/animal/d), rosemary essential oil (1.33 g/animal/d),

433 clove essential oil (1.33 g/animal/d).

434 ^f SEM: Standard error of mean.

435 ^g FE_{DM}: Dry matter feeding efficiency.

436 ^h FE_{NDF}: Neutral detergent fiber feeding efficiency.

437 ⁱ RE_{DM}: Dry matter rumination efficiency.

438 ^j RE_{NDF}: Neutral detergent fiber rumination efficiency.

439 ^{ab} Values with different letters in the same row statistically different by Duncan test.

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

442

443 **Short Title:** Natural ingredients to finished feedlot steers

444

445 **Journal:** Animal Production Science

446

447 **ABSTRACT**

448

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

465

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

467 **Summary**

468
469 Leaves of *Baccharis dracunculifolia in natura* used as potential substitute for the antibiotics
470 offered in the steers feed feedlot with the objective of fermentative modulators. The performance,
471 ingestive behavior, and blood parameters were not affected by the inclusion of up to 15 grams of
472 the *Baccharis*, proving that this product can be used in the beef cattle feed. But more studies are
473 needed in the area to define the dosages and the true functioning of this plant in animal feed.

475 **Introduction**

476
477 The efficiency in the beef cattle production is considered a great challenge and has been
478 target of innumerable research and discussions over the years, indicating the need to maximize
479 production through the developed of the entire meat production chain. The finishing phase in
480 feedlot of the animals, which was been studied in this work is one of the phases more important
481 on the production cycle. This is an onerous phase due to the high costs of a quality feed, thus
482 allowing the animals to express their full genetic potential. Thus, the use of new alternatives to
483 increase the productivity of the Brazilian cattle herd has been studied more frequently, being the
484 class of growth promoters in the highlighted (Ornaghi *et al.* 2017).

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

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

495 This includes the plant of *Baccharis dracunculifolia*, being a native plant from Brazil,
496 commonly known as "Alecrim do campo". This extract is composed of aliphatic hydrocarbons,
497 cyclic hydrocarbons, terpenes (baccharin), isopropenol, flavonoids (isosakuranetin,

498 aromadendrin-4'-methyl ether) and phenolic acid (artepelin C, caffeic acid, *p*-coumaric acid,
499 ferulic acid) (Kumazawa *et al.* 2003; Campos *et al.* 2016), however having the artepelin C as the
500 principal compound (Veiga *et al.* 2017), besides presenting potential as antioxidants (Burdock
501 1998), classifying themselves as biological, antimicrobial, antioxidant and anti-inflammatory
502 agents (Tiveron *et al.* 2016).

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

506

507 **Materials and Methods**

508

509 *Animals and experimental diet*

510 Forty Nellore purebred steers with a mean initial body weight of 412.9 ± 22.0 kg were used in
511 this study. Steers were distributed randomly in individual pens, with dimensions of 10 m² for
512 each animal, partially covered and equipped with automatic drinkers and masonry feeders. The
513 period of adaptation to the feedlot and concentrate diet was 14 days; afterwards, the experimental
514 period was extended to 56 days until animals reached a mean FBW of 499.9 ± 25.6 kg. During
515 the experimental period, Nellore steers were weighed monthly in order to record weight gain and
516 productivity variables.

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

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

534

535 *Chemical analyses*

536 The chemical compositions of ingredients and experimental diets were presented as g/kg of
537 DM (Table 2). DM was estimated after oven drying at 65 °C for 24 h and milling through a 1-mm
538 screen following method ID 934.01 (AOAC 2005). Ash content was measured by combustion at
539 550 °C for 16 h according to method ID 942.05 (AOAC 2005). Nitrogen concentration was
540 determined by the Kjeldahl method (ID 988.05) (AOAC 2005). Following the determination of
541 nitrogen concentration, crude protein was calculated by multiplying the nitrogen content by a
542 factor of 6.25. Ether extract content was determined by method ID 920.39 (AOAC 2005). The
543 neutral detergent fiber (NDF) content was measured according to the recommendations of
544 Mertens (2002) using α -amylase and was expressed inclusive of residual ash. The acid detergent
545 fiber was measured by using method ID 973.18 (AOAC 2005) and was expressed inclusive of
546 residual ash. The non-nitrogen extract was obtained by equation according to (AOAC 2005). The
547 digestible energy was calculated according to the recommended equations (NRC 2000). Total
548 digestible nutrients (TDN) content of diets was obtained by the methodology described by Kears
549 (1982), using the equation for pre-dried:

550

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

552

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

555

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

558

559 *Animal performance*

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

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

572

573 *Ingestive behavior activities*

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

581

582 *Blood analyses*

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

588 (2007). The activities of muscle injury indicative enzymes aspartate aminotransferase (AST) and
589 creatine kinase (CK) were measured in spectrophotometer (Spectrophotometer UV-Vis-Evolution
590 200, Massachusetts, United State of America) by means of commercial enzymatic dosage kits
591 (Bioclin, Belo Horizonte, Brazil) according the manufacturer's instructions. The gamma glutamyl
592 transferase (GGT) was performed using Roche assay reagents in the Roche 900 series automated
593 clinical chemical analyzer (Roche Diagnostics, Indiana, United State of America).

594

595 *Statistical Analyses*

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

599

$$600 \quad Y_{ijk} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \alpha_{ijk} + \epsilon_{ijk}$$

601

602 where: Y_{ijk} , β_0 , X_{ijk} , α_{ijk} , and ϵ_{ijk} are Dependent variables (plant levels); Regression coefficient;
603 Independent variables; Regression deviations; and Residual error, respectively.

604

605 **Results**

606

607 The inclusion of up to 15 grams per animal/day of the leaves of *Baccharis dracunculifolia in*
608 *nature* in the steers' diets finished in the feedlot did not affect ($P \geq 0.47$) final body weight,
609 average daily gain, dry matter intake, and feed efficiency (Table 3). No treatment effects were
610 detected ($P \geq 0.23$) for ingestive behavior activities during the 12 uninterrupted hours for
611 activities drinking, feeding, rumination, idleness (Table 4). No treatment effects were detected (P
612 ≥ 0.12 ; Table 5) for plasma concentrations of urea, creatine, aspartate aminotransferase, gamma
613 glutamyl transferase, and creatine kinase.

614

615 **Discussion**

616

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

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

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

636 Even without the differentiation between the treatments from animals that received or not the
637 supplementation with the plant extracts, the average daily gain can be considered satisfactory for
638 feedlot animals fed a high grain diet on Nellore breed (Maggioni *et al.* 2009; Françoço *et al.*
639 2013). The final body weight, dry matter intake, and feed efficiency had similar results between
640 treatment animals throughout the experimental period (Table 3). The plant *in nature* presents
641 high levels of flavonoids and phenol (Kumazawa *et al.* 2003; Paula *et al.* 2017), consequently,
642 these concentrations in steers' diet negatively influenced ruminal dynamics, justifying the lack of
643 effect detection for feed efficiency.

644 Ingestive behavior activities (feeding, drinking, ruminating, and idle; Table 4) were similar
645 among the treatments that received the vegetal extract or not, this resemblance is possibly
646 explained due to the similarity between the feedlot pens, as well as the basal diet offered to the
647 animals. Corroborating with these results another works carried out under similar conditions and

648 with inclusion of natural additives (essential oils) as those from our research group (Ornaghi *et*
649 *al.* 2017) who did not detect either effect for ingestive behavior on young bulls receiving a high
650 concentrated diet.

651 Results for feeding and ruminating are in according with Missio *et al.* (2010); Eiras *et al.*
652 (2013); Ornaghi *et al.* (2017) who also evaluated ingestive behavior on beef cattle feedlot
653 supplemented with a high concentrated diet. The low levels for these activities are understood
654 due to the greater energetic support that this type of diet provides, thus the animals reach their
655 nutritional requirements and cease their consumption. According Van Soest *et al.* (1994), a diet
656 with a higher percentage of forage increase the time used for rumination, that is, high concentrate
657 diet due to the size of its particles may have reduced the rumination capacity of the present study
658 steers. Another factor that may have compromised the rumination rate are the high levels of
659 phenolic substances found in *B. dracunculifolia* (Park *et al.* 2002; Tiveron *et al.* 2016), which
660 adversely affected the use of feed by ruminal bacteria.

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

666 Stresses being by transport, dehydration, or nutrient-poor diets have an effect on metabolism,
667 through changes in plasma concentrations of urea, total protein, and creatine kinase (Tarrant *et al.*
668 1992; Earley and O'Riordan 2006; Buckham Sporer *et al.* 2008). Corroborating with this
669 affirmation Bershauer *et al.* (1983) report that with increasing feed intake there is a decrease in
670 blood urea concentrations. In the present study the results for urea, creatinine, and creatine kinase
671 were above what is classified as a reference for cattle (Table 5). Therefore, these results indicate
672 that the steers did not suffer any type of metabolic alteration during the experimental period, but
673 in the period before the experiment.

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

679 neurological or metabolic diseases, which could negatively influence the performance and health
680 of the steers.

681

682 **Conclusion**

683

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

688

689 **Acknowledgements**

690

691 This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
692 – CAPES (Brasília, Brazil) for the scholarship, Conselho Nacional de Desenvolvimento
693 Científico e Tecnológico – CNPq (Brasília, Brazil), and the company Safeeds Nutrição Animal.

694

695 **Conflict of interest**

696

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

699

700 **Ethics statement**

701

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

704

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840 animal performance and carcass characteristics. *Journal of Animal and Feed Sciences* **20**, 16-
841 25.

842 **Table 1** Ingredient composition of total mixed ration offered during the experiment¹

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
<i>Baccharis dracunculifolia</i>	-	0.05	0.10	0.15

843 ¹TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g
844 animal⁻¹ day⁻¹); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal⁻¹ day⁻¹); and
845 BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal⁻¹ day⁻¹).

846 ²Mineral mix composition (kg): calcium, 50 g; magnesium, 57 g; sodium, 81 g; sulfur, 3.75 g;
847 cobalt, 20 mg; copper, 500 mg; iodine, 25 mg; manganese, 1.500 mg; selenium, 10 mg; zinc,
848 2.000 mg; vitamin A, 400.000 UI; vitamin D3, 50.000 UI; vitamin E, 750 UI; ether extract, 168
849 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	-	-	-	-	-	-	-
<i>Baccharis dracunculifolia</i>	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

851 **Table 3** Performance parameters of steers supplemented or not with plant *in nature* during the
 852 feedlot finishing period¹

Parameters	TEST	BAC05	BAC10	BAC15	SEM	P-value	
						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

853 ¹TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g
 854 animal⁻¹ day⁻¹); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal⁻¹ day⁻¹); and
 855 BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal⁻¹ day⁻¹).

856 ²kg average daily gain/kg dry matter feed intake.

857 **Table 4** Ingestive behavior activities parameters of steers supplemented or not with plant *in*
 858 *nature* during the feedlot finishing period¹

Activities	TEST	BAC05	BAC10	BAC15	SEM	P-value	
						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

859 ¹TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g
 860 animal⁻¹ day⁻¹); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal⁻¹ day⁻¹); and
 861 BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal⁻¹ day⁻¹).

862 **Table 5** Concentrations of plasma urea (mg/dL), creatine (mg/dL), aspartate aminotransferase
 863 (U/L), gamma glutamyl transferase (U/L), creatine kinase (U/L) in steers supplemented or not
 864 with plant *in nature* during the feedlot finishing period¹

Parameters	TEST	BAC05	BAC10	BAC15	SEM	P-value	
						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

865 ¹TEST = no supplement ingredients; BAC05 = Leaves of *B. dracunculifolia in nature* (5 g
 866 animal⁻¹ day⁻¹); BAC10 = Leaves of *B. dracunculifolia in nature* (10 g animal⁻¹ day⁻¹); and
 867 BAC15 = Leaves of *B. dracunculifolia in nature* (15 g animal⁻¹ day⁻¹).

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

870

871 **Short Title:** Alternative feed ingredients to feedlot cattle

872

873 **Journal:** Animal Journal

874

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

891 XTRACT Ruminants 7065; Pancosma SA) from day 0 to 32, and XTRACT only (800
892 mg/calf daily) from day33 to 60 (**EG**), or 3) no supplemental ingredients (**CON**; day 0 to
893 60). Calves were assessed for bovine respiratory disease (**BRD**) signs and DMI was
894 recorded from each pen daily. Calves were vaccinated against BRD pathogens on day
895 0 and 22. Shrunken BW was recorded on day 61, and blood samples collected on days 0,
896 6, 11, 22, 33, 43, and 60. Calf ADG was greater ($P = 0.04$) in PC vs. EG and tended (P
897 = 0.09) to be greater in PC vs. CON. Feed efficiency also tended ($P = 0.09$) to be
898 greater in PC vs. CON, although main treatment effect for this response was not
899 significant ($P = 0.23$). Mean serum titers against *bovine respiratory syncytial virus* were
900 greater in EG vs. PC ($P = 0.04$) and CON (tendency; $P = 0.08$). Collectively, inclusion of
901 alternative feed ingredients prevented the decrease in feed efficiency when
902 chlortetracycline and ionophores were not added to the initial feedlot diet, and improved
903 antibody response to vaccination against *bovine respiratory syncytial virus* in newly-
904 weaned cattle.

905

906 **Key Words:** beef cattle; growth; immunity; nutrition

907 **Implications**

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

918

919 **Introduction**

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

930 Prophylactic medication with feed-grade antimicrobials, including ionophores and
931 chlortetracycline, is often effective in mitigating incidence of BRD and other health
932 syndromes during feedlot receiving (Duff and Galylean, 2007; Wilson *et al.*, 2017). With
933 increased regulations regarding the use of feed-grade antimicrobials in livestock
934 systems (US Food and Drug Administration, 2015), alternative dietary strategies that
935 enhance immunity of receiving cattle are warranted. These include the use of sodium
936 saccharin-based sweetener in feedlot receiving diets to increase cattle DMI (Ponce *et al.*,
937 2014). Another strategy is supplementing plant extracts containing organic compounds
938 known to enhance rumen function and immunity in cattle, such as cinnamaldehyde,
939 eugenol, and capsicum oleoresin (Yang *et al.*, 2010a; Yang *et al.*, 2010b; Ayrle *et al.*,
940 2016). Based on this information, we hypothesized that plant extracts and sodium
941 saccharin-based sweetener are alternatives to feed-grade antimicrobials in enhancing
942 cattle immunocompetence and productivity during feedlot receiving. Hence, this
943 experiment compared performance, health, and physiological responses of newly-
944 weaned cattle supplemented with feed-grade antibiotics, the aforementioned alternative
945 ingredients, or without such supplements during a 60-day feedlot receiving period.

946

947 **Materials and Methods**

948 This experiment was conducted at the Oregon State University – Eastern Oregon
949 Agricultural Research Center (Burns, OR, USA) from April to June, 2017. During the
950 experiment, environmental temperature ranged from 35 to -5°C, with an average of
951 12°C and 54% humidity, and 16 mm of total precipitation as rain. For all management
952 procedures that required cattle to be restrained, a Silencer Chute (Moly Manufacturing,

953 Lorraine, KS, USA) mounted on Avery Weigh-Tronix load cells (Fairmount, MN, USA;
954 readability 0.45 kg) was utilized.

955

956 *Animals and treatments*

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

971 On day -1, calves were unloaded (0600 h) at the Eastern Oregon Agricultural
972 Research Center, immediately weighed (initial shrunk BW = 197 ± 3 kg), and
973 maintained in a single paddock (160 × 100 m) with *ad libitum* access to alfalfa-grass
974 hay, water, and a commercial mineral mix (described in Table 1) for 24 h. On day 0,
975 calves were ranked according to sex, source and shrunk BW, and allocated to 1 of 21

976 drylot pens (7 × 15 m; 3 steers and 2 heifers per pen) in a manner that pens had
977 equivalent initial shrunk BW and calves from both sources to stimulate the stress of
978 comingling (Step *et al.*, 2008). Pens were assigned to receive 1 of 3 treatments: 1)
979 lasalocid (360 mg/calf daily of Bovatec; Zoetis, Florham Park, NJ, USA) +
980 chlortetracycline (350 mg/calf of Aureomycin at cycles of 5-day inclusion and 2-day
981 removal from diet; Zoetis) from day 0 to 32, and monensin only (360 mg/calf daily of
982 Rumensin; Elanco Animal Health, Greenfield, IN, USA) from day 33 to 60 (**PC**; n = 7), 2)
983 sodium saccharin-based sweetener (Sucram at 0.04 g/kg of diet DM; Pancosma SA;
984 Geneva, Switzerland) + plant extracts containing eugenol, cinnamaldehyde, and
985 capsicum (800 mg/calf daily of XTRACT Ruminants 7065; Pancosma SA) from day 0 to
986 32, and XTRACT only (800 mg/calf daily) from day 33 to 60 (**EG**; n = 7), or 3) no
987 supplemental ingredients (**CON**; n = 7). The inclusion and administration rate of the PC
988 and EG ingredients were according to manufacturer's recommendations for growing
989 cattle. Ionophores and chlortetracycline were chosen based on traditional U.S. feedlot
990 practices (Samuelson *et al.*, 2016). Chlortetracycline was supplemented from day 0 to
991 32 when elevated BRD incidence was expected (Snowder *et al.*, 2006; Lippolis *et al.*,
992 2017), whereas lasalocid was used during this period because it is approved for use in
993 combination with chlortetracycline (US Food and Drug Administration, 2017). In turn,
994 monensin was supplemented from day 33 to 60 when chlortetracycline supplementation
995 ended, given that monensin is the primary ionophore used by U.S. commercial feedlots
996 (Samuelson *et al.*, 2016). Sucram was supplemented from day 0 to 32 to stimulate DMI
997 upon feedlot arrival (McMeniman *et al.*, 2006), whereas XTRACT was supplemented
998 throughout the receiving period as an immunostimulant and dietary alternative to

999 ionophores (Yang *et al.*, 2010a; Yang *et al.*, 2010b).

1000 From day 0 to 60, calves had free-choice access to water and total mixed ration
1001 (**TMR**; Table 1), which was offered twice daily (0800 and 1300 h). Sucram was mixed
1002 daily in 4 L of water, whereas 2 L were mixed with the morning and 2 L with the
1003 afternoon TMR allocation of each EG pen (day 0 to 32). The PC and CON pens received
1004 the same amount of water without the addition of Sucram (day 0 to 32). Lasalocid,
1005 chlortetracycline, monensin, and XTRACT were mixed with soybean meal and top-
1006 dressed daily into the morning TMR feeding of respective PC or EG pens during the
1007 supplementation period (0.25 kg of mixture/calf daily). Moreover, chlortetracycline was
1008 supplemented to PC calves on day 0 to 4, day 7 to 11, day 14 to 18, day 21 to 25, and
1009 day 28 to 32. Soybean meal was also top-dressed into the morning TMR feeding of
1010 CON pens (0.25 kg/calf daily) from day 0 to 61, without the addition of the experimental
1011 ingredients. Based on daily visual observations, all pens consumed the top-dress within
1012 5 min after feeding.

1013 On day 0, calves were vaccinated against *Clostridium* and *Mannheimia*
1014 *haemolytica* (One Shot Ultra 7; Zoetis), *bovine respiratory syncytial virus (BRSV)*,
1015 *bovine herpesvirus-1 (BHV-1)*, *bovine viral diarrhea virus (BVD) 1 and 2*, and
1016 *parainfluenza-3 virus (PI3; Bovi-Shield Gold 5; Zoetis)*, and were administered an
1017 anthelmintic (Dectomax; Zoetis). On day22, calves were re-vaccinated against
1018 *Clostridium* (Ultrabac 8; Zoetis), BRSV, BHV-1, BVD 1 and 2, and PI3 (Bovi-Shield Gold
1019 5; Zoetis), following the manufacturer's recommendation for revaccination against these
1020 pathogens (Zoetis).

1021

1022 *Sampling*

1023 Samples of TMR ingredients were collected weekly, pooled across all weeks,
1024 and analyzed for nutrient content by a commercial laboratory (Dairy One Forage
1025 Laboratory, Ithaca, NY, USA). All samples were analyzed by wet chemistry procedures
1026 for concentrations of CP, ADF, and NDF as described by Lippolis *et al.* (2017).
1027 Calculations for net energy for maintenance and gain were calculated with the
1028 equations proposed by the NRC (2000). Nutrient profile of TMR is described in Table 1.

1029 Full BW was recorded on days 0, 5, 11, 22, 33, 43, and 60 of the experiment at
1030 0700 h, prior to the first TMR feeding of the day. Shrunk BW was recorded on day 61,
1031 after 16 h of water and feed withdrawal. Shrunk BW values from days -1 and 61 were
1032 used to calculate calf ADG during the experiment. Intake of TMR (DM basis) was
1033 evaluated daily from day 0 to 60 from each pen by collecting and weighing offered and
1034 non-consumed TMR. All samples were dried for 96 h at 50°C in forced-air ovens for DM
1035 calculation. Total TMR intake of each pen was divided by the number of calves within
1036 each pen, and expressed as kg per calf/day. Total BW gain and TMR intake of each
1037 pen were used for feed efficiency calculation. Calves were observed daily for BRD signs
1038 according to the DART system (Zoetis), and received antimicrobial treatment as in
1039 Lippolis *et al.* (2017).

1040 Blood samples were collected from all calves, concurrently with full BW
1041 evaluation into commercial blood collection tubes (Vacutainer, 10 mL; Becton
1042 Dickinson, Franklin Lakes, NJ, USA) containing no additive or containing freeze-dried
1043 sodium heparin for serum and plasma collection, respectively. During each sampling
1044 day, approximately 16 mL of blood was collected from each calf, being 8 mL in each

1045 collection tube. After collection, all blood samples were placed immediately on ice,
1046 centrifuged ($2\ 500 \times g$ for 30 min; 4°C) for plasma or serum harvest, and stored at -
1047 80°C on the same day of collection.

1048

1049 *Laboratorial analyses.*

1050 Plasma samples collected from day 0 to 33 were analyzed for cortisol (Immulite
1051 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) and haptoglobin
1052 concentrations (Cooke and Arthington, 2013), given that adrenocortical and acute-
1053 phase protein responses return to baseline levels in receiving cattle within 4 wk after
1054 feedlot entry (Cooke, 2017). Plasma samples collected on days 0, 33 and 60 were
1055 analyzed for IGF-I concentrations (Immulite 1000; Siemens Medical Solutions
1056 Diagnostics) to metabolically assess calf nutritional status throughout the experimental
1057 period (Lippolis et al., 2017). The intra- and inter-assay CV for haptoglobin were,
1058 respectively, 2.4 and 10.8%. Plasma IGF-I and cortisol were analyzed within single
1059 assays, and the intra-assay CV were, respectively, 7.4 and 1.9%.

1060 Serum samples collected from 2 calves/pen not observed with BRD signs during
1061 the experiment were selected for analysis of antibody titers against BRD pathogens, to
1062 ensure that this response was associated with vaccine efficacy rather than pathogenic
1063 infection (Callan, 2001). More specifically, samples collected on days 0, 5, 11, 22, 33,
1064 and 43 were analyzed for antibody titers against BRSV, BHV-1, BVD-1, and PI3 using
1065 virus neutralization tests, and for antibodies against *M. haemolytica* via a quantitative
1066 agglutination test (Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX,
1067 USA). It is not certain if selected calves were indeed healthy or just asymptomatic to

1068 BRD, although none of them exhibited BRD signs and clinical symptoms throughout the
1069 experimental period as mentioned previously.

1070

1071 *Statistical analysis.*

1072 Pen was considered the experimental unit for all analyses. Quantitative data
1073 were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA),
1074 whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst.
1075 Inc.) with a binomial distribution and logit link function. All data were analyzed using
1076 Satterthwaite approximation to determine the denominator df for tests of fixed effects,
1077 with pen (treatment) and calf (pen) as random variables, but for DMI and feed efficiency
1078 that used pen (treatment) as random variable. Model statements for initial and final BW,
1079 ADG, feed efficiency, and morbidity-related results contained the effects of treatment
1080 and calf sex as independent covariate. Model statements for DMI, cumulative BRD
1081 incidence, full BW change, and blood variables contained the effects of treatment, day,
1082 the resultant interaction, and calf sex as independent covariate. Blood variables were
1083 analyzed using results from day 0 as independent covariate, whereas calf source was
1084 also included as independent covariate for antibody titers against BRD pathogens. The
1085 specified term for all repeated statements was day, with pen (treatment) as subject for
1086 DMI and calf (pen) as subject for all other analyses. The covariance structure used was
1087 first-order autoregressive, which provided the smallest Akaike information criterion and
1088 hence the best fit for all variables analyzed. All results are reported as covariately-
1089 adjusted least square means. Significance was set at $P \leq 0.05$ and tendencies were

1090 determined if $P > 0.05$ and ≤ 0.10 . Repeated measures are reported according to main
1091 treatment effect if the treatment \times day interaction was $P > 0.10$.

1092

1093 **Results**

1094 A tendency for a treatment effect was detected ($P = 0.10$) for ADG, which was
1095 greater ($P = 0.04$) in PC vs. EG calves, and tended ($P = 0.09$) to be greater in PC vs.
1096 CON calves (Table 2). However, no main treatment effects were detected ($P \geq 0.55$) for
1097 final shrunk BW (day 61; Table 2) or full BW during the 60-day receiving period (Figure
1098 1). No treatment effects were detected for DMI ($P = 0.52$; Table 2 and Figure 2) and
1099 feed efficiency ($P = 0.23$; Table 2). Despite the lack of main treatment effect for feed
1100 efficiency, it should be noted that this response tended to be greater ($P = 0.09$) in PC
1101 vs. CON calves, and did not differ ($P \geq 0.38$) in EG vs. PC and CON calves.

1102 No treatment differences were detected ($P = 0.94$) for BRD incidence (Table 3),
1103 which were only observed during the initial 15 days of feedlot receiving (Figure 2; day
1104 effect, $P < 0.01$). No treatment differences were detected ($P \geq 0.39$; Table 3) for other
1105 morbidity reasons (physical injury), number of antimicrobial treatments required upon
1106 BRD diagnosis, and percentage of cattle that required ≥ 1 antimicrobial treatment upon
1107 BRD diagnosis. No incidence of mortality was observed during the experiment.

1108 No treatment effects were detected ($P \geq 0.56$) for plasma concentrations of
1109 cortisol, haptoglobin, and IGF-I (Table 4), whereas day effects were detected ($P \leq 0.01$)
1110 for these variables (Table 5). No treatment effects were detected ($P \geq 0.35$) for serum
1111 titers against *M. haemolytica*, PI3, BVD-1, and BHV-1 (Table 4). A tendency for a
1112 treatment effect was detected ($P = 0.09$) for serum titers against BRSV, which was

1113 greater ($P = 0.04$) in EG vs. PC and tended to be greater ($P = 0.08$) in EG vs. CON
1114 calves, and were similar ($P = 0.80$) between CON and PC calves. Moreover, day effects
1115 were also detected ($P \leq 0.01$) for all serum titers against BRD pathogens (Table 5).

1116

1117 **Discussion**

1118 Calves utilized in this experiment were considered high-risk, given that their prior
1119 management and health history were not fully known (Wilson *et al.*, 2017). Moreover,
1120 cattle experienced the stress of weaning, auction, transportation, commingling,
1121 vaccination, and feedlot entry within a 72-h period, whereas the combination of these
1122 stressors impacts cattle immunocompetence and performance (Cooke, 2017). Hence,
1123 the experimental model adopted herein represented the stress and health challenges
1124 that commercial feeder cattle typically experience during feedlot receiving in the U.S.
1125 (Duff and Galyean, 2007).

1126 Inclusion of chlortetracycline and ionophores in the receiving diet tended to
1127 improve cattle ADG as previously reported by others (Perry *et al.*, 1986; Duffield *et al.*,
1128 2012), although such difference was not sufficient to impact final BW on day 61 (Table
1129 2; Figure 1). This outcome should be primarily attributed to the tendency for improved
1130 feed efficiency in PC vs. CON cattle (Perry *et al.*, 1986; Birkelo, 2003), given that DMI,
1131 BRD incidence, as well as physiological responses were similar among all treatment
1132 groups (Table 2, 3, and 4). Accordingly, ionophores and chlortetracycline have been
1133 shown to increase feed efficiency in cattle by, respectively, improving rumen fermentation
1134 efficiency (Russell and Strobel, 1989; Callaway *et al.*, 2003), and increasing nutrient
1135 supply due to reduced intestinal mass and energy loss as methane (Vissek *et al.*, 1978;

1136 Zinn, 1993; Baldwin *et al.*, 2000). It also should be noted that DMI in the present
1137 experiment was not depressed by ionophore inclusion (Table 2; Figure 1), either
1138 lasalocid or monensin according to the experimental schedule, despite previous
1139 research reporting such outcome in feedlot cattle (Zinn, 1987; Duff *et al.*, 1995; Duff and
1140 Galyean, 2007).

1141 Cattle DMI and ADG were not improved by inclusion of sodium saccharin-based
1142 sweetener and plant extracts (Table 2; Figure 1). Contrary to our findings, Ponce *et al.*
1143 (2014) reported that supplementing the same sweetener utilized herein increased DMI
1144 by 17% during feedlot receiving, although this effect was observed when sweetener was
1145 included at 200 g/ton of diet DM. McMeniman *et al.* (2006) also included sodium
1146 saccharin-based sweetener into a feedlot receiving diet at 200 g/ton of diet DM, and
1147 reported a trend in increased DMI from day 29 to 56 after feedlot arrival. Yet, both
1148 authors also reported that ADG and feed efficiency were not impacted by inclusion of
1149 sodium saccharin-based sweetener. Perhaps DMI was not improved in EG cattle herein
1150 because the sweetener was included at a different dose than Ponce *et al.* (2014) and
1151 McMeniman *et al.* (2006), and was only fed from day 0 to 32 when receiving DMI is
1152 inconsistent and often inadequate (Duff and Galyean, 2007). Others have also reported
1153 that supplementing plant extracts also failed to improve ADG in ruminants consuming
1154 concentrate-based diets (Yang *et al.*, 2010a; Geraci *et al.*, 2012). It should be noted that
1155 the EG treatment prevented the decrease in feed efficiency when chlortetracycline and
1156 ionophores were not included into the diet, based on similar feed efficiency between EG
1157 vs. PC and tendency for greater feed efficiency in PC vs. CON calves (Table 2). Plant-
1158 derived organic compounds such as eugenol, cinnamaldehyde, and capsicum appear to

1159 enhance rumen fermentation in cattle consuming high-concentrate diets (Cardozo *et al.*,
1160 2005; Cardozo *et al.*, 2006), and may be used as alternatives to ionophores such as
1161 monensin (Fandiño *et al.*, 2008). Accordingly, Geraci *et al.* (2012) reported similar feed
1162 efficiency in feedlot cattle supplemented with monensin or a mixture of eugenol,
1163 cinnamaldehyde, and capsicum during an 84-day feeding period. In contrast, no
1164 research has compared the effects of plant extracts and/or sodium saccharin-based
1165 sweetener with chlortetracycline in feedlot receiving diets to further debate the
1166 performance results reported herein.

1167 Morbidity and BRD-related responses were similar among treatments (Table 3;
1168 Figure 2), which does not support our experimental hypothesis and the use of
1169 chlortetracycline supplementation to reduce morbidity during feedlot receiving (Duff *et*
1170 *al.*, 2000; Edwards, 2010; Samuelson, 2016). Others have also reported that dietary
1171 inclusion of sodium saccharin-based sweetener failed to mitigate BRD incidence in
1172 receiving cattle (McMeniman *et al.*, 2006; Ponce *et al.*, 2014), whereas research
1173 investigating the effects of plant extracts on BRD is lacking. It should be noted, however,
1174 that BRD incidence observed herein were not as elevated compared with previous
1175 research from our group (Lippolis *et al.*, 2017), as well as research conducted at
1176 commercial receiving yards reporting up to 43% of BRD incidence (Snowder *et al.*,
1177 2006). In fact, Lippolis *et al.* (2017) commingled cattle obtained from 7 cow-calf
1178 operations, which is typical of U.S. commercial feed yards, and reported BRD incidence
1179 at 66% during an 80-day receiving period. In this experiment, cattle were obtained from
1180 2 different sources due to market availability, which likely reduced commingling-elicited
1181 stress (Step *et al.*, 2008) and resulted less BRD incidence compared with previous

1182 research (Snowder *et al.*, 2006; Lippolis *et al.*, 2017). Hence, the reduced prevalence of
1183 BRD in the present experiment may have hindered proper assessment of feedlot
1184 receiving morbidity, and contributed to the lack of treatment effects on BRD-related
1185 responses.

1186 Supplementing sodium saccharin-based sweetener and plant extracts improved
1187 acquired humoral immunity against BRSV during the 60-day receiving period (Table 4).
1188 Serum antibody titers against all other BRD pathogens were not impacted by treatments
1189 but increased during the experiment (Table 5), denoting that cattle effectively acquired
1190 humoral immunity against these pathogens upon vaccination (Richeson *et al.*, 2008). It
1191 should be noted that calves were not revaccinated against *M. haemolytica* based on
1192 recommendations by the manufacturer (Zoetis), explaining why concentrations of serum
1193 titers against this pathogen did not increase beyond day 22. The exact mechanisms by
1194 which the EG treatment improved efficacy to BRSV vaccination (Callan, 2001) warrants
1195 investigation, and could be attributed to potential immunomodulatory effects of plant
1196 extracts (Ayrle *et al.*, 2016). Yet, such outcome was also not sufficient to alter BRD
1197 incidence in the present experiment, which corroborates with the lack of treatment
1198 differences on acquired humoral immunity against *M. haemolytica*, parainfluenza-3
1199 virus, BHV-1, BVD-1, and PI3.

1200 Similar concentrations of plasma cortisol, haptoglobin, and IGF-I among PC,
1201 CON, and EG cattle (Table 4) indicate that none of the experimental treatments
1202 modulated the physiological and acute-phase responses typically associated with
1203 feedlot receiving (Cooke, 2017). In turn, the specific impact of ingredients evaluated
1204 herein on these plasma variables are either variable or mostly undetermined. As

1205 examples, monensin has either increased or failed to change circulating IGF-I
1206 concentrations in growing beef cattle (Vendramini *et al.*, 2015; Vendramini *et al.*, 2016).
1207 Supplementing cinnamaldehyde to feedlot steers did not impact serum haptoglobin
1208 concentrations, but reduced concentrations of the acute-phase protein serum amyloid A
1209 (Yang *et al.*, 2010a). Nonetheless, day effects reported for plasma variables (Table 5)
1210 corroborate that cattle were exposed to the stress and nutritional changes associated
1211 with feedlot entry. Plasma haptoglobin concentrations transiently increased across all
1212 treatments upon feedlot arrival, corroborating that calves experienced an acute-phase
1213 protein response elicited by weaning, transport, vaccination, and feedlot entry (Cooke,
1214 2017). Plasma IGF-I concentrations increased across all treatments during feedlot
1215 receiving, mainly due to increased nutrient intake (Table 1) and growth (Table 2) during
1216 the experimental period (Lippolis *et al.*, 2017). Plasma cortisol concentration also
1217 increased across all treatments as the experiment progressed; the exact reason for this
1218 outcome is unknown, but may be associated with increasing concentrate inclusion in the
1219 TMR (Enemark, 2008). Hence, the tendencies for improved ADG and feed efficiency in
1220 PC calves were not associated with altered cortisol, IGF-I, and acute-phase responses
1221 elicited by transport and feedlot entry, although these responses influence nutrient
1222 utilization and growth in beef cattle (Cooke, 2017). Collectively, plasma variables
1223 evaluated herein failed to elucidate biological mechanisms by which chlortetracycline
1224 and ionophore supplementation benefited performance of receiving cattle; perhaps
1225 these occurred without substantial impacts on systemic inflammatory and metabolic
1226 responses.

1227 **Conclusions**

1228 Beef cattle supplemented with feed-grade antibiotics (lasalocid, chlortetracycline,
1229 and monensin) during feedlot receiving tended to have improved ADG and feed
1230 efficiency compared with non-supplemented cohorts. Replacing feed-grade antibiotics
1231 with plant extracts and sodium saccharin-based sweetener prevented the decrease in
1232 feed efficiency when feed-grade antibiotics were not included in the receiving diet.
1233 Moreover, cattle supplemented with these alternative feed ingredients had greater
1234 serum antibody response to BRSV, suggesting improved humoral response to
1235 immunization against this pathogen compared to all other treatments. Yet,
1236 supplementing feed-grade antibiotics or plant extracts and sodium saccharin-based
1237 sweetener did not reduce BRD incidence, which was not as prevalent as typically
1238 observed in commercial feedlot systems. Nonetheless, results from this experiment
1239 suggest that plant extracts and sodium saccharin-based sweetener may replace feed-
1240 grade antibiotics in feedlot receiving diets without substantially impairing feed efficiency.

1241

1242 **Acknowledgements**

1243 Financial support for this research was provided by Pancosma SA (Geneva,
1244 Switzerland). Dr. Reinaldo Cooke is also affiliated as permanent professor to the
1245 Programa de Pós-Graduação em Zootecnia / São Paulo State University (UNESP),
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1247

1248 **Declaration of interest**

1249 No conflict of interest to report.

1250

1251 **Ethics statement**

1252 All animals were cared for in accordance with acceptable practices and
1253 experimental protocols reviewed and approved by the Oregon State University,
1254 Institutional Animal Care and Use Committee (#4937).

1255

1256 **Software and data repository resources**

1257 No software, data, or models were deposited in official repositories.

1258

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1371 **Table 1** *Ingredient composition and nutrient profile of total mixed ration offered during*
 1372 *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

1373 ¹A = day 0 to 7; B = day 8 to 18; C = day19 to 32; and D = day 33 to 60. Calves had
 1374 free-choice access to the total mixed ration and water throughout the experimental
 1375 period.

1376 ²Cattleman's Choice (Performix Nutrition Systems, Nampa, ID, USA) containing 14%
 1377 Ca, 10% P, 16% NaCl, 1.5% Mg, 3 200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn,
 1378 140 mg/kg of Se, 6 000 mg/kg of Zn, 136000 IU/kg of vitamin A, 13000 IU/kg of vitamin
 1379 D3, and 50 IU/kg of vitamin E.

1380 **Table 2** Performance parameters from beef calves supplemented or not (**CON**; $n = 7$)
 1381 with feed-grade antibiotics (**PC**; $n = 7$) or alternative feed ingredients (**EG**; $n = 7$) during
 1382 a 60-day feedlot receiving¹

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

1383 ¹PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60;
 1384 EG = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant
 1385 extracts only from day 33 to 60; CON = no supplemental ingredients. Within rows,
 1386 values with different superscripts differ ($P \leq 0.05$).

1387 **Table 3** Morbidity and mortality parameters in beef calves supplemented or not (**CON**; $n = 7$) with feed-grade antibiotics
 1388 (**PC**; $n = 7$) or alternative feed ingredients (**EG**; $n = 7$) during a 60-day feedlot receiving¹

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	-	-

1389 ¹PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60; EG = sodium saccharin-based
 1390 sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to 60; CON = no supplemental
 1391 ingredients.

1392 ²All non-BRD related morbidity were due to physical injury.

1393 **Table 4** *Physiological and humoral responses from beef calves supplemented or not*
 1394 *(CON; n = 7) with feed-grade antibiotics (PC; n = 7) or alternative feed ingredients (EG;*
 1395 *n = 7) during a 60-day feedlot receiving¹*

Item	CON	PC	EF	SEM	P-value
<i>Physiological variables</i>					
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
<i>Serum antibody variables (titer log 2)</i>					
<i>Mannheimia haemolytica</i>	9.34	9.53	9.42	0.19	0.79
<i>Parainfluenza-3 virus</i>	6.60	5.89	6.05	0.35	0.35
<i>Bovine respiratory syncytial virus</i>	2.82 ^y	2.62 ^b	3.82 ^{ax}	0.42	0.09
<i>Bovine viral diarrhea virus-1</i>	2.62	2.87	3.42	0.43	0.39
<i>Bovine herpesvirus-1</i>	1.07	1.37	1.58	0.30	0.51

1396 ¹PC = lasalocid + chlortetracycline from day 0 to 32, and monensin from day 33 to 60;
 1397 EG = sodium saccharin-based sweetener + plant extracts from day 0 to 32, and plant
 1398 extracts only from day 33 to 60; CON = no supplemental ingredients. Within rows,
 1399 values with different superscripts differ at $P = 0.04$ (a,b) or $P = 0.08$ (x,y).

1400 **Table 5** Concentrations of plasma cortisol (ng/mL), haptoglobin (mg/mL), IGF-I (ng/mL), and serum titers against
 1401 *Mannheimia haemolytica* (**MH**), parainfluenza-3 virus (**PI3**), bovine respiratory syncytial virus (**BRSV**), bovine viral
 1402 diarrhea virus-1 (**BVD-1**), and bovine herpesvirus-1 (**BHV**) in beef cattle during an 60-day feedlot receiving ¹

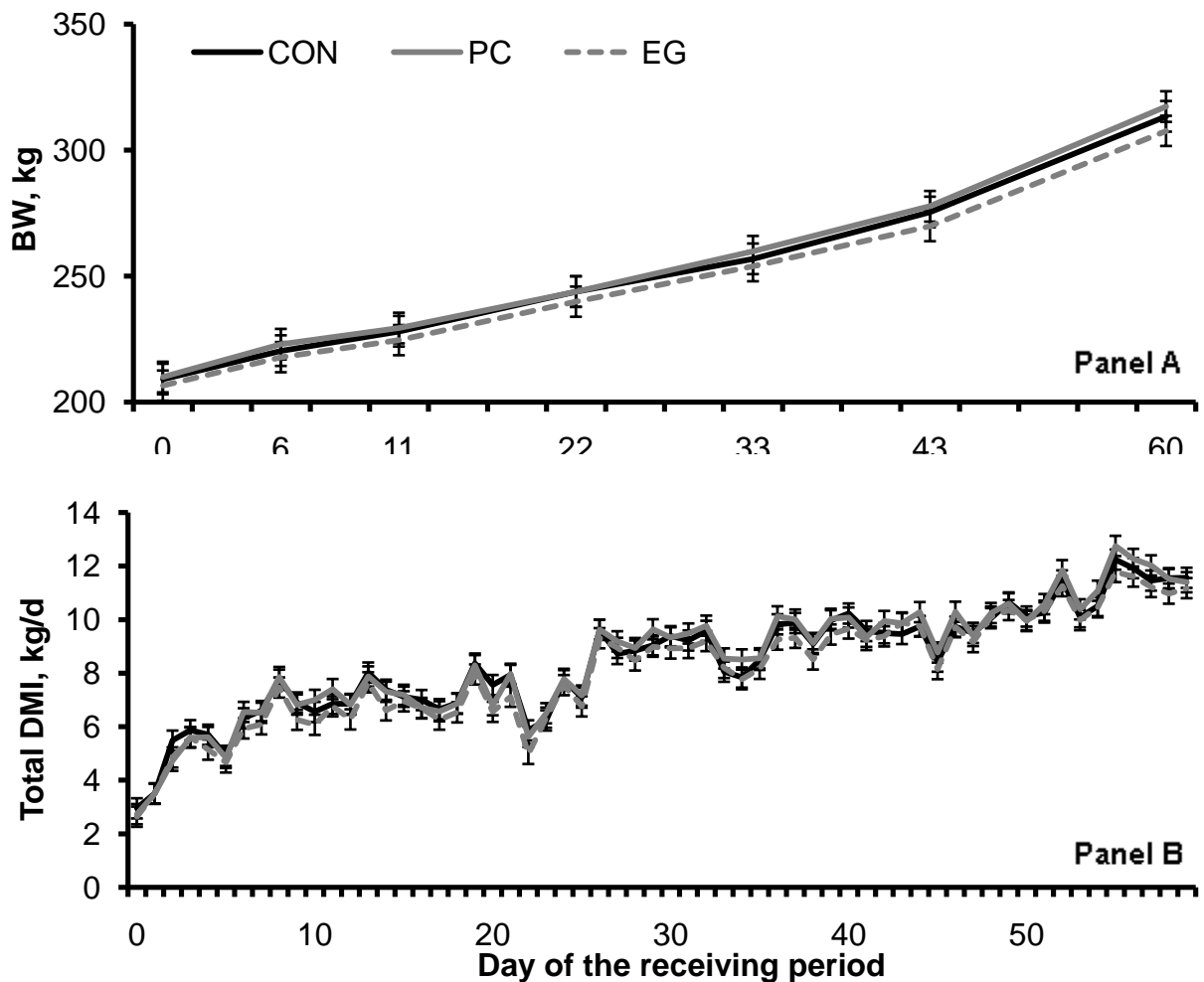
Day	Plasma variables			Serum antibody titers				
	Cortisol	Haptoglobin	IGF-I	MH	PI3	BRSV	BVD	BHV
0	41.5 ^d	0.151 ^d	88 ^c	6.05 ^e	4.86 ^d	0.63 ^d	0.76 ^d	0.19 ^d
5	40.4 ^d	0.383 ^a	-	7.34 ^d	5.31 ^{cd}	3.11 ^b	0.63 ^d	0.52 ^{cd}
11	54.0 ^c	0.278 ^{bc}	-	9.61 ^c	5.94 ^b	3.07 ^b	0.81 ^d	0.79 ^{bc}
22	57.6 ^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
<i>P</i> -value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

1403 ¹ Within columns, values with different superscripts differ ($P \leq 0.05$).

1404 **Figure captions**

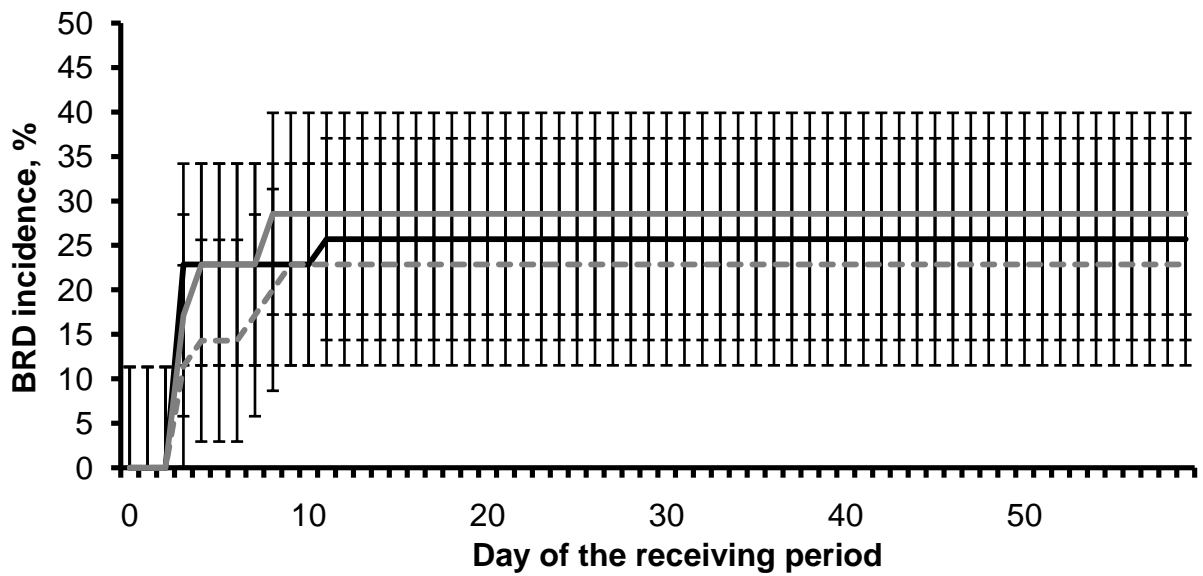
1405

1406 **Figure 1.** Body weight (Panel A) and DMI (total mixed ration; Panel B) during a 60-
 1407 day feedlot receiving from beef cattle assigned to: **PC** = lasalocid + chlortetracycline
 1408 from day 0 to 32, and monensin from day 33 to 60; **EG** = sodium saccharin-based
 1409 sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to
 1410 60; **CON** = no supplemental ingredients. Values reported are least square means \pm
 1411 SEM. No treatment effect was detected ($P \geq 0.52$).



1412

1413 **Figure 2.** Cumulative incidence of bovine respiratory disease (**BRD**) signs during a
1414 60-day feedlot receiving in beef cattle assigned to: **PC** = lasalocid + chlortetracycline
1415 from day 0 to 32, and monensin from day 33 to 60; **EG** = sodium saccharin-based
1416 sweetener + plant extracts from day 0 to 32, and plant extracts only from day 33 to
1417 60; **CON** = no supplemental ingredients. Values reported are least square means \pm
1418 SEM. No treatment effect or treatment \times day interaction were detected ($P \geq 0.94$).



1419