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ESTADO REDOX, PERFIL HEMATOLÓGICO E SAÚDE DE BEZERROS EM ALEITAMENTO RECEBENDO EXTRATOS VEGETAIS

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ESTADO REDOX, PERFIL HEMATOLÓGICO E SAÚDE DE BEZERROS EM ALEITAMENTO RECEBENDO EXTRATOS VEGETAIS[1]

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Resumo: Objetivou-se avaliar o efeito do fornecimento de extratos vegetais sobre o estado redox, perfil hematológico e sanidade de bezerros do nascimento aos 60 dias de vida. No primeiro estudo 23 bezerros foram alimentados com o leite proveniente de vacas que receberam diariamente as seguintes dietas: controle, sem a adição de extratos vegetais (CON), adição de 10 g de extrato de orégano (EO) e adição de 5,0 g de extrato de chá verde (ECV). Foram avaliados o escore fecal a cada dois dias, e, nos dias 1, 30 e 60 em relação ao nascimento, os atributos do perfil hematológico e do estado redox no sangue. Os extratos vegetais promoveram o aumento na concentração de neutrófilos em relação ao CON. Ocorreram interações significativas entre tratamentos e dia para Glutationa peroxidase (GPx) e TIOIS. Ao nascimento, maior atividade da GPx foi observada para os bezerros ECV, enquanto no dia 60, GPx foi inferior para ECV e EO em relação ao CON. Ao nascimento houve maior concentração de TIOIS no tratamento CON em relação aos extratos vegetais; enquanto no dia 30, foi superior para os bezerros EO e ECV comparados ao CON. Verificou-se menor concentração de diclorofluoresceína oxidada nos eritrócitos (DCFE) para os bezerros EO em relação aos demais e maiores concentrações de carbonilas, GSH e atividade da enzima catalase (CAT) para ECV em relação ao CON e EO. O fornecimento de leite proveniente de vacas suplementadas com extratos vegetais a bezerros pode melhorar alguns dos biomarcadores do estado redox e perfil hematológico. No segundo estudo, 38 bezerros leiteiros (17 no experimento I e 21 no experimento II) receberam diariamente os tratamentos: CON, sem adição de extratos vegetais no leite; EO, adição no leite de 70 mg de EO por kg de PC e ECV, adição no leite de 35 mg de ECV por kg de PC. Foram avaliados escores de fezes a cada dois dias e parâmetros de estado redox no sangue nos dias 1, 30 e 60 em relação ao nascimento. Interações significativas entre tratamentos e blocos (experimento) foram observadas para CARBO, SOD, GPx e DCFP, e entre tratamentos e dias para DCFP e DCFE. Os bezerros EO tenderam a ter menor concentração do antioxidante não-enzimático GSH em relação aos ECV, mas não diferiram dos CON. Os extratos vegetais não influenciaram na concentração de TIOIS, na atividade eritrocitária da enzima CAT e na frequência de diarreia. O fornecimento de ECV e EO a bezerros durante o aleitamento não foi efetivo em melhorar o estado redox e reduzir a frequência de diarreias.

Palavras-chave: chá verde, enzimas antioxidantes, período de aleitamento, orégano, saúde

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[1]Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (89p.), março de 2019.

REDOX STATE, HEMATOLOGICAL PROFILE AND HEALTH OF BEZERS IN SICKNESS RECEIVING VEGETABLE EXTRACTS [1]

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Abstract: The objective of this study was to evaluate the supply of plant extracts on the redox status, hematological profile and health of calves from birth 60 days of age. In the first study, 23 calves received maternal milk from cows that received the following diets: control, without the addition of plant extracts (CON), addition of 10 grams of oregano extract (OE) and addition of 5.0 grams of green tea extract (GT). The fecal score was evaluated at two day intervals and on days 1, 30 and 60 in relation to the birth the attributes of the hematological profile and the redox state in the blood. Plant extracts promoted an increase in neutrophil concentration in relation to the CON. There were significant interactions between treatments and day for Glutathione peroxidase (GPx) and TIOIS. On day 1, higher activity of GPx was observed for calves GT, while on day 60, GPx was lower for GT and OE in relation to CON. On day 1 there was a higher concentration of TIOIS in the CON treatment in relation to the plant extracts; while on day 30, it was superior for OE and GT calves compared to the CON. There was a lower concentration of oxidized dichlorofluorescein in erythrocytes (DCFE) for OE calves in relation to the other and higher concentrations of carbonyls, GSH and catalase enzyme activity (CAT) for GT in relation to CON and OE. The supply of milk from cows supplemented with extracts to calves may improve some of the redox biomarkers and hematological profile. In the second study 38 dairy calves (17 in experiment I and 21 in experiment II) received treatments daily: CON, without addition of vegetable extracts in milk; OE, addition in the milk of 70 mg of OE per kg of BW and GT, addition in the milk of 35 mg of GT per kg of BW. The fecal score was evaluated at two day intervals and on days 1, 30 and 60 in relation to the birth the redox status in the blood were evaluated. Significant interactions between treatments and blocks (experiment) were observed for CARBO, SOD, GPx and DCFP, and between treatments and days for DCFP and DCFE. OE calves tended to have a lower concentration of the GSH in relation to GT, but did not differ from the CON. Plant extracts did not influence TIOIS concentration, CAT erythrocyte activity and frequency of diarrhea. The supply of GT and OE to calves during lactation was not effective in improving redox status and reducing the frequency of diarrhea.

Key-words: antioxidant enzymes, green tea extract, health, oregano extract, pre-weaning period

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AGV: ácidos graxos voláteis
BCS: body condition score
BIC: critério de informação bayesiano
BW: body weight
C: catequinas simples
CARBO: carbonilas
CAT: catalase
CON: controle
DCFE: diclorofluoresceína nos eritrócitos
DCFH-DA: diclorodihidrofluoresceína-diacetato
DCFP: diclorofluoresceína no plasma
EC: epicatequinas
ECC: escore de condição corporal
ECG: galatoepicatequinas
ECV: extrato de chá verde
EDTA: ácido etilenodiamino tetra-acético
EGC: epigalocatequinas
EGCG: galato epigalocatequinas
EO: extrato de orégano
ERO: especies reativas de oxigênio
GCG: galocatequinas-galato
GMD: ganho médio diário
GPD: ganho de peso diário
GPx: glutationa-peroxidase
GSH: glutationa
GT: green tea extract
H_2O_2: peróxido de hidrogênio
O_2: oxigênio
O_2^-: superóxido
OE: oregano extract
OH: hidroxila
PC: peso corporal
ROS: oxygen reactive species
SEM: standard error of the mean
SH: sulfidrila
SOD: superóxido-dismutase

CAPÍTULO I

1. INTRODUÇÃO

A crescente preocupação com a presença de traços de contaminantes nos produtos de origem animal, somada à resistência bacteriana aos antibióticos convencionais, tem estimulado a busca por alternativas aos agentes químicos, utlizando produtos naturais, potencialmente mais seguros, que poderiam ser utilizados em sistemas de produção. Nesse âmbito tem-se aumentando as pesquisas acerca do uso de extratos vegetais na alimentação animal.

Catequinas e carvacrol são os principais metabólitos secundários produzidos por plantas de chá verde e orégano, respectivamente. Em ruminantes eles são basicamente usados como aditivos alimentares devido ao seu potencial para modular a fermentação ruminal e atuar na capacidade antioxidante.

O extrato de chá verde apresenta ações benéficas, incluindo caracterísitcas antiinflamatórias e antioxidantes. O chá verde pode reduzir os radicais livres, como as espécies reativas de oxigênio, quelando-os e desempenha um papel importante no combate a diferentes microrganismos como *Escherichia coli, Salmonella*, *Staphylococcus aureus*, alguns fungos e vírus. O extrato de oregano é conhecido por sua ação antimicrobiana, tanto em bactérias gram positivas quanto em gram negativas, bem como atuação como anti-inflamatório e antioxidante. No entanto existem poucos estudos em bezerros, o que motivou a presente pesquisa.

Os extratos vegetais podem ser utilizados como aditivos na suplementação de bezerros leiteiros na fase de aleitamento, sendo essa fase desafiadora aos animais lactentes, momento em que o animal está passando por alterações relacionadas aos aspectos do sistema imunológico e digestivo dos mesmos. Na fase de pseudoruminante, os anticorpos oriundos da transferência passiva estão em baixas concentrações, e seu sistema imune ainda é imaturo, desde que o bezerro está apenas começando a ter suas próprias respostas de anticorpos à microbiota ambiental.

Diversas doenças são associadas com o aumento da formação de espécies reativas de oxigênio, causando estresse oxidativo. Em bezerros lactentes, em casos de enfermidades, ocorre o aumento do estresse oxidativo, afetando negativamente o sistema antioxidante, prejudicando o desenvolvimento e a sobrevivência dos animais.

O perfil hematológico e bioquímico de bezerros passa por alterações fisiológicas importantes em adaptação à vida extrauterina, cuja natureza complexa dificulta a diferenciação entre os eventos normais e aqueles ocasionados por processos mórbidos. O hemograma é uma ferramenta de avaliação importante uma vez que, quando correlacionado ao exame clínico, pode fornecer informação relevante quanto à natureza e evolução da enfermidade.

Entretanto, existe uma deficiência de estudos relacionados aos efeitos desses extratos sobre as respostas hematológicas e o estado redox de bezerros leiteiros, se as vacas alimentadas com esse composto poderiam passar maior capacidade antioxidante à progênie e se haveria resposta

diferencial no metabolismo, estresse oxidativo e incidência de diarreia caso os extratos vegetais fossem ministrados diretamente no leite ou via transferência pelo leite da vaca.

O objetivo desse estudo foi avaliar indicadores do estado redox e das condições de saúde, de bezerros leiteiros em aleitamento, suplementados com extrato de chá verde (*Camellia sinensis*) ou de orégano (*Origanum vulgare*) fornecidos indiretamente via leite materno (proveniente de vacas suplementadas com os extratos comparadas com o controle não suplementado) e via direta, quando os bezerros receberam os extratos no momento de sua alimentação.

2. REVISÃO BIBLIOGRÁFICA

2.1. Criação de bezerras leiteiras

A criação da bezerra é fundamental para repor as vacas de descarte, melhorar o patrimônio genético do rebanho e elevar a produtividade e os índices econômicos da propriedade leiteira. A correta criação de bezerras durante a fase de aleitamento é o primeiro passo para o sucesso da atividade leiteira, já que a maior mortalidade em bovinos leiteiros é verificada no primeiro mês de vida (Oliveira et al., 2009). As principais metas para o sucesso na criação de bezerros devem ser minimizar a incidência de doenças e mortalidade nos primeiros quatro meses de vida, dobrar o peso ao nascimento na desmama, atingir a puberdade e maturidade sexual precocemente (50% do peso adulto) e ser economicamente viável (Coelho, 2009).

2.2. Sistema imunológico de bezerros

As bezerras recém-nascidas são agamaglobulinêmicas (Kampen et al., 2006), em virtude do tipo de placenta presente nos ruminantes (epitélio-corial), que não permite a transferência de anticorpos da mãe para o feto durante a gestação. Os bezerros dependem totalmente do colostro da mãe para adquirir imunidade inicial (Kertz et al., 2017). Nesse sentido, o colostro é considerado essencial para a sobrevivência do neonato (Church, 1974) e consiste da combinação de secreções lácteas e constituintes de soro sanguíneo, chamadas de imunoglobulinas e outras proteínas séricas, que se acumulam na glândula mamária no período pré-parto.

O mecanismo de defesa de transferência de imunoglobulinas da vaca para o bezerro é chamado de imunidade passiva (USDA, 2008), e é responsável pela proteção inicial contra infecções (Black, 2003), na qual as imunoglobulinas inativam ou destroem os antígenos que ameaçam a integridade do hospedeiro (Logan, 1974). No colostro bovino são encontradas três importantes imunoglobulinas: IgG (e seus isotipos IgG1, de maior ocorrência, e IgG2), IgM e IgA. No que se refere à composição das imunoglobulinas presentes no colostro, 70-80% são de IgG, 10-15% de IgM e 10-15% de IgA. Cada uma tem uma função específica, sendo a IgG responsável pela imunidade sistêmica, identificando e destruindo os patógenos. A IgM e a IgA estão relacionadas com a imunidade local do intestino do bezerro, servindo como primeira linha de defesa e protegendo a mucosa, respectivamente.

As variações nas concentrações das imunoglobulinas no soro dos bezerros estão associadas com a mortalidade neonatal (Mcguire et al., 1976; Burton et al., 1989), pois a falha nessa transferência predispõe o recém-nascido ao desenvolvimento da doença (Weaver et al., 2000).

A defesa contra microrganismos é mediada por reações iniciais da imunidade inata e por respostas tardias da imunidade adquirida. A imunidade inata consiste na linha de defesa inicial contra micro-organismos, composto por (1) barreiras físicas e químicas como os epitélios; (2) células fagocitárias, dentríticas e *natural killer*; (3) proteínas do sangue e (4) proteínas denominadas citocinas, que regulam e coordenam muitas das atividades das células da imunidade inata (Abbas et al., 2011).

A imunidade inata é uma rede de subsistemas conectados formados por diversos mecanismos de defesa. O mais importante é o processo de inflamação que concentra as células de defesas, como os leucócitos, que compreendem as células fagocitárias, como os monócitos, macrófagos e neutrófilos (Roitt, 1999).

Além dos mecanismos inatos, existe outro tipo de resposta do sistema imunológico: a imunidade adaptativa ou adquirida. Esse sistema é complexo e responsável pela proteção final do organismo, possuindo duas linhas de defesas, a resposta imune humoral e a resposta imune celular (tizard, 2008). Esse sistema compreende um grupo importante de leucócitos conhecidos como linfócitos, que estão enquadrados em duas categorias básicas, os linfócitos T e os linfócitos B (Roitt, 1999). Os linfócitos B produzem anticorpos, enquanto que os linfócitos T funcionam na regulação imune e na imunidade citotóxica (Jones & Alisson, 2007). As principais características da resposta adquirida são: especificidade e diversidade de reconhecimento, memória, especialização de resposta, autolimitação e tolerância a componentes do próprio organismo (Cruvinel et al., 2010).

2.3. Desenvolvimento do sistema digestivo em bezerros

O estômago dos ruminantes é, na verdade, um estômago simples, modificado pela expansão acentuada da região esofágica em três divertículos distintos e volumosos (o rúmen, o retículo e o omaso), conhecidos como compartimentos gástricos anteriores. Esses compartimentos não são glandulares e compreendem uma série de câmaras, nas quais o alimento é submetido à digestão por microrganismos antes de passar, através do trato digestório, para a parte menor e glandular do estômago dos ruminantes, o abomaso (Frandson et al., 2011). O desenvolvimento desses compartimentos ocorre em três fases: 1) compreende do nascimento até a terceira semana de idade, quando o bezerro é considerado um não ruminante; 2) engloba a fase de transição dos compartimentos estomacais, da terceira a oitava semana; e 3) quando o bezerro está com mais de oito semanas de vida e passa a ser considerado um ruminante (Church, 1974). Durante essas fases, o trato gastrointestinal do bezerro passa por várias alterações anatômicas e fisiológicas, desde o desenvolvimento do rúmen até sua total funcionalidade (Suárez et al., 2006).

Durante as três a quatro semanas iniciais do período de aleitamento os ruminantes se comportam fisiologicamente como animais monogástricos. Através da goteira esofágica, o leite ingerido é conduzido do esôfago direto ao abomaso. Nessa fase a atividade digestiva é exercida pelo abomaso. Essa é a fase mais desafiadora em relação à nutrição, devido às limitações enzimáticas e à ausência de síntese microbiana.

Os bezerros nascem com o rúmen essencialmente afuncional e, por isso, dependem dos nutrientes oriundos do leite, os quais são digeridos pelas enzimas no abomaso, absorvidos no intestino e transferidos para a corrente sanguínea, sendo essa a forma como os bezerros recebem energia para manutenção e crescimento (Baldwin et al., 2004; Drackley, 2008).

Geralmente, a alimentação sólida é fornecida durante o aleitamento e é incrementada após o manejo de desmame dos bezerros. Com isso, a mudança mais significativa no desenvolvimento dos microrganismos ruminal e intestinal ocorre nesse período, marcada pelo início dos processos fermentativos no rúmen, no qual o bezerro deixa de ser um psedouruminante e passa a ser um ruminante (Meale et al., 2016). Nesse momento de transição, ocorre o estabelecimento de bactérias amilolíticas, no primeiro momento, seguido dos microoganismos celulolíticos e metanogênicos (Bittar et al., 2011). No entanto, a introdução de grãos antes de um mês de idade permite que o rúmen amadureça mais rapidamente (Longenbach & Heinrichs, 1998).

O desenvolvimento do intestino em bezerros ocorre no período fetal e perinatal (Baldwin et al., 2004). Até o nascimento, o trato gastrointestinal é estéril; após o nascimento, os microrganismos intestinais são introduzidos a partir de microbiota fecal, vaginal e ambiental (Soto et al., 2011). Nas primeiras 24 horas, se verifica a colonização por bactérias (LI et al., 2012). A microbiota intestinal é estabilizada nas primeiras semanas de vida (Bunešová et al., 2015) e qualquer falha na ingestão de colostro pode ser prejudicial para o equilíbrio do ecossistema intestinal em bezerros. O crescimento e a diferenciação das células epiteliais do intestino de bezerros neonatais são desencadeados pela ingestão do colostro. O colostro de melhor qualidade (imunoglobulinas presentes devem ser maiores do que 50 mg de IgG/mL) pode ajudar os bezerros a estabelecerem seu próprio mecanismo de defesa imunológica imediatamente após o nascimento, o que pode auxiliar na redução dos efeitos de microrganismos nocivos, promovendo o desenvolvimento intestinal e, consequentemente, diminuindo a morbidade e a mortalidade de bezerros (Yang et al., 2015).

2.4. Estado redox

O termo utilizado para definir qualquer espécie de átomo ou molécula que possua um ou mais elétrons de valência desemparelhados é radical livre (RL). Este desparelhamento pode decorrer pela perda (oxidação) ou ganho (redução) de um elétron de uma substância. São altamente instáveis, quimicamente muito reativos e com meia vida curta (Halliwell & Gutteridge,

2007). Os radicais livres podem ser gerados no citoplasma, mitocôndria ou membranas celulares.

Porém, quando há um desequilibrio entre a produção de radicais livres e os mecanismos antioxidantes, ocorre um processo denominado estresse oxidativo (EO). Este desequilíbrio pode ser resultante de um aumento na produção de RL, ou ainda, devido à diminuição de mecanismo antioxidante (Fang et al., 2002). Os radicais livres em excesso reagem com qualquer componente celular como proteínas, lipídios e ácido nucléico, dependendo do seu sítio de formação (Rosenfeldt et al., 2013). Essas reações desencadeiam danos celulares que, dependendo da intensidade do estresse, podem acionar o mecanismo de apoptose ou necrose celular (Culotta., 2000).

O oxigênio é essencial para a oxidação de compostos orgânicos e a produção de energia para o metabolismo celular (Comhair & Erzurum, 2002). Uma pequena quantidade do oxigênio consumido (2 a 5%) é parcialmente reduzido e produz uma variedade de substâncias químicas altamente reativas, denominadas espécies reativas do oxigênio (ERO) (Halliwell & Gutteridge, 1999; Damasceno et al., 2002).

As EROs são encontradas em praticamente todos os sistemas biológicos. No metabolismo aeróbico, o oxigênio (O_2) sofre redução tetravalente, formando reativos intermediários, como o superóxido ($O_2^{-\bullet}$), a hidroxila ($^{\bullet}OH$) e o peróxido de hidrogênio (H_2O_2) (Halliwell, 2007). As EROs podem ser mediadoras de doenças, embora nem sempre sua formação seja prejudicial, pois podem induzir a defesa dos organismos. A formação de radicais livres *in vivo* pode ocorrer via ação catalítica de enzimas, tais como NADPH-oxidase e xantina-oxidase, durante os processos de transferência de elétrons que acontecem no metabolismo mitocondrial normal, bem como na reação inflamatória, por exemplo. Contudo, a concentração desses radicais pode aumentar devido à maior geração intracelular ou à deficiência dos mecanismos antioxidantes.

Neste contexto, existem os biomarcadores, os quais podem ser utilizados para avaliar o equilíbrio redox e estimar os riscos e danos causados pelo estresse oxidativo ainda verificar a deficiência das defesas antioxidantes. Os principais biomarcadores de estado redox e estresse oxidativo são a concentração de compostos carbonílicos e sulfidrílicos, os quais mensuram o dano às proteínas; atividade das enzimas antioxidantes como a superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GPx), além da concentração de glutationa reduzida (GSH) e a oxidação de diclorodihidrofluoresceína-diacetato (DCFH-DA).

Antioxidantes podem ser definidos como qualquer substância endógena ou exógena capaz de neutralizar um radical livre (Vannucchi et al., 1998), em geral pela doação de um elétron. Os mecanismos de inibição ou redução dos danos causados pelos radicais livres podem ser preventivos, ou seja, impedindo a formação destes, ou ainda, reconstrutivos, favorecendo o reparo das estruturas lesadas (Koury & Donangelo, 2003). Os antioxidantes podem ser de origem enzimática, como a SOD, a CAT e a GPx. E também podem ser de origem não enzimática, incluindo a glutationa (GSH) e as vitaminas A, C e E (Sies, 1997).

A SOD é uma metaloenzima que contém cobre e zinco (Cu-Zn-SOD) em seu sítio ativo, quando presente no citosol, manganês (Mn-SOD) quando presente na mitocôndria e ferro (Fe-SOD) na isoforma extracelular. Ela apresenta capacidade de interceptar o ânion superóxido gerado pelo metabolismo celular ou por fontes externas, atuando sobre aminoácidos, lipídios e bases de ácido desoxirribonucleico (DNA), impedindo a formação de lesões. Sua atividade consite em converter o ânion superóxido em peróxido de hidrogênio, o qual será eliminado por outras enzimas antioxidantes. Essa enzima é encontrada no citosol de praticamente todas as células eucariontes, e sua ação é importante para o funcionamento do metabolismo, a reprodução, a diferenciação celular e a defesa imunológica, entre outros (Goodsel, 2007).

A CAT é uma enzima que degrada o peróxido de hidrogênio em água e oxigênio livre de forma extremamente rápida. Está presente em animais, vegetais e em algumas bactérias. Nos mamíferos, sua maior concentração encontra-se principalmente nos peroxissomos. A atividade da CAT, nos tecidos humanos, é maior no fígado, no rim, no pulmão e no músculo, respectivamente (Halliwell; Gutteridge, 1999). Os órgãos que não possuem peroxissomos estão mais expostos aos danos celulares provocados por EROs, entre eles o coração, os pulmões e o cérebro.

A GPx é responsável pela detoxificação de peróxidos orgânicos e inorgânicos. Sua ação é dependente da GSH, que é oxidada em GSSG durante a conversão de peróxido de hidrogênio em água. Os níveis de GSH reduzido são mantidos por meio da oxidação do NADPH resultante do ciclo das pentoses-fosfato. Ela é encontrada no citosol e nas mitocôndrias, representando um importante papel na defesa antioxidante, pois reduz os hidroperóxidos pelo selênio presente em sua composição. A hiperglicemia diminui a atividade da GPx no endotélio vascular, nas células dos rins e nas mitocôndrias do cérebro (Ballatori, 2009), o qual é muito sensível à redução da atividade da GPx.

A GSH é um tripeptídeo formado por glutamato, cisteína e glicina e possui atividade química redutiva através do seu grupo sulfidrila (SH). É considerada a primeira linha de defesa do organismo contra as EROs, de modo que sua redução está ligada diretamente ao aumento do estresse oxidativo. Possui importantes funções no organismo, pois mantém o funcionamento do sistema imune, é antioxidante e removedor de radicais livres, atua na reparação de proteínas e lipídios e no transporte transmembrana, e sua ação mais importante ocorre nas mitocôndrias. É uma molécula essencialmente antioxidante e destoxificante, mas também está envolvida no transporte de aminoácidos através da membrana, na proteção contra radiações solares e em muitos processos metabólicos, incluindo a apoptose. Sua depleção ou redução implica danos celulares, que são diretamente proporcionais a sua quantidade e viabilidade celular (Forman et al., 2009). O aumento da concentração de GSH nos tecidos promove a prevenção dos danos provocados pelo estresse oxidativo, enquanto que a deficiência de GSH está relacionada a maior vulnerabilidade do organismo a esses danos provocados resultando em danos metabólicos.

A produção intracelular de ROS pode ser detectada utilizando-se o composto não fluorescente diclorofluoresceína-diacetato (DCFH-DA, do inglês

dichlorofluorescein diacetate) o qual é permeável à membrana celular. O DCFH-DA é hidrolisado pelas esterases intracelulares tornando-se diclorofluoresceína não fluorescente (DCFH), a qual é então oxidada pela ação de espécies reativas intracelulares gerando o composto fluorescente diclorofluoresceína (DCF) (Almeida et al., 2008).

2.5. Extratos vegetais na alimentação animal

Todas as plantas produzem compostos químicos como parte de suas atividades metabólicas normais. Estes compostos são divididos em metabólitos primários - tais como açúcares e gorduras, encontradas em todas as plantas, essenciais para o desenvolvimento das plantas; e metabólitos secundários, ou fitoquímicos - compostos não essenciais para a função básica de desenvolvimento, responsáveis por proporcionar o cheiro e a cor das plantas, atuando também nos processos de defesa da planta contra patógenos e herbivoria, na reprodução e resistência a situações adversas (Hashemi & Davoodi, 2011).

Dentre os principais compostos sintetizados podem-se citar os óleos essenciais, as saponinas, as substâncias picantes e amargas, os flavonoides e os polifenóis, que variam em sua disponibilidade de acordo com a espécie e parte da planta da qual são extraídos, além da forma de extração (Burt, 2004). De acordo com Christaki et al. (2012), os compostos produzidos pelas plantas podem ser aplicados tanto na nutrição e promoção de saúde humana quanto animal, sendo igualmente importantes, uma vez que a melhoria da saúde animal pode traduzir-se na melhoria da segurança e da qualidade dos alimentos consumidos pelos humanos.

2.5.1. Chá verde *(Camellia sinensis)*

O chá verde foi introduzido na China e no Japão no início do século VIII, sendo usado como remédio (Ishihara et al., 2001). É derivado de *Camellia sinensis* L., e é uma das bebidas mais consumidas no mundo (Senanayake, 2013). É cultivada nas regiões tropicais e temperadas da Ásia, como a China, Índia, Sri Lanka e Japão. As diferentes idades das folhas produzem qualidades variadas do chá, com composições químicas distintas, sendo as folhas imaturas preferencialmente colhidas e processadas para a produção de chá e extrato (Senanayake, 2013), as quais são comercialmente disponíveis em forma de folha seca ou pó.

As catequinas são os principais flavonóides presentes no chá verde. As quatro principais catequinas são (-)-epigallocatequina-3-gallato (EGCG), que representa aproximadamente 59% das catequinas totais; (-)-epigallocatequina (EGC) (19% aproximadamente); (-)-epicatequina-3-gallato (ECG) (13,6% aproximadamente); e (-)-epicatequina (EC) (6,4% aproximadamente) (McKay e Blumberg, 2002). Embora as catequinas sejam os compostos fenólicos dominantes (Kilmartin e Hsu, 2003), diversos flavonóis

(até 4%) e flavonas (em traços) também estão presentes nas folhas de chá. Outros compostos relacionados encontrados no chá verde são ácido gálico, cumárico e cafeico, bem como os alcalóides de purinas, teobromina e cafeína (Rusak et al., 2008).

Os polifenóis são metabólitos secundários de plantas, geralmente envolvidos na defesa contra a radiação ultravioleta ou agressão por patógenos (Manach et al., 2004) e podem ser classificados em grupos diferentes em função do número de anéis fenólicos que contêm os elementos estruturais que os ligam entre si. Um desses grupos é o dos flavonóides, que partilham uma estrutura comum composta por dois anéis aromáticos ligados entre si por três átomos de carbono, formando um heterociclo oxigenado, e podem ser divididos em flavonóis, flavonas, isoflavonas, flavanonas, antocianidinas, e flavanóis (catequinas e proantocianidinas – Figura 1), em função do tipo de heterociclo envolvido. Cerca de 88% dos polifenóis presentes no chá verde são catequinas e seus derivados (Cyboran et al., 2015). A atividade antioxidante dos polifenóis do chá verde é primariamente atribuida à combinação de anéis aromáticos e grupos hidroxil que são os responsáveis por neutralizar os radicais livres (Senanayake, 2013). Dessa forma, a epicatequina galato e a epilocatequina galato são as catequinas mais importantes para promoção de saúde (Khan & Mukhtar, 2007; Senanayake, 2013), devido ao número de anéis aromáticos além do número e posição dos grupos hidroxil na molécula (Farkas et al., 2004).

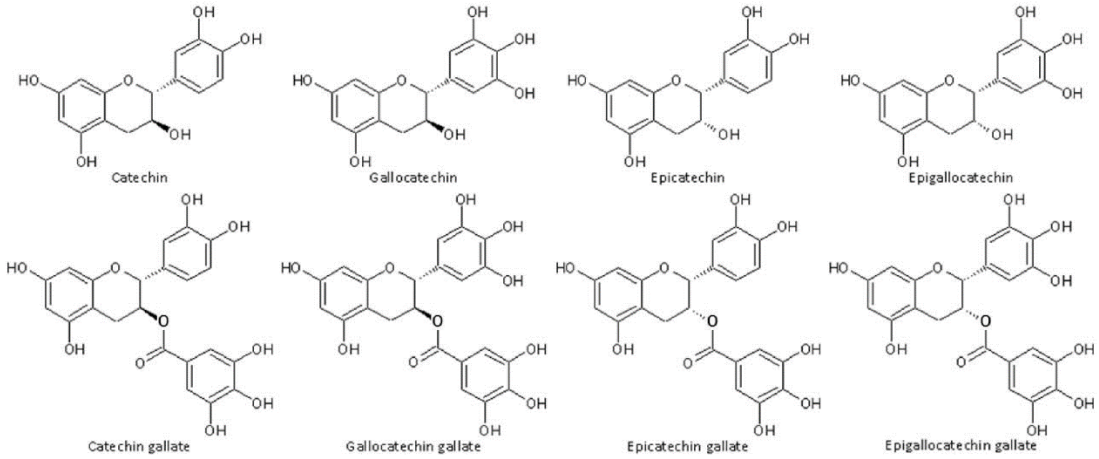

FIGURA 1 – Estrutura química das principais catequinas do chá verde (Fonte: Wein et al., 2016).

As propriedades promotoras de saúde das catequinas são relacionadas à sua atividade antioxidante e anti-inflamatoria (Ishihara et al., 2001; Nishida et al., 2006; Gonçalves et al., 2015; Cyboran et al., 2015; Wein et al., 2016), sendo os efeitos sobre a saúde dependentes da quantidade consumida e da biodisponibilidade (Manach et al., 2004; Khan & Mukhtar, 2007).

A estabilidade das catequinas presentes no chá verde depende do pH e da temperatura sendo estáveis em pH ácido (1,8 a 6,4), como as encontradas no estômago, mas são extensivamente degradadas em pH alcalino semelhante ao encontrado no intestino delgado (Zhu et al., 1997; Lamothe et al., 2014). Entretanto, a epigalocatequina galatto é estável até um pH de 7,4 (Yoshino et al., 1999).

Em animais monogástricos, as catequinas são parcialmente degradadas e absorvidas no intestino delgado por meio de difusão passiva simples e difusão facilitada, com auxílio de proteínas transportadoras na membrana (Yoshino et al., 1999; Starp et al., 2006). Em ruminantes, foi evidenciado que as catequinas são extensivamente degradadas no rúmen de ovinos (Gladine et al., 2007) e vacas leiteiras (Wein et al., 2016). De acordo com Lambert & Yang (2003) e Khan & Mukhtar (2007), as principais vias metabólicas para as catequinas do chá verde são glucuronidação, sulfatação e metilação. Isso gera diferentes metabólitos intermediários, os quais são convertidos aos ácidos fenilacético e fenilpropiônico no intestino, passam à circulação e atingem os tecidos. Em seres humanos e outros animais monogástricos, as catequinas foram detectadas no plasma após administração oral de chá verde (Nakagawa et al., 1997; Manach et al., 1999; Lambert & Yang, 2003; Manach et al., 2004; Abrahamse et al., 2005; Cyboran et al., 2015).

Em ruminantes, existem apenas dois estudos onde a biodisponibilidade das catequinas do chá verde foi avaliada (Gladine et al., 2007; Wein et al., 2016). Wein et al, (2016) avaliaram a absorção sistêmica das catequinas do chá verde após aplicação intraruminal e intraduodenal em vacas, bem como a degradação ruminal das catequinas e produção de gás *in vitro*. Após aplicação intraruminal, os autores observaram níveis de catequinas no plasma abaixo do limite de detecção, mas detectaram picos de prováveis metabólitos das catequinas. Quando o chá verde foi aplicado no duodeno, as catequinas foram detectadas no plasma entre 1,5 a 2,5 horas após a administração, sugerindo que esses compostos são degradados pela microflora ruminal. Para comprovar essa hipótese, os autores testaram a degradação ruminal *in vitro* com 24 horas de incubação utilizando fluído ruminal ativo e inativo e observaram que a concentração de catequinas se reduziu continuamente apenas quando fluído ruminal ativo foi utilizado. Como a microbiota ruminal impacta de forma negativa a biodisponibilidade das catequinas, os autores sugerem que os metabólitos gerados sejam investigados quanto ao seu potencial benéfico à saúde tanto de forma sistêmica, quanto nos tecidos do trato gastro intestinal, e sugerem a possibilidade de uso do extrato na forma protegida.

No entanto o processo digestivo em ruminantes não inibe as propriedades benéficas dos polifenois de chá verde, como aumento no status antioxidante no plasma de ovinos (Gladine et al., 2007) e de novilhas (Nishida et al., 2006); e redução *in vitro* do dano ao tecido mamário de vacas leiteiras causado pela mastite (Lauzon et al., 2005), redução no quadro de diarreia em bezerros (Maciej et al., 2016) e segundo Elshahawy (2018), o chá verde foi efetivo no combate ao estresse oxidativo e danos produzidos pelo metabolismo celular em bezerros leiteiros.

Os resultados descritos até o momento com o uso dos polifenois em ruminantes são limitados e de difícil comparação, devido à variação das doses e produtos. Dessa forma, mais estudos são ncessários visando esclarecer o potencial desses compostos em atuar na capacidade antioxidante e melhorar o estado de saúde, especialmente em bezerros na fase de aleitamento.

2.5.2. Orégano (*Origanum vulgare*)

O orégano (*Origanum vulgare*) é uma planta perene que pertence à família *Lamiacea*. Várias espécies do gênero *Origanum* são nativas do Mediterrâneo Europeu e muito utilizada na cozinha como tempero, e contém compostos odorosos, voláteis, hidrofóbicos e altamente concentrados chamados de óleos essenciais (Christaki et al., 2012; Cobellis et al., 2016). Os óleos essenciais são metabólitos secundários produzidos pelas plantas, que têm como principal componente os terpenos, os quais apresentam diferentes estruturas e funções.

Os terpenos são classificados de acordo com o número de carbonos existentes em sua cadeia principal, sendo os monoterpenos (C10) as moléculas mais representativas, constituindo cerca de 90% dos principais óleos essenciais (Cobellis et al., 2016). O orégano é composto por aproximadamente 20 óleos essenciais diferentes, sendo os monoterpenos carvacrol e timol aqueles encontrados em maior quantidade, 59,7 e 13,7% dos óleos essenciais, respectivamente (Lagouri et al., 1993; Utlee et al., 2002; Busquet et al., 2006). Ambos são estruturalmente muito semelhantes (Figura 1), variando apenas na posição do grupo hidroxil no anel fenólico (Lambert et al., 2001).

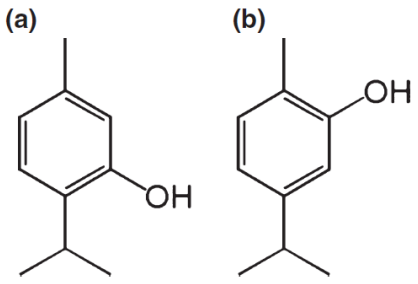

FIGURA 2 – Estrutura molecular do timol (a) e do carvacrol (b) (Fonte: Peixoto-Neves et al., 2009).

Numerosas publicações da medicina humana e algumas com animais de produção, principalmente monogástricos, mostram que o carvacrol, presente no orégano, possui consideráveis atividades antimicrobianas (Cowan, 1999; Lambert et al., 2001; Calsamiglia et al., 2007), antifúngicas (Basilico & Basilico, 1999), anti-inflamatórias e antioxidantes (Cervato et al., 2000; Gabbi et al.,

2009a; Christaki et al., 2012; Peraskevakis, 2015). O interesse no estudo deste composto como aditivo na nutrição animal é devido à necessidade da obtenção de um produto alternativo ao uso de antibióticos na produção animal, e as suas propriedades antibacterianas, antifúngicas (Banias et al., 1992) e antiparasitárias (Didry et al., 1994), antioxidantes (AeschbacH et al., 1994; Fasseas et al., 2008).

Na produção de ruminantes, Chaves et al. (2008, 2011) verificaram que o carvacrol pode aumentar a proporção de propionato, um dos precursores da glicose, com potencial para aumentar o ganho de peso dos animais. O modo de ação antimicrobiana do carvacrol ainda não foi totalmente esclarecido, porém, sua ação pode ser atribuída principalmente à capacidade aumentar a permeabilidade das membranas das bactérias, especialmente das bactérias gram-positivas (Lambert et al., 2001; Lambert et al., 2004; Benchaar et al., 2008), ao reagir com os lipídeos e os radicais hidroxila convertendo-os em produtos instáveis (POKORNY; YANISHLIEVA; GORDON, 2001). Por outro lado, o carvacrol também parece ser capaz de alterar a membrana externa das bactérias gram-negativas, aumentando a sua permeabilidade (Ultee et al., 2000; Burt, 2004). A alteração dos gradientes de íons conduz à deterioração dos processos essenciais da célula como transporte de elétrons, translocação de proteínas, etapas da fosforilação e outras reações dependentes de enzimas, resultando em perda do controle quimiosmótico da célula afetada e, consequentemente, a morte bacteriana (Dorman & Deans, 2000).

Lejonklev et al. (2016) observaram que o carvacrol estava presente nas amostras de leite de vacas que receberam 0,2 e 1 g/kg de MS de óleo de orégano na dieta, sugerindo que existe alguma forma de proteção contra alterações metabólicas. Peraskevakis (2015), por sua vez, forneceu 30 g de planta seca de orégano para cabras e observou aumento significativo nas atividades da GPx e da glutationa redutase tanto no sangue como no leite evidenciando assim melhora nas defesas antioxidantes. Segundo Cervato et al. (2000) o extrato de folha de orégano pode ser eficaz na prevenção do estresse oxidatico de todas as fases do processo peroxidativo.

Em relação ao efeito estimulante dos óleos essenciais utilizados na alimentação animal sobre o sistema imunológico, Alexander (2002) e Fujiwara et al. (2002) associaram os efeitos benéficos com o maior número de leucócitos. Estudos sobre o perfil hematológico de ruminantes submetidos a dietas com óleos essenciais ainda são incipientes. Gabbi et al. (2009) analisaram parâmetros hematológicos em novilhas Jersey e verificaram um aumento significativo nos leucócitos, linfócitos e monócitos para animais que receberam 1g/dia de uma mistura de óleos essenciais na dieta, quando comparado com os animais não suplementados.

No estudo de Katsoulos et al. (2017), foi testado o efeito da suplementação de OE de orégano (12,5 mg/kg de PV) sobre o escore de fezes e incidência de diarreia em bezerros até os 10 dias de vida. Os escores fecais médios ao longo do experimento, a incidência de diarreia, a duração e a severidade dos episódios de diarreia foram significativamente menores nos bezerros suplementados com OE em comparação aos bezerros controle. Os autores concluíram que a suplementação com OE de orégano possui um efeito preventivo contra a síndrome diarreica neonatal.

Froehlich et al. (2017), testando diferentes doses (1,25, 2,5 e 3,75 g de OE por alimentação – duas alimentações diárias com 270 g de ração cada) de uma mistura comercial de OE (isto é, carvacrol, cariofileno, *p*-cimeno, cineole, terpimeno e timol) em bezerros, observaram que os bezerros que consumiram a dose de 1,25 g/dia apresentaram maior ganho de peso diário e, consequente, maior peso corporal, quando comparados às demais doses de OE e ao controle negativo. Neste estudo, os autores também observaram que essa mesma dose elevou as quantidades de IgA e IgG, o que corresponde ao desempenho destes bezerros e, que os escores fecais foram melhorados com a suplementação de OE.

As pesquisas com foco no potencial de utilização dos OE na alimentação de bezerros leiteiros são escassas, havendo poucas publicações. Os resultados publicados são variáveis, porém demonstram muitos benefícios promissores. No entanto, mais pesquisas precisam ser feitas sobre a dosagem ideal e as formas de suplementação de óleos essenciais.

3. HIPÓTESES E OBJETIVOS

As hipóteses deste estudo são:

O fornecimento de leite oriundo de vacas suplementadas com extratos de orégano ou de chá verde 1) reduz o desequilíbrio do estado redox, 2) diminui a ocorrência e/ou severidade de diarreias, e 3) modifica o perfil hematológico de bezerros durante o seu aleitamento.

O fornecimento de extrato de chá verde ou orégano a bezerros em aleitamento 1) melhora o estado redox e 2) reduz a ocorrência e/ou severidade de diarreias.

O objetivo geral foi:

Avaliar os biomarcadores do estado redox, o perfil hematológico e a ocorrência de diarreias de bezerros leiteiros, os quais consumiram extrato de chá verde (*Camellia sinensis*) ou de orégano (*Origanum vulgare*) ou receberam leite oriundo de vacas suplementadas com os mesmos extratos, do nascimento aos 60 dias de vida.

Os objetivos específicos foram:

1. Avaliar os biomarcadores do estado redox e o perfil hematológico e de bezerros, durante o aleitamento, recebendo ou não leite de vacas suplementadas com extratos vegetais;
2. Avaliar biomarcadores do estado redox de bezerros suplementados ou não com extratos vegetais durante o aleitamento;
3. Avaliar a ocorrência e a severidade de diarreia de bezerros recebendo ou não extratos vegetais durante o aleitamento.

CAPÍTULO II

Calves fed with milk from cows receiving plant extracts improved redox status

Este capítulo é apresentado de acordo com as normas de publicação do periódico **Animal**

Calves fed with milk from cows receiving plant extracts improved redox status

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Short title: Plant extracts used as antioxidants by calves

Abstract

The objective of this study was to evaluate the biomarkers of the redox state, hematological profile, and health of preweaned Jersey dairy calves that consumed milk from cows supplemented with green tea extract (*Camellia sinensis*) or oregano (*Origanum vulgare*). A completely randomized design was used with repeated measures in time; 23 calves received milk from cows fed on a basal diet without addition of plant extracts (CON), or with addition of 10.0 g of

oregano (OE) extract or 5.0 g of green tea extract (GT). On 1, 30, and 60 days of life hematological profile and redox state biomarkers were evaluated. Body temperature and occurrence diarrhea severity were evaluated every two days during the whole period. On day 1, calves receiving GT had higher plasma glutathione peroxidase activity (GPx), however the reverse occurred on day 60. On the day of birth, calves in the CON group presented higher concentrations of TIOIS than those in GT and OE groups, with reverse occurring on day 30. Calves in the OE group had lower oxidation of dichlorofluorescein in erythrocytes (DCFE) when compared to the others; while calves in the GT group presented higher GSH and higher activity of the catalase enzyme (CAT) in relation to the others. Plant extracts fed to cows increased the concentration of neutrophils in relation to CON calves. The frequency of diarrhoea was similar between treatments. Preweaned calves fed with milk of cows supplemented with extracts of green tea and oregano present some improvements in antioxidant system biomarkers without significantly altering the hematological profile and the frequency of diarrhea.

Keywords: antioxidant enzymes, catechins, carvacrol, health status

Implications

Dairy calves are challenged with separation of their dams, early weaning and the need to change from a non pre-ruminant state to a full ruminant digestion few weeks after birth. These physiologic challenges may reduce their antioxidant capacity and predispose them to a higher incidence of diseases. In the present study, calves fed with milk from cows supplemented with green tea

extract or oregano increased the concentrations of some antioxidant enzymes. The indirect feeding of planta extracts may be a valuable tool in free-range raising calves systems.

Introduction

The plant extracts have a wide variety of compounds (Burt et al., 2004), that may present antimicrobial, anti-inflammatory and antioxidant actions (Aristatile et al., 2015; Cyboran et al., 2015, Gonçalves et al., 2015), and their inclusion into the diet may enhance animals performance and health. Previous studies reported that the use of oregano and green tea extracts improve the redox state in goats (Peraskevakis, 2015) without negative effects on behavior, voluntary intake and milk production (Maciej et al., 2016; Kolling et al., 2016; 2018).

If the redox status is imbalanced favoring the pro-oxidant state, it may trigger adverse clinical effects (Sies, 2018). The enzymatic system is the primary route of antioxidant defense, being represented mainly by the antioxidant enzymes GPx, CAT and SOD (Gutteridge and Halliwell, 2000; Belló, 2002) that can decrease reactive oxygen species (ROS) and, consequently, the damage to biological structures (Belló, 2002).

Diseases may be associated with increased reactive species, causing redox disequilibrium (Halliwell, 1991). In calves, imbalance of the redox state was observed to increase when the animals were diseased (Ahmed and Hassan, 2007) and on the first day of life (Alexeyrovich and Antonovna, 2009). Poor management, such as incorrectly supplying colostrum to newborn calves, possibly affects adversely the antioxidant system (Blum et al., 1997).

In this context, the use of plant extracts that act as an antioxidant becomes essential since the digestive process in ruminants virtually catabolizes the catequines but does not seem to inhibit the beneficial properties of green tea polyphenols (Gladine et al., 2007; Maciej et al., 2016; Ibrahim I. Elshahawy 2018). On the other hand, carvacrol seems to resist the digestive process as its presence has been detected in milk (Lejonklev et al., 2016).

The knowledge about calves fed milk of cows receiving plant extracts is scarce, being the main contribution of this research. Our hypotheses are the addition of extracts into the diet of the cows 1) reduces the imbalance of redox status of calves, 2) decreases the occurrence and / or severity of diarrhea, and 3) modifies the hematological profile of pre-weaned calves. The present study aimed to evaluate the biomarkers of the hematological profile, redox status, the occurrence and severity of diarrhea in dairy calves fed milk from dairy cows supplemented with green tea extract (*Camellia sinensis*) or oregano (*Origanum vulgare*) from birth to 60 days of age.

Material and methods

Location Description, Animals and Management

This study was approved by the Ethics Committee for the Use of Farm Animals of the Universidade Federal do Rio Grande do Sul, protocol number 30756. The experiment was conducted at the Embrapa Clima Temperado Experimental Station, in Rio Grande do Sul State, between October 2015 and

January 2016. Region's climate is classified by Köppen as subtropical humid (Köppen, 1900; Köppen 1901).

During the study, mean values of air temperature, relative air humidity and wind speed were, respectively, 20.1 ± 0.5 ºC (mean ± SEM), 85.3 ± 1.6% and 14.2 ± 0.9 km/h, accumulated rainfall over the entire experimental period was 847.0 mm (Table 1).

Twenty-three Jersey dairy calves (13 males and 10 females) received milk, from birth to 60 days of age, from cows fed with the same basal diet and divided in three treatment groups. The groups were: Control (CON) - without addition of plant extracts into the diet, oregano extract (OE) - addition of 10 g per cow per day of dietary oregano extract, and green tea extract (GT) - addition of 5 g per cow per day of green tea extract into the diet. Cows were fed with experimental diets in the period from 21 days before calving to 60 days after calving. The plant extracts were ministered in powder, homogenized in 500 grams of concentrate. Oregano extract (Orego Stim®) has a minimum concentration of 50 g/kg, containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2-5.0% Y-Terpinene, and green tea extract (glycolic extract, marketed by Seiva Bazilis) has a concentration of approximately 56% (± 2.5%) of polyphenols. Cows were distributed to dietary treatments according to number of births, body weight (BW) and body condition score (BCS).

Calves were kept in the same dietary treatments as their progenitors. On the day of birth, the calves were separated of the cows, identified according to treatments and allocated to individual shelters. The control group consisted of 3 male and 5 female calves with mean birth weight of 29.9 ± 2.3 kg of BW; GT

group consisted of 5 male and 2 female calves with mean birth weight of 30.1 ± 2.3 kg of BW and OE group by 4 male calves and 3 female calves with mean birth weight of 28.4 ± 2.4 kg of BW.

From birth to 60 days of life, each calf received 4 liters of milk/day, divided into two 2-liter meals, fed between 8:00 AM to 8:30 AM and 5:00 PM to 5:30 PM. During the first five days of life, milk was supplied with the aid of bottles and came from their respective mothers. After the fifth day, milk was supplied in individual buckets from each group of cows (separated by dietary treatments), mixed and supplied to the calves. Water was supplied from third day of life in individual buckets. Concentrated feed was ofered *ad libitum* from fifth day of life.

Haematological profile and antioxidant profile and redox state

On 1, 30, and 60 days of life, in the morning but after feeding, blood samples were collected from the jugular vein of each animal in 5 mL tubes containing EDTA anticoagulant (Vacutainer; Becton-Dickinson, Rutherford, NJ); tubes were immediately stored, cooled and taken to the laboratory. Hematological variables evaluated were erythrocytes, hemoglobin, leukocytes, monocytes, platelets, and eosinophils.

Analysis were performed using the Poch-100iy automated method for blood count analysis and the xs-100i (sysmex/Roche) automated/interface method for platelet analysis using impedance and flow cytometry methodology (Lopes, Biondo, & Santos, 2007).

On days 1, 30 and 60 post-birth, in the morning but after feeding, blood samples were collected from the jugular vein of each animal in 5 mL tubes containing heparin as anticoagulant (Vacutainer; Becton-Dickinson, Rutherford, NJ).

Preparation of samples for biochemical tests

After blood collection, plasma and erythrocytes were separated by centrifugation at 1000 g for 10 min at 4 °C. Plasma fraction was transferred to a microtube and stored at -80 ºC for further analysis. In order to isolate the erythrocytes, the fractions of platelets and leukocytes, corresponding to the intermediate fraction, were removed and discarded. Remaining fraction containing the isolated erythrocytes was diluted 1:10 (v/v) with 0.9% commercial saline solution and centrifuged at 1000 g for 10 min at 4 °C, 3 times; and at each step the supernatant was removed and discarded. At the end of the last centrifugation, the erythrocytes were resuspended in saline solution at a final dilution of 1:10, and then stored at -80 ºC until the biochemical assays described below were performed (Kakgniashvili, 2004).

Determinations of redox state biomarkers

Dichlorofluorescein Oxidation (DCFH)

Reactive oxygen and nitrogen species were measured in erythrocytes and plasma using 2 ', 7'-dichlorofluorescein diacetate (DCFH2-DA) according to Lebel et al (1992). The DCFH2-DA is cleaved by esterase enzymes producing

DCFH2, which is oxidized by reactive species present in the sample, giving rise to DCF fluorescence. Fluorescence was measured at excitation and emission wavelengths of 488 nm and 525 nm, respectively, using the SpectraMax Gemini XS Fluorescence Reader (Molecular Devices, Sunnyvale, CA, USA). Standard DCF curve ranging from 0.25 to 10 μM was performed in parallel. Data are expressed as nmol DCF / mg protein.

Superoxide dismutase activity (SOD)

Total SOD enzyme activity was measured in erythrocytes by quantification of the superoxide inhibition dependent autooxidation at 480 nm (Misra and Fridovich, 1972). Absorbance was measured on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The activity of SOD is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. Data are expressed as SOD units/mg protein.

Catalase Activity (CAT)

The enzymatic activity of CAT was measured in erythrocytes and tested according to Aebi (1984), which was adapted for microplates. Decrease in absorbance at 240 nm was measured in a medium containing 20 mM hydrogen peroxide and 10 mM potassium phosphate buffer pH 7.0 using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The CAT unit is defined as 1 μmol H_2O_2 consumed per minute. The specific activity data are expressed as CAT units/mg protein.

Activity of glutathione peroxidase (GPx)

The activity of GPx enzyme in erythrocytes was tested according to Wendel (1981), which was adapted for microplates. The medium contained 100 mM potassium phosphate buffer, pH 7.7, 1 mM EDTA, 2 mM reduced glutathione (GSH), 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.1 mM NADPH and 0.5 mM tert-butyl hydroperoxide as the enzymatic substrate. The disappearance of NADPH was monitored at 340 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The GPx unit is defined as 1 µmol of NADPH consumed per minute and the specific activity is represented as GPx units/mg of protein.

Reduced glutathione (GSH)

Concentration of GSH in erythrocytes was measured according to Browne and Armstrong (1998). Initially, proteins in the supernatant were precipitated with meta-phosphoric acid (1: 1) and centrifuged at 5,000 g for 10 min at 25 °C. GSH present in the supernatant is reacted with the fluorophore o-phthaldialdehyde present in the medium at a concentration of 7.5 mM in addition to 100 mM sodium phosphate buffer pH 8.0 containing 5 mM EDTA. Fluorescence was measured at excitation and emission wavelengths of 350 nm and 420 nm, respectively, using the SpectraMax Gemini XS Fluorescence (Molecular Devices, Sunnyvale, CA, USA) microplate reader. The standard GSH curve ranging from 0.001 to 1 mM was prepared and a blank sample was run in parallel. Data are expressed as nmol GSH/mg protein.

Thiol levels

The thiol content was measured in plasma according to Aksenov and Markesbery (2001), adapted for microplates. The assay is based on the reduction of 50-dithiobis-2-nitrobenzoic acid (DTNB) by thiols, which become oxidized (disulphide), yielding a yellow derivative (TNB). Absorbance was measured at 412 nm in a medium containing 20 mM sodium phosphate buffer pH 7.4 and 10 mM DTNB prepared in a 0.2 M potassium phosphate solution pH 8.0 using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol TNB/mg protein.

Protein carbonyl (CARBO) content

Carbonylated protein content was measured in plasma according to Reznick and Packer (1994) and adapted by Stone et al. (2016) for reading in 96-well microplates. Protein carbonyls react with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that was measured at 370 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol carbonyls/mg protein.

Proteins

Protein concentration was measured according to Lowry et al. (1951), which was adapted for microplates using bovine albumin as standard. The absorbance was measured at 750 nm using the SpectraMax M5 microplate

reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as mg protein/mL.

Frequency of diarrhea

The occurrence of diarrhea was monitored every two day throughout the experimental period. In these days, on morning and afternoon, body temperature was measured with the aid of a digital clinical thermometer inserted into the animal's rectal ampulla at a depth of approximately 3.5 cm for 3 minutes.

The consistency and appearance of the faeces were observed daily and the score was assigned according to their appearance, following classification from 0 to 3 according to Ishihara et al. (2001): (0) normal faeces, (1) soft faeces, (2) muddy faeces, and (3) watery faeces.

The incidence of diarrhea were calculated according to Ishihara et al. (2001) following formula: Frequency of diarrhoea (%): (total number of days suffered from diarrhea/total number of days inspected) X 100.

Experimental design and statistical analysis

The experimental design was a completely randomized, with repeated measures in time (days), three treatments (n = 3, control, oregano extract and green tea extract) and 8 replicates (calves) for control and green tea treatments and 7 replicates for oregano treatment (due to the death at birth of one calf). Statistical analysis considered treatments, days of evaluation (1, 30 and 60) and treatment x day interaction as fixed effects, and animal and residue as random

effects, using the SAS® MIXED procedure, version 9.4. A structural selection test was performed using the Bayesian information criterion (BIC). Covariance structures tested were compound symmetry, first-order autoregressive, toeplitz and unstructured. Analysis of variance was performed to test interaction effect. The contrasts CON x OE, CON x GT and OE x GT with adjusted one-tailed side Dunnett *P*-values were used to compare the means between treatments for all variables. The variables CARBO and GSH were not normally distributed, therefore, they were logarithmically transformed. Significant differences were declared when $P < 0.05$ and a trend considered to exist if $0.05 < P < 0.10$.

Results

The interaction between treatment and day variables was significant ($P < 0.05$) for the biomarkers, GPx activity, thiols levels, and DCFE oxidation. On the day 1, calves in GT group presented higher activity of the GPx enzyme in relation to the CON group, whereas those in the OE group were not statistically different from the others. On day 60, activity of GPx was lower for the calves in GT and OE groups compared to the CON group, whereas on day 30 GPx activity did not differ between groups (Figure 1A).

On day 1, calves in GT group had a higher thiol concentration than calves in CON; however, on day 30 the reverse occurred, and on day 60 there were no significant differences between treatments (Figure 1B). There was lower oxidation of DCFE in OE group in relation to GT and CON groups on all evaluated days (Figure 1C).

Calves in the GT group presented higher higher GSH and CAT activity, whereas OE group calves showed lower values of DCFP and DCFE than CON (Table 2).

The addition of oregano extract tends to be larger than the control and is larger than the green tea extract for the neutrophil concentration, and there were no differences in the other parameters of the hematological profile between treatments (table 3).

Plant extracts did not influence the frequency of diarrhea (P> 0.10) which was on average 36.4 ± 3.7%, 42.1 ± 3.7%, and 39.1 ± 4.0% for CON, GT, and OE, respectively. No occurrences of other diseases (based on observation of clinical symptoms) were recorded in calves during the experimental period.

Discussion

Catechins, carvacrol and thymol are secondary metabolites produced by green tea and oregano plants, respectively (Manach et al., 2004, Oh et al., 2017). Many diseases are related to the unbalanced increase of the reactive species of oxygen and nitrogen, that can cause oxidative stress (Halliwell, 1991). In cells of aerobic organisms the generation of reactive species is a normal process resulting from the cellular metabolism itself. In calves, previous studies reported that supplementation with oregano essential oil has a preventive effect against neonatal diarrheal syndrome (12.5 mg/kg of essential oil of Oregano (Katesoulos et al., 2017)). Also previous reports showed influences on metabolism and improvement of antioxidant state in dairy calves receiving flavonoids up to 26 days of age (Maciej et al 2016). Moreover, Maciej

et al. (2016) reported decreased diarrhea condition in those calves. Diarrhea causes about 80% of morbidity and mortality cases in dairy calves (Fruscalso, 2018).

The main contribution of the present study was to highlight some positive effects of maternal supplementation during the transitional and early lactation period with green tea and oregano extracts on the redox status of preweaned calves.

In general, all groups of calves showed a variation in the redox state during the preweaning period. On postnatal day 1, all bioindicators evaluated in plasma and erythrocytes of calves born to cows fed with green tea extract were similar to the control group. On the other hand, both plasma and erythrocytes of calves born to cows supplemented with oregano extract showed a significant reduction in ROS levels and a reduction of GPx activity in the erythrocytes compared to the control group. These results are promising as the supplementation of pregnant cows with plant extract may improve the redox status in newborn calves.

It was expected that calves born to cows fed green tea extract would show a greater reduction of ROS due to the antioxidant capacity attributed to green tea (Guo et al 1996), but it was not observed. The authors of the present study acknowledge that one of the limitations for the results' interpretation is that the secondary compounds were not evaluated in the milk of the cows in order to verify their passage to the milk. There is evidence that essential oils are poorly or not degraded in the ruminal environment, being detected in the blood

and milk of cows (Lejonklev et al., 2016), what could explain the positive results observed at birth in OR group.

In agreement to the present study, Maciej et al (2016) reported that supplementation with flavonoids (quercetin, rutin, catechin) added to the diet of newborn calves did not modify the antioxidant capacity or activity of oxidative damage biomarkers in the early postnatal days using doses of 10 mg/kg of BW of green tea extract. This dosage was similar to that used in the present study (10.9 mg/ kg BW of cows) but these authors ministered the supplement directly to the calves while in our study the supply was indirect (by maternal route).

We also hypothesized that calves present differences in the redox state at 30 days of age, because 1) the passive transfered antibodies are in low concentrations, and calves are still developing their active immunity (Hulbert et al. Moses, 2016); 2) solid diet consumption increases, amd may increase production of ROS (Alexandrovich, K.N, Antova, S. E, 2009). The challenge faced by calves between 1 and 30 postnatal may explained by the significant increase in plasma levels of reactive species in all groups. However, OE treatment calves presented lower levels of plasma reactive species in the erythrocytes (DCFE) compared to control group on postnatal day 30, that remained reduced on postnatal day 60.

Thiol groups react with reactive species to maintain the redox state of the cell, and its reduced levels may indicate oxidative stress (Halliwell and Gutteridge, 2007). The inverse behavior of the thiol concentration, being higher at the day of birth in control treatment, while at day 30 it was superior in GT and OE groups, demonstrates the potential of oregano and green tea extracts in

reducing the oxidative stress. In this sense, the increase in the enzymatic activity of CAT observed in calves in the green tea group corroborates with the results of redox state improvement. Both catechins and carvacrol are known as stimulants of antioxidant system and reducers of free radical production on cells and tissues (Gladine et al., 2007; Maciej et al., 2016).

Plant extracts can increase the activation of immune cells, rising the rate at which neutrophils migrate to the affected site, accelerating phagocytosis, aiding in elimination of pathogens or inhibiting their multiplication, and thereby reducing the inflammatory process (Alexander, 2002). With regard to the effect of stimulation to the immune system, the present study showed that the calves in GT and OE groups had higher concentration of neutrophils when compared to the animals that did not receive plant extracts, indicating a greater defense potential. Neutrophils are part of the innate immune system and their presence is directly involved in protection against infections since they are the first defense cells to migrate to infection sites where they are able to destroy pathogens by phagocytosis (Brinkmann and Zychlinsky, 2007).

Diarrhea is a frequent problem that affects calves during the first weeks of life, and is one of the main causes of mortality (Fruscalso, 2018, Doepel, Bartier, 2014, Wudu et al., 2008, USDA, 2007). The absence of significant differences between the three groups of calves with respect to the frequency of diarrhea may have been due to the limited number of animals and variability among the individuals, which resulted in a coefficient of variation of 26.4%, and probably prevented the detection of significant effect between treatments. Diarrhea is a complex disease that can be triggered by both infectious and non-

infectious causes (Cho & Yoon, 2014; Meganck et al., 2014), thus other factors may have influenced its incidence, limiting the effectiveness of the extracts.

Katsoulos et al. (2017) ministered oregano essential oil at 12.5 mg/kg of BW to calves (in our study mothers received 10 g of oregano extract containing 80-82% carvacrol, i.e. 19.1 to 19.5 mg carvacrol/kg cow BW). These authors concluded that supplementation with oregano essential oil has a preventive effect against neonatal diarrheal syndrome, attributed to its antimicrobial properties (Benchaar and Greathead, 2011).

The results open new perspectives to investigate the possible effects of maternal supplementation with oregano extract during the transition period and initial stage of lactation of dairy cows on metabolic parameters of preweaned calves. Researches focusing on the potential use of plant extracts in feeding dairy calves are still scarce. The published results are variable, but demonstrate potential benefitial effects. More research is needed to establish the optimal and effective dosage and ways of supplementing the green tea and oregano extracts.

Conclusions

Feeding milk from cows supplemented with green tea or oregano extract to pre-weaned calves improves some of the redox biomarkers and haematological profile, but is not sufficient to prevent or reduce the frequency of diarrhea.

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Ethics statement

This study was approved by the Ethics Committee for the Use of Farm Animals from the Federal University of Rio Grande do Sul, protocol number 30756.

References

Aebi, H 1984. Catalase in vitro. Methods Enzymol, 105, 121-126.

Aksenov MY, Markesbery WR 2001. Change in thiol content and expression of glutathione redox system gene in the hippocampus and cerebellum in Alzheimer's disease. Neuroscience Letters 302, 141–145.

Alexander, M 2002. Aromatherapy and immunity: how the use of essential oils aids immune potentiality. International Journal of Aromatherapy 12, 49-56.

Aristatile B, Al-Numair KS, Al-Assaf A, Veeramani C and Pugalendi KV 2015. Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UVB irradiation in human peripheral lymphocytes. Journal of Biochemical and Molecular Toxicology 29, 497-507.

Belló A 2002. Dano oxidativo e regulação biológica pelos radicais livres. In: MARRONI, N. P. et al. Estresse Oxidativo e Antioxidantes. Porto Alegre: Editora Ulbra

Benchaar C, Greathead H 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Animal Feed of Science Technolology 166, 338–355.

Browne RW, Armstrong D 1998. Reduced glutathione and glutathione disulfide. Methods MolBiol, 108, 347-52.

Burt S 2004. Essential oils: their antibacterial properties and potential applications in foods- A review. International Journal of Food Microbiology 94, 223-53.

Cho Y, Yoon KJ 2014. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. Journal of Veterinary Science 15, 1–17.

Christaki E, Bonos E, Giannenas L, Florou-Paneri PC 2012. Aromatic plants as a source of bioactive compounds. Agriculture 2, 228-243.

Clement P, Guatteo R, Delaby L, Rouillé B, Chanvallon A, Philipot JM, Bareille N 2014. Short communication: Added value of rumination time for the prediction of dry matter intake in lactating dairy cows. Journal of Dairy Science, Champaign, 97, 6531–6535.

Cyboran S, Strugała P, Włoch A, Oszmiański J, Kleszczyńska H 2015. Concentrated green tea supplement: Biological activity and molecular mechanisms. Life sciences. 126, 1-9.

Doepel L, Bartier A, 2014. Colostrum Management and Related to Poor Calf Immunity. WCDS Advanced Dairy Science and Technology 26, 137–149.

Fruscalso V, Antillón GO, Hötzel MJ 2017. Smallholder family farmers' perceptions, attitudes and choices regarding husbandry practices that influence performance and welfare of lactating dairy calves. Ciència rural 47, 4-25.

Fruscalso, V. Caracterização socioambiental dos sistemas de criação de bezerras leiteiras no Rio Grande do Sul. 2018. Tese (Doutorado em Agrossistemas) – Programa de Pós-Graduação em Agrossistemas, Universidade Federal de Santa Catarina, Florianípolis.

Gladine C, Rock E, Morand C, Bauchart D, Durand D 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. British Journal of Nutrition 98, 691-701.

Gonçalves G, Sá-Nakanishi AB, Wendt MMN, Comar JF, Amado CAB, Bracht A, Peralta RM 2015. Green tea extract improves the oxidative state of the liver and brain in rats with adjuvant-induced arthritis. Food & function 6, 2701-2711.

Gutteridge J & Halliwell B 2000. Free radicals and antioxidants in the year 2000: a historical look to the future. Annals of the New York Academy of Sciences, 899, 136-147.

Halliwell B 1991. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. American Journal of Medicine 91,14-22.

Halliwell B, Gutteridge J 2007. Free Radicals in Biology and Medicine. Oxford University Press, USA: Oxford University Press, 4.

Hashemzadeh FC, Ghorbani GR, Khorvash M, Riasi A, Taghizadeh A, Zebeli Q 2014. Supplementation of herbal plants differently modulated metabolic profile, insulin

sensitivity, and oxidative stress intransition dairy cows fed various extruded oil seeds. Preventive Veterinary Medicine 11, 45-55.

Hulbert L, Moisá SJ 2016. Stress, immunity, and the management of calves. Journal of Dairy Science 99, 1-18.

Ibrahim I Elshahawy 2018. Oxidantantioxidant Status in Calves Supplemented with Green Tea Extract. Journal of Animal and Veterinary Sciences 12.

Lowry OH, Rosebrough NJ, Farr AL Randall RJ 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

Kakhniashvili DG, Bulla AL. Goodman SR 2004. The Human Erythrocyte Proteome. Molecular & Cellular Proteomics 3, 5001-509.

Katsoulos PD, Karatzia MA, Dovas CI, Filioussis G, Papadopoulos E, Kiossis E, Arsenopoulos K, Papadopoulos T, Boscos C, Karatzias H 2017. Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves. Research in Veterinary Science 115, 478-483.

Köppen, W., 1900: Versuch einer Klassifikation der Klimate, vorzugweise nach ihren Beziehungen zur Pflanzenwelt. – Geogr. Z. 6, 657–679.

Köppen, W., 1901: Versuch einer Klassifikation der Klimate, vorzugweise nach ihren Beziehungen zur Pflanzenwelt. – Meteorol. Z. 18, 106–120.

LeBel CP, Ischiropoulos H, Bondy SC 1992. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chemical Research Toxicology 5, 227-231.

Lejonklev J, Kidmose U, Jensen S, Petersen MA, Helwing ALF, Mortensen G and Larsen MK 2016. Effect of oregano and caraway essential oils on the production and flavor of cow milk. Journal of Dairy Science 99, 7898-7903.

Maciej J, Schäff CT, Kanitz E, Tuchscherer A, Bruckmaier RM, Wolffram S and Hammon HM 2016. Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. Journal of Dairy Science 99, 805-811.

Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L 2004. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition 79, 727-747.

Meganck V, Hoflack G, Opsomer G 2014. Advances in prevention and therapy of neonatal dairy calf diarrhoea: a systematical review with emphasis on colostrum management and fluid therapy. Acta Veterinaria Scandinavica 56, 75.

Misra HP, Fridovich I 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247, 3170-3175.

Nishida T, Eruden B, Hosada K, Matsuyama H, Nakagawa K, Miyazawa T and Shioya S 2006. Effects of Green Tea (*Camellia sinensis*) Waste Silage and Polyethylene Glycol on Ruminal Fermentation and Blood Components in Cattle. Asian-Austalasian Journal Animal Science 19, 1728–1736.

Oh J and Hristov AN 2016. Effects of plant-derived bio-active compounds on rumen fermentation, nutrient utilization, immune response, and productivity of ruminant animals. Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization. Oxford University Press Inc, Madson, NY, USA.

Paraskevakis, N. 2015. Effects of dietary dried Greek Oregano (Origanum vulgare ssp. hirtum) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. Animal Feed Science and Technolog 209, 90-97.

Rashidinejad A, Birch JE, Everett DW 2016.Interactions between milk fat globules and green tea catechins. Food Chemistry 199, 347–355.

Reznick AZ, Packer L 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233, 357-63.

Saeed m, Hach MEA, Alagawany M, Naveed M, Arain MA, Soomro RN, Manzoor R, Tiwari R, Khandia R, Munial A, Kasthik K, Dhama K, Igbal HMN, Sun C 2017. Phytochemistry, Modes of Action and Beneficial Health Applications of Green Tea (Camellia sinensis) in Humans and Animals. International Journal of Pharmacology 13, 698-708.

Sies H. On the history of oxidative stress: Concept and some aspects of current development 2018. Current Opinion in Toxicology 7, 122– 126.

Silva CS, Souza EJO, Pereira GFC, Cavalcante EO, Lima EIM, Torres TR, Silva DC 2016. Plant extracts as phytogenic additives considering intake, digestibility, and feeding behavior of sheep. Tropical animal health and production 49, 353-359.

Stone V, August, PM, Stocher DP, Klein CP, Couto PR, Silva YD, Ssalomon TB, Benfato MS, Matté C 2016. Food restriction during pregnancy alters brain's antioxidant network in dams and their offspring. Free radical research 50, 530-541.

USDA, 2007. Dairy 2007. Part I: Reference of Dairy Cattle Health and Management Practices in the United States, 2007. Fort Collins, CO.

Wendel, A. 1981. Glutathione peroxidase. Methods Enzymol 77, 325-33.

Wudu T, Kelay B, Mekonnen HM, Tesfu K 2008. Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia. Tropical Animal Health and Production 40, 369–376.

Table 1 *Mean and amplitude of air temperature (ºC), relative air humidity (%), wind speed (km/h) and precipitation (mm/day) in Pelotas Rio Grande do Sul - Brazil from October to January.*

Month	Temperature		Humidity		Wind speed		Precipitation
	Mean	Amplitude	Mean	Amplitude	Mean	Amplitude	
October	16.3	21.1 – 4.1	88.9	97.0 – 73.3	35.1	54.7 – 24.1	315.2
November	18.9	22.6 – 15.6	84.3	96.4 – 72.0	8.6	17.0 – 3.5	192.3
December	22.1	25.3 – 17.3	83.7	94.9 – 52.8	6.9	12.8 – 0.0	262.0
January	23.2	25.1 – 21.1	84.5	95.9 – 70.8	6.2	13.4 – 2.0	77.5

Table 2 *Mean values of redox biomarkers and hematological profile of calves fed milk from cows consuming control diet (CON) or containing green tea extract (GT) or oregano (OE).*

Variables[2]	Treatments[1]			SEM	*P* – values for Contrast		
	CON	GT	OE		CON x GT	CON x OE	GT x OE
Redox status profile							
TIOIS (nmol/mg)	0.23	0.25	0.27	0.25	0.4258	0.1920	0.5373
CARBO (nmol/mg)	2.42	3.33	2.55	2.76	0.2213	0.3329	0.0386
DCFP (nmol/mg)	48686	46658	30463	41935	<0001	<0001	0.8283
DCFE (nmol/mg)	4839	4840	3255	4311	<0001	<0001	0.0523
GSH (U/mg)	0.09	0.14	0.06	0.09	0.0355	0.3896	0.0070
SOD (U/mg)	56.58	58.20	52.32	55.7	0.6531	0.1969	0.0947
CAT (U/mg)	2.17	2.68	2.10	2.31	0.0026	0.6868	0.0022
GPx (U/mg)	7.35	7.16	6.96	7.15	0.8736	0.3787	0.4632

[1]Treatments: CON =control; GT = 5 g / day green tea extract; OR = 10 g / day oregano extract.

[2]Variables: CARBO = carbonyl; DCFP = oxidation of dichlorofluorescein in plasma; DCFE = oxidation of dichlorofluorescein in erythrocytes; GSH = reduced glutathione; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase.

SEM = standard Error of the Mean.

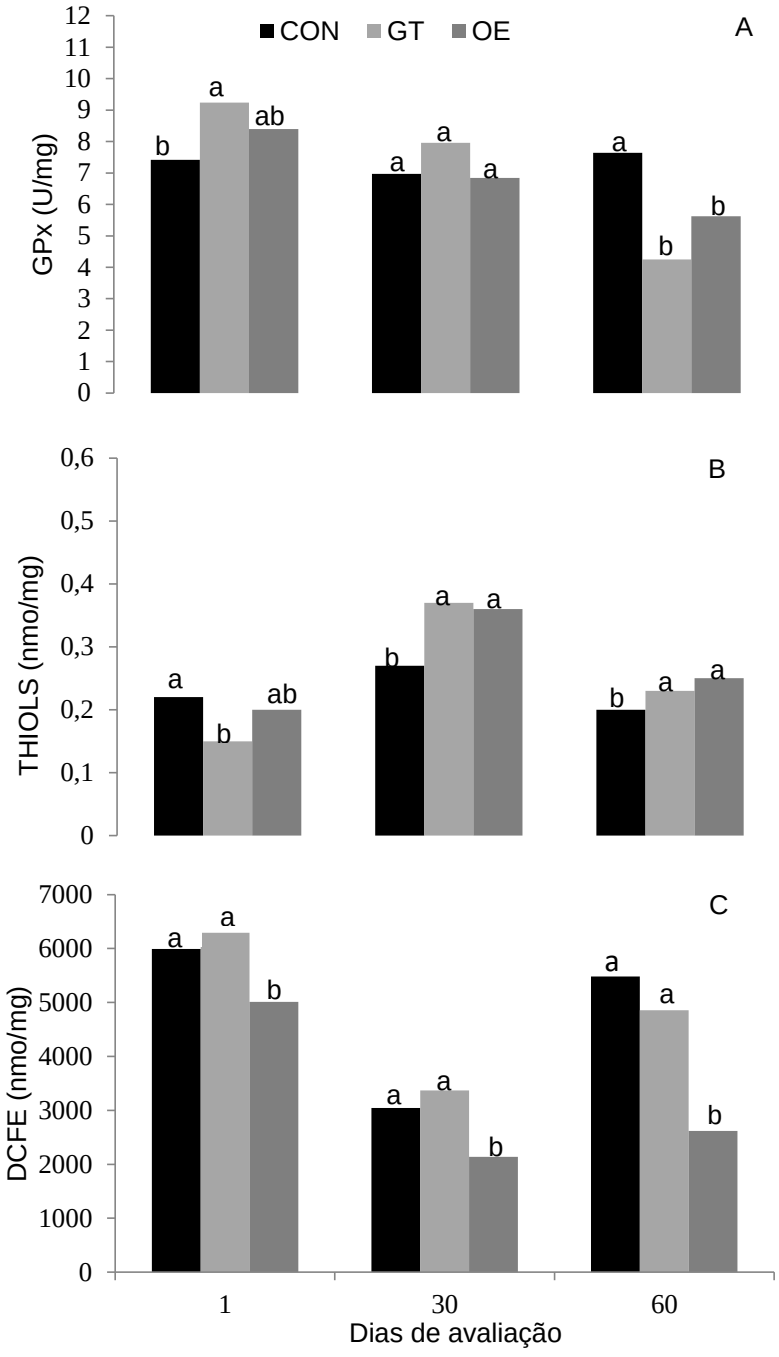
Table 3 *Averages for the hematological profile of calves fed milk from cows consuming control diet (CON) or containing green tea extract (GT) or oregano extract (OE).*

Variables	Treatments[1]			SEM	*P* – values for Contrast		
	CON	GT	OE		CON x GT	CON x OE	GT x OE
Hematologic Profile							
Erythrocytes (x10³/mm³)	5.25	5.37	5.05	5.22	0.7835	0.7020	0.5351
Platelets (x10^3/µL)	477706	478869	479340	478638	0.9748	0.9683	0.9909
Leukocytes (µL)	8496	7210	7815	7840	0.2065	0.5573	0.6092
Eosinophils (mm³)	125.42	94.54	23.16	81.04	0.6911	0.2892	0.4650
Lymphocytes (µL)	5213	5068	4834	5038	0.7886	0.5624	0.7240
Monocytes (mm³)	243.74	309.94	291.47	281.72	0.2795	0.5308	0.8109
Neutrophils (µL)	2738	2358	3921	3005	0.0456	0.0571	0.0142
Neutrophils to Lymphocytes Ratio	1.00	0.85	1.62	1.15	0.6594	0.1301	0.0653

[1]Treatments: CON =control; GT = 5 g / day green tea extract; OR = 10 g / day oregano extract.

SEM = Standard Error of the Mean.

Figure 3 *Mean values of glutathione peroxidase activity in erythrocytes (GPx; A), plasma thiois concentration (B) and oxidation of dichlorofluorescein in erythrocytes (DCFE; C) according to control treatments (CON), green tea extract (GT) and oregano extract (OE) on days 1, 30 and 60 after birth.*



CAPÍTULO III

Plant extracts supplied to pre-weaned dairy calves influence their redox status

Este capítulo é apresentado de acordo com as normas de publicação do periódico **Animal**

Plant extracts supplied to pre-weaned dairy calves influence their redox status

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Short title: Plant extracts affect the redox status of dairy calves

Abstract

The objective of this study was to evaluate the effect of the supply of green or oregano tea extracts on the biomarkers of the redox state and health condition in pre-weaned dairy calves, from birth to 60 days of life. Two experiments were carried out, following the randomized block design, with

measures repeated in time, using 38 Jersey calves (17 and 21 calves in experiments 1 and 2, respectively). Animals were distributed according to date of birth into three groups: control (**CON**) - without addition of vegetable extracts in the diet, oregano extract (**OE**) - addition of 70 mg/kg of body weight (BW), green tea extract (**GT**) - addition of 35 mg/kg of BW. The biomarkers of the redox state were evaluated on 1, 30, and 60 days of life. Body temperature and occurrence of diarrhea were evaluated every two days. The interaction treatments x blocks (experiments) was significant for the variables CARBO, SOD, and GPx. The activity of the GPx enzyme during experiment 1 was reduced when the plant extracts were added into the diet, while in the experiment 2, OE was effective in increasing the erythrocyte activity of GPx. There was no difference in CARBO levels in plasma (experiment 1) while CARBO plasma levels was lower in calves that received OE in relation to CON (experiment 2). The activity of the SOD enzyme in OE group was 19.2% higher in relation to the calves fed GT during the experiment 1. In the experiment 2 the addition of the vegetal extracts reduced the activity of the SOD enzyme when compared to CON. Calves supplemented with plant extracts in the milk presented lower concentration of DCF in the plasma in relation to the control animals. Calves did not show clinical symptoms of diseases except for diarrhea. The frequency of diarrhea was not altered by treatments or during the days of evaluation. The supply of green tea extract or oregano to pre-weaned calves improved some of the biomarkers of the redox state, but without consistency between the two experiments except for DCF in plasma, and did not change the health condition.

Keywords: carvacrol, catechins, diarrhea, redox state

Implications

Pre-weaned calves are challenged with cow separation, the transition of pre-ruminant to ruminant digestion and milk withdrawl. A large number of free radicals are continuously produced in the body's cells and may cause oxidative damage to the internal organs and increase health problems. Plant extracts such oregano and green tea have alleged antioxidant properties that might be useful during preweaning phase. The present study showed some improvements in redox status, especially when oregano extract was fed although it was not consistent between trials. Health status was not changed with the addition of green tea or oregano extracts into the milk.

Introduction

The use of plant extracts as feed additives for dairy cattle has been increased due to concerns on the health and the antioxidant status of the animals (Maciej et al., 2016). Diarrhea has been reported as one of the most prevalent diseases in calves worldwide (Cho, 2014; Santos, Bittar, 2015), accounting for about more than 50% (Cho, 2014) or 80% of cases of morbidity and mortality in dairy calves in the south of Brazil (Fruscalso, 2018).

A large number of free radicals are continuously produced in the body’s cells causing oxidative damage to internal organs. Excessive generation and/or inadequate removal of free radicals results in destructive, degenerative and irreversible damage to exposed cells (Nazifi et al., 2009). The increase in

oxidative stress in calves was observed in the following situations: on the first day of life, in sick calves and in poor colostrum management (Maciej et al., 2016).

The green tea and oregano plants produce secondary metabolites such as essential oils and polyphenols, respectively (Manach et al., 2004; Oh et al., 2017), that may decrease reactive oxygen species (Guo et al., 1996; Gladine et al., 2007). Green tea extract was effective in increasing the values of serum antioxidative parameters in dairy calves. These effects were attributed to the chelation of free radicals such as harmful oxygen species (Elshahawy, 2018). Peraskevakis (2015) provided 30 g of dried oregano plant for goats and observed a significant increase in the activities of GPx and glutathione reductase in both blood and milk thus evidencing an improvement in antioxidant defenses.

The use of oregano essential oils or green tea poliphenols decreased the frequency or the severity of diarrhea in dairy calves (Maciej et al., 2016; Katsoulos et al., 2017). To our knowledge, there are few (green tea extract) or none study (oregano extract) about feeding plant extracts to pre-weaned calves. The hypotheses of the present study are: the supply of green tea or oregano extract in the diet 1) increases the antioxidant capacity of pre-weaned calves; and 2) improves health status of dairy calves from birth to 60 days of age.

The objective of this research was to evaluate blood biomarkers of the redox state and the health status in pre-weaned dairy calves supplemented with green tea or oregano extracts from birth at 60 days of life.

Material and methods

Location Description, Animals and Management

This study was approved by the Ethics Commission on the Use of Animals of the Federal University of Rio Grande do Sul (CEUA/UFRGS), project number 30756. The experiments were conducted at Embrapa Clima Temperado Experimental Station in Pelotas, Rio Grande do Sul, Brazil, between March 2016 and June 2017. The climate of the region is temperate, classified by Köppen as subtropical humid (Köppen, 1900; Köppen, 1901). The mean values of air temperature, relative air humidity and wind speed were, respectively, 16.2 ± 4.94 and 18.5 ± 2.9 °C (mean ± SD), 88.6 ± 1.86 and 84 , 9 ± 3.8% and 5.9 ± 0.8 and 5.2 ± 2.3 km/h, accumulated rainfall was 843.7 ± 147.0 and 633.2 ± 41.9 mm, respectively (Table 1).

Two experiments were conducted, the first between March and June 2016 and the second between March and June 2017, totaling 38 Jersey calves. In the trial 1, 17 calves were enroled while in the trial 2 we used 21 calves, from birth to 60 days of age. The treatments and experimental protocol were the same for the two experiments.

In each experiment, calves were blocked by birth date and randomly assigned to one of three treatments: Control (CON) - without addition of plant extracts into the diet, Oregano Extract (OE) - addition of 70 mg/kg BW of oregano extract into the diet, Green Tea Extract (GT) - addition of 35 mg/kg BW of green tea extract into the diet. Adjustment in the concentrations of the plant extracts was performed every 15 days according to the BW of the animals.

Plant extracts were given to calves in the form of powder, diluted in milk. The commercial product (Orego Stim®) had a minimum concentration of 50 g / kg of oregano extract, containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2-5.0% Y-Terpinene, and the green tea extract (glycolic extract, marketed by Seiva Bazilis), a concentration of approximately 56% (± 2.5%) of polyphenols for study I.

For study II The commercial product (OregonOL®) had a minimum concentration of 65 g / kg of oregano extract, containing 80-82% Carvacrol, 2.5-3.0% Thymol, 3.5-9.0% p-Cymene and 2-5.0% Y-Terpinene, and the green tea extract (glycolic extract, marketed by Seiva Bazilis), a concentration of approximately 56% (± 2.5%) of polyphenols. In both studies it was adjusted to maintain the same concentration of carvacrol.

After birth, the calves were separated from the mothers, identified and housed in individual hutches bedded with straw. Hutches were placed in a flat, grassy area and in East-West orientation to make shade permanently available. Hutches were displaced three times a week to assure cleanliness and dryness.

In the experiment 1, at birth, CON and GT groups were composed of 3 male calves and 3 female calves each, with (mean ± SE) BW of 28.4 ± 0.9 kg and 28.3 ± 1.0 kg , respectively, whereas OE group consisted of 2 calves males and 3 females with BW of 29.2 ± 1.1 kg. In the experiment 2, the CON group consisted of 2 male and 4 female calves with BW of 28.2 ± 1.3 kg ; the GT group by 2 male and 5 female calves with BW of 29.1 ± 1.1 kg of CP and the OE group by 5 male and 3 female calves with BW of 30.4 ± 1.0 kg.

Calves received colostrum in bottles from birth to the fifth day of age and further on, they were fed with 4 liters of milk/day, twice a day in buckets between 8:00 AM and 8:30 AM, as well as between 5:00 PM and 5:30 PM.

Haematological profile and antioxidant profile and redox state

On 1, 30, and 60 days of life, in the morning and after feeding, blood samples were collected from the jugular vein of each animal in 5 mL tubes containing EDTA anticoagulant (Vacutainer; Becton-Dickinson, Rutherford, NJ); tubes were immediately stored, cooled and taken to the laboratory. Hematological variables evaluated were erythrocytes, hemoglobin, leukocytes, monocytes, platelets, and eosinophils.

Analysis were performed using the Poch-100iy automated method for blood count analysis and the xs-100i (sysmex/Roche) automated/interface method for platelet analysis using impedance and flow cytometry methodology (Lopes, Biondo, & Santos, 2007).

On the same days more blood samples were collected from the jugular vein of each animal in 5 mL tubes containing heparin as anticoagulant (Vacutainer; Becton-Dickinson, Rutherford, NJ).

Preparation of samples for biochemical tests

After blood collection, plasma and erythrocytes were separated by centrifugation at 1000 g for 10 min at 4 °C. Plasma fraction was transferred to a microtube and stored at -80 ºC for further analysis. In order to isolate the

erythrocytes, the fractions of platelets and leukocytes, corresponding to the intermediate fraction, were removed and discarded. Remaining fraction containing the isolated erythrocytes was diluted 1:10 (v/v) with 0.9% commercial saline and centrifuged at 1000 g for 10 min at 4 °C, 3 times, and at each step the supernatant was removed and discarded. At the end of the last centrifugation, the erythrocytes were resuspended in saline at a final dilution of 1:10, and then stored at -80 ºC until the biochemical assays described below were performed (Kakgniashvili, 2004).

Determinations of redox state biomarkers

Dichlorofluorescein Oxidation (DCFH)

Reactive oxygen and nitrogen species were measured in erythrocytes and plasma using 2 ', 7'-dichlorofluorescein diacetate (DCFH2-DA) according to Lebel et al (1992). The DCFH2-DA is cleaved by esterase enzymes producing DCFH2, which is oxidized by reactive species present in the sample, giving rise to DCF fluorescence. Fluorescence was measured at excitation and emission wavelengths of 488 nm and 525 nm, respectively, using the SpectraMax Gemini XS Fluorescence Reader (Molecular Devices, Sunnyvale, CA, USA). Standard DCF curve ranging from 0.25 to 10 µM was performed in parallel. Data are expressed as nmol DCF / mg protein.

Superoxide dismutase activity (SOD)

Total SOD enzyme activity was measured in erythrocytes by quantification of the superoxide inhibition dependent autooxidation at 480 nm (Misra and Fridovich, 1972). Absorbance was measured on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The activity of SOD is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. Data are expressed as SOD units/mg protein.

Catalase Activity (CAT)

The enzymatic activity of CAT was measured in erythrocytes and tested according to Aebi (1984), which was adapted for microplates. Decrease in absorbance at 240 nm was measured in a medium containing 20 mM hydrogen peroxide and 10 mM potassium phosphate buffer pH 7.0 using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The CAT unit is defined as 1 μmol H_2O_2 consumed per minute. The specific activity data are expressed as CAT units/mg protein.

Activity of glutathione peroxidase (GPx)

The activity of GPx enzyme in erythrocytes was tested according to Wendel (1981), which was adapted for microplates. The medium contained 100 mM potassium phosphate buffer, pH 7.7, 1 mM EDTA, 2 mM reduced glutathione (GSH), 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.1 mM NADPH and 0.5 mM tert-butyl hydroperoxide as the enzymatic substrate. The disappearance of NADPH was monitored at 340 nm using the SpectraMax M5

microplate reader (Molecular Devices, Sunnyvale, CA, USA). The GPx unit is defined as 1 µmol of NADPH consumed per minute and the specific activity is represented as GPx units/mg of protein.

Reduced glutathione (GSH)

Concentration of GSH in erythrocytes was measured according to Browne and Armstrong (1998). Initially, proteins in the supernatant were precipitated with meta-phosphoric acid (1: 1) and centrifuged at 5,000 g for 10 min at 25 °C. GSH present in the supernatant is reacted with the fluorophore o-phthaldialdehyde present in the medium at a concentration of 7.5 mM in addition to 100 mM sodium phosphate buffer pH 8.0 containing 5 mM EDTA. Fluorescence was measured at excitation and emission wavelengths of 350 nm and 420 nm, respectively, using the SpectraMax Gemini XS Fluorescence (Molecular Devices, Sunnyvale, CA, USA) microplate reader. The standard GSH curve ranging from 0.001 to 1 mM was prepared and a blank sample was run in parallel. Data are expressed as nmol GSH/mg protein.

Thiol levels

The thiol content was measured in plasma according to Aksenov and Markesbery (2001), adapted for microplates. The assay is based on the reduction of 50-dithiobis-2-nitrobenzoic acid (DTNB) by thiols, which become oxidized (disulphide), yielding a yellow derivative (TNB). Absorbance was measured at 412 nm in a medium containing 20 mM sodium phosphate buffer pH 7.4 and 10 mM DTNB prepared in a 0.2 M potassium phosphate solution pH

8.0 using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol TNB/mg protein.

Protein carbonyl (CARBO) content

Carbonylated protein content was measured in plasma according to Reznick and Packer (1994) and adapted by Stone et al. (2016) for reading in 96-well microplates. Protein carbonyls react with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that was measured at 370 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as nmol carbonyls/mg protein.

Proteins

Protein concentration was measured according to Lowry et al. (1951), which was adapted for microplates using bovine albumin as standard. The absorbance was measured at 750 nm using the SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). Data are expressed as mg protein/mL.

Frequency of diarrhea

Health status was monitored every two day throughout the experimental period. Animals were observed to detect clinical symptoms of diseases and diarrhea. In the same days, body temperature was measured with the aid of a digital clinical thermometer inserted into the animal's rectal ampulla at a depth of approximately 3.5 cm for 3 minutes ion the morning and afternoon.

The consistency and appearance of the faeces were observed daily and the feacal score was assigned according to their appearance, following classification from 0 to 3 according to Ishihara et al. (2001): (0) normal faeces, (1) soft faeces, (2) muddy faeces, and (3) watery faeces. The incidence of diarrhea were calculated according to Ishihara et al. (2001) following formula: Frequency of diarrhea (%): (total number of days suffered from diarrhea/total number of days inspected) X 100.

Experimental Design and Statistical Analysis

Data were analyzed considering the randomized complete block design with repeated measures in time (days), and the experiments were considered as the blocks. Data were analyzed considering six replicates (calves) per block for the control treatment (in both experiments), six and seven replications per block for treatment of green tea extract (for experiments 1 and 2, respectively) and five and eight replications per block for treatment of oregano extract (for experiments 1 and 2, respectively). Uneven number of replicates per block was due deaths of calves, not related to the treatments. Statistical analysis considered treatments (n = 3, control, oregano extract and green tea extract), days (1, 30 and 60), blocks (n=2, experiments) and their interactions as fixed effects, and animal and residue as random effects, using the SAS® MIXED procedure, version 9.4. A structural selection test was performed using the Bayesian information criterion (BIC). Covariance structures tested were compound symmetry, first-order autoregressive, toeplitz, and unstructured. The contrasts CON x OE, CON x GT and OE x GT with adjusted one-tailed side

Dunnett *P*-values were used to compare the means between treatments and blocks for all variables. All variables were previously submitted to the normality test. The significant differences were declared when $P < 0.05$ and a trend considered to exist if $0.05 < P < 0.10$.

Results

The interaction between treatments x blocks x days of evaluation was not significant (P> 0.05) for any of the evaluated variables. The interaction between treatments x blocks (experiments) was significant (P <0.05) for the variables CARBO, SOD, GPx and DCFP. In experiment 1, the use of plant extracts did not modify (P> 0.10) the CARBO concentration. On the other hand, in experiment 2, the CARBO concentration was 0.69 (nmol/mg) lower (P <0.05) in calves fed oregano extract compared to CON and tended (0.05 <P < 0.10) to be 0.36 (nmol/mg) lower in the OE group compared to the GT (Table 2). The activity of the SOD enzyme in the OE group calves was 19.15% higher (P <0.05) than in the GT group calves during the experiment 1, but without differences in relation to the CON; while in the experiment 2 the addition of the plant extracts reduced (P <0.05) the activity of SOD in relation to the CON (Table 2).

The erythrocyte activity of the GPx enzyme during experiment 1 was reduced (P <0.05) by 25.1% and 28.6% due to the addition of extracts of green tea and oregano, respectively in relation to the CON group (Table 2). In the experiment 2 the extract of oregano increased (P <0.05) the erythrocyte activity of this enzyme, when compared with the CON (Table 2).

In experiment 1 the value of plasma oxidized DCF was lower (P <0.05) in calves of the CON group than in the calves of the GT group and tended to be lower (0.05 <P <0.10) for the group CON in relation to OE. In experiment 2, OE group calves tended to present lower concentration of plasma oxidized DCF (0.05 < P < 0.10) than in the CON group.

The interaction treatments x days of evaluation was significant (P <0.05) for the variable DCFP and DCFE. The value of the DCFP was higher in the calves of the CON and OE groups compared to the calves of the GT group at 60 days of life, while on the other evaluated days there was no difference between the treatments (Figure 1A). The DCFE value was higher in calves of the CON group compared to the calves of the GT and OE groups at 30 days of life, while on the other evaluated days there was no difference between the treatments (Figure 1B).

Plant extracts did not influence the concentration of thiols and the erythrocyte activity of the CAT enzyme (P> 0.10 Table 2). The calves receiving oregano extract tended to have a lower erythrocyte concentration of the GSH enzyme compared to the calves that received green tea extract but did not differ from control (Table 2).

We did not detect clinical symptoms of diseases except for diarrhea. Plant extracts did not influence the frequency of diarrhoea (P> 0.10) which was on average 37.9 ± 2.2%, 38.5 ± 2.1%, and 40.6 ± 2.1% for CON, GT and OE, respectively.

Discussion

Calves at birth are totally dependent on the colostrum of the mother to acquire initial immunity, called passive immunity (Kertz et al., 2017), and have the rumen essentially afuncional, and therefore, depend on the nutrients coming from the milk, which are digested by enzymes in the abomasum, absorbed in the intestine and transferred to the bloodstream (Baldwin et al., 2004).

Disturbances in the redox state of the animals can be evaluated with the use of biomarkers (Zwart et al., 1999). Oxidative damage to proteins is induced by direct attack by reactive species or indirectly involving attack by products of lipid peroxidation (Halliwell & Gutteridge, 2007). The attack of reactive species on proteins can generate amino acid radicals, which can react with O_2 forming the peroxyl and alkoxyl radicals. Peroxyl radicals are capable of capturing hydrogen atoms producing peroxides of proteins, while alkoxyl radicals can fragment the protein through beta-cleavage to form carbonyls (Halliwell & Whiteman, 2004). In this sense, the most commonly used marker to evaluate oxidative damage to proteins is the carbonyl determination assay, performed by spectrophotometry and enzyme immunoassay (ELISA) techniques (Dalle-Donne et al., 2003; Levine, 2002; Reznick & Packer, 1994).

In this sense, the results of the present study showed some benefit in the use of oregano extract (but not for the green tea extract), to reduce the level of protein oxidation, verified by the lower carbonyl and the DCFP concentration in relation to control. This positive result was found only in experiment 2.

On the other hand, the enzymatic defense system includes the enzymes Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). These enzymes act preventing the oxidation of cell biomolecules,

controlling the levels of free radicals and non-radical species involved in the initiation of chain reactions that culminate in the propagation and amplification of the process and, consequently, the occurrence of oxidative damage (Ferreira & Matsubara, 1997).

The present study did not evidence a clear overall beneficial effect of feeding green tea and oregano extract on SOD and CAT, and the beneficial effect of the oregano extract, increasing the activity of the GPx in relation to the control, was verified only in the experiment 2. We do not have a plausible reason to this lack of effect of plant extracts on antioxidant enzymes activity inrta and inter trials because the experiments were runned at the same season (but on different years) and under similar meteorological conditions.

According to Hulbert and Moisá (2016), there are two critical aspects during the pre-weaning period: the decrease of passive immunity and the transition from pre-ruminant to ruminants. In this period, the overall positive effect of oregano extract reducing the concentration of DCFE compared to control signaled that the oregano extract was effective in oxidizing the reactive species (Halliwel & Whiteman, 2004). It is worth to notice that the effects plant extracts were depended on the day of measurement, what is probably related to distinct susceptibility of calves to the already mentioned challenges calves usually face. In this sense, green tea was effective in reducing DCF in plasma at the end of the trial (day 60) while both plant extracts were effective in reducing DCP in the erythrocytes on day 30 after birth.

Other studies in the literature have also shown controversial results on stimulating the immune and antioxidant systems of ruminants supplemented

with green tea extract and oregano. Peraskevakis (2015) added 30 g of dried oregano leaves (equivalent to 1 mL of essential oil) to the diet of dairy goats and observed an improvement in enzymatic and non-enzymatic antioxidant defenses in blood and milk. On the other hand, Maciej et al. (2016) reported that calves supplemented with 10 mg/day of quercitin (a secondary compound present in green tea) did not alter the metabolism and antioxidant status. Zhong et al. (2011) found that lower dosages (2 g/kg DM) of catechin supplementation promoted a better antioxidant action when compared to higher dosages (3 or 4 g/kg DM).

Plant extracts had few beneficial effects on the redox status of dairy calves, and, therefore, did not influence the health status of calves, e.g. the frequency of diarrhea. It should be noticed that calves present a good health status without clinical symptoms of diseases, except for diarrhea. Variations between studies about the supplementation of green tea and oregano extracts to calves could be related to the doses used, type of product and intrinsic aspects of animals.

Conclusions

The supply of green tea or oregano extract to Jersey calves during the pre-weaning period improved some biomarkers of the redox state and did not influence health status. However, benefitial results on antioxidant status are promising, requiring further studies on the performance of antioxidant enzymes in calves in the lactation phase.

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Ethics statements

This study was approved by the Ethics Commission for the Use of Animals from the Universidade Federal do Rio Grande do Sul (CEUA/UFRGS), protocol number 30756.

References

Aebi H 1984. Catalase in vitro. Methods Enzymol, 105, 121-126.

Aksenov MY, Markesbery WR 2001. Change in thiol content and expression of glutathione redox system gene in the hippocampus and cerebellum in Alzheimer's disease. Neuroscience Letters 302, 141–145.

Baldwin RL, Mcleod KR, Klotz JL, Heitmann RN 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. Journal of Dairy Science, 87, 55-65.

Berlett BS, Stadtman ER 1997. Protein oxidation in aging, disease, and oxidative stress. Journal of Biological Chemistry, 272, 20313–20316.

Browne RW, Armstrong D 1998. Reduced glutathione and glutathione disulfide. Methods MolBiol, 108, 347-52.

Cecarini V, Gee J, Fioretti E, Amici M, Angeletti M, Eleuteri AM, Keller JM 2007. Protein oxidation and cellular homeostasis: Emphasis on metabolism. Biochimica et Biophysica Acta, 2, 93-104.

Dalle-Donne, I. Giustarini D, Colombo R, Rossi R, Milzani A 2003. Protein carbonylation in human diseases. Trends in Molecular Medicine, 9, 169-76.

Drackley JK 2008. Calf nutrition from birth to breeding. Veterinary Clinics of North America: Food Animal Practice, 24, 55-86.

Elshahawy II 2018. Oxidantantioxidant Status in Calves Supplemented with Green Tea Extract. International Scholarly and Scientific Research & Innovation 12.

Fruscalso V, Antillón GO, Hötzel MJ 2017. Smallholder family farmers' perceptions, attitudes and choices regarding husbandry practices that influence performance and welfare of lactating dairy calves. Ciència rural 47, 4-25.

Gladine C. Rock E, Morand C, Bauchart D, Durand D 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. British Journal of Nutrition 98, 691-701.

Guo Q, Zhao B, Li M, Shen S, Xin W 1996. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochimica et Biophysica Acta 1304, 210 - 222.

Halliwell B, Whiteman M 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? British Journal Of Pharmacology, 142, 231-255.

Halliwell, B.; Gutteridge, JMC. 2007.Free Radicals in Biology and Medicine. Fourth ed., Oxford University Press. 851.

Hulbert L, Moisá SJ 2016. Stress, immunity, and the management of calves. Journal of Dairy Science 99, 1-18.

Katsoulos PD, Karatzia MA, Dovas CI, Filioussis G, Papadopoulos E, Kiossis E, Arsenopoulos K, Papadopoulos T, Boscos C, Karatzias H 2017. Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves. Research in Veterinary Science 115, 478-483.

Kertz AF, Hill TM, Quigley JD, Heinrichs AJ, Linn JG, Drackley JK 2017. A 100-Year Review: Calf nutrition and management. Journal of Dairy Science, 100,10151-10172.

Kondo MK, Kita K, Yokota HO 2007. Ensiled or oven-dried green tea by-product as protein feedstuffs: Effects of tannin on nutritive value in goats. Asian-Australasian Journal of Animal Sciences. 20, 880–886.

Köppen, W., 1900: Versuch einer Klassifikation der Klimate, vorzugweise nach ihren Beziehungen zur Pflanzenwelt. – Geogr. Z. 6, 657–679.

Köppen, W., 1901: Versuch einer Klassifikation der Klimate, vorzugweise nach ihren Beziehungen zur Pflanzenwelt. – Meteorol. Z. 18, 106–120.

LeBel CP, Ischiropoulos H, Bondy SC 1992. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chemical Research Toxicology, 5, 227-231.

Levine, RL 2002. Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radical Biology and Medicine, 32, 790-796, .

Maciej J, Schäff CT, Kanitz E, Tuchscherer A, Bruckmaier RM, Wolffram S and Hammon HM 2016. Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. Journal of Dairy Science 99, 805-811.

Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L 2004. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition 79, 727-747.

Misra HP, Fridovich I 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247, 3170-3175.

Nazifi SM, Saeb N, Ghafari I, Razeghian M, Razavi F, Vosoughi and H. 2009. Orangi: Reference values of oxidative stress parameters in adult native Iranian goats. Bulgarian Journal of Veterinary Medicine. 12, 119-124

Oh J and Hristov AN 2016. Effects of plant-derived bio-active compounds on rumen fermentation, nutrient utilization, immune response, and productivity of ruminant animals. Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization. Oxford University Press Inc, Madson, NY, USA.

Paraskevakis, N. 2015. Effects of dietary dried Greek Oregano (Origanum vulgare ssp. hirtum) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. Animal Feed Science and Technolog 209, 90-97.

Reznick AZ, Packer L 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233, 357-63.

Santos G dos, Bittar CMM 2015. A survey of dairy calf management practices in some producing regions in Brazil. Revista Brasileira Zootecnia. 44, 361–370.

Serafini M, Del Rio D, Yao DN, Bettuzzi S, Peluso I 2011. “Health benefits of tea”, in Herbal Medicine: Biomolecular and Clinical Aspects, 2nd Edn, Chapter 12, eds I. F. F. Benzie and S. Wachtel-Galor (Boca Rotan, FL: CRC Press), 239–262.

Silva CS, Souza EJO, Pereira GFC, Cavalcante EO, Lima EIM, Torres TR, Silva DC 2017. Plant extracts as phytogenic additives considering intake, digestibility, and feeding behavior of sheep. Tropical animal health and production, 49, 353-359.

Stone V, August, PM, Stocher DP, Klein CP, Couto PR, Silva YD, Ssalomon TB, Benfato MS, Matté C 2016. Food restriction during pregnancy alters brain's antioxidant network in dams and their offspring. Free radical research 50, 530-541.

Wendel, A. 1981. Glutathione peroxidase. Methods Enzymol 77, 325-33.

Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE 1999. Free Radical Biology and Medicine, 26, 202.

Table 4 *Mean and amplitude of air temperature (ºC), relative air humidity (%), wind speed (km/h) and precipitation (mm/day) during the experimental period.*

Month	Temperature		Humidity		Wind speed		Precipitation
	Mean	Amplitude	Mean	Amplitude	Mean	Amplitude	
				Experiment I			
March	20.9	17.8-25.5	86.2	68.3-97.2	6.8	0.5-18.1	321.5
April	19.8	9.8-29.1	89.6	68.9-97.7	6.4	1.1-15.5	339
May	13.6	9.6-17.7	90.1	74.8-98.7	5.4	0.1-15.6	153.8
June	10.6	7.7-14.5	87.3	65.5-98.9	5.1	0.5-13.6	29.4
				Experiment II			
March	21.6	15.9-27.3	83.2	67.8-91.9	6.1	0.1-10.7	139.6
April	19	13.9-23.4	81.3	51.8-94.9	6.8	2.5-15.8	135.3
May	16.7	13.2-21	90.1	79.8-97	1.7	0.3-5.2	221.1
June	14.9	7.8-22.8	85.1	66.1-97.6	6.2	1.7-12.8	137.2

Table 5 *Averages for the redox state profile of calves that received or not green tea extract (GT) or oregano extract (OE).*

Variables[2]	Block[3]	Treatments[1]			SEM	*P* – values for Contrast		
		CON	GT	OE		CON x GT	CON x OE	GT x OE
THIOLS (nmol/mg)		0.37	0.39	0.39	0.02	0.52	0.54	0.98
CARBO (U/mg)	Exp I	1.93	1.74	2.02	0.16	0.40	0.70	0,15
DCFP (nmol/mg)	Exp II	1.45	1.12	0.76	0.13	0.10	0.001	0,05
DCFP (nmol/mg)	Exp I	2680.98	3071.91	3033.37	146.36	0.04	0.08	0.85
SOD (U/mg)	Exp II	1978.86	1765.00	1676.99	120.99	0.17	0.05	0.53
SOD (U/mg)	Exp I	26.21	23.30	28.82	1.80	0.23	0.32	0.04
GPx (U/mg)	Exp II	58.87	51.59	48.74	5.14	0.01	0.01	0.75
CAT (U/mg)		2.16	2.14	2.21	0.14	0.93	0.78	0.71

[1]Treatments: CON = control; GT = green tea extract; OR = oregano extract.

[2]Variables: CARBO = carbonyl; DCFP = oxidation of dichlorofluorescein in plasma; SOD = superoxide dismutase; GPx = glutathione peroxidase.

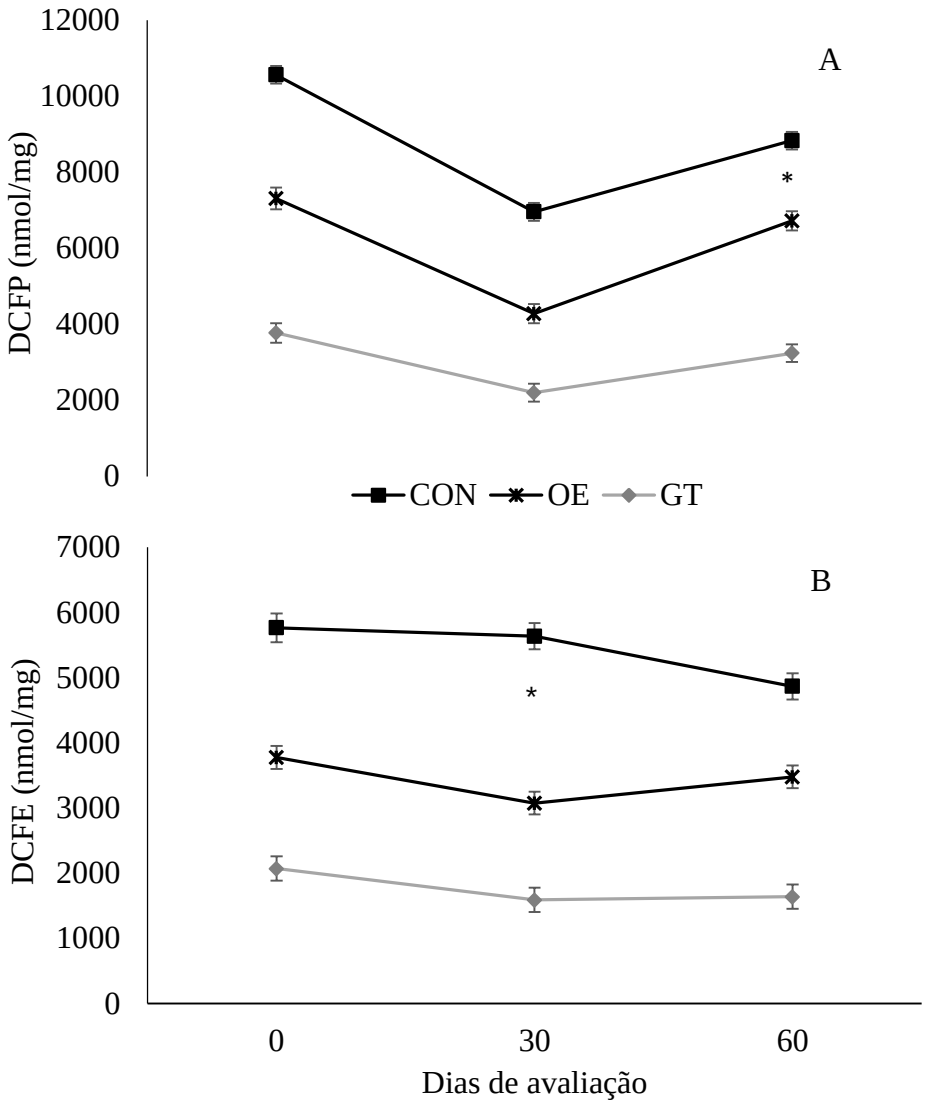
Variables without interaction: DCFE = oxidation of dichlorofluorescein in erythrocytes; GSH = reduced glutathione;

[3]Block: Exp I = experiment I; Exp II = experiment II.

SEM = Standard Error of the Mean.

Figures

Figure 4 Mean and variation for dichlorofluorescein in plasma (DCFP; A) and dichlorofluorescein in erythrocytes (DCFE; B) according to control treatments (CON), green tea extract (GT) and oregano extract (OE).



CONSIDERAÇÕES FINAIS

A contribuição do presente estudo foi avaliar o estado redox, perfil hematológico e estado de saúde em bezerros leiteiros, os quais consumiram extrato de chá verde (*Camellia sinensis*) ou de orégano (*Origanum vulgare*) ou receberam leite oriundo de vacas suplementadas com os mesmos extratos, do nascimento aos 60 dias de vida.

No primeiro estudo, de maneira geral, pode-se observar que bezerros alimentados com leite de vacas suplementadas com extrato de chá verde ou orégano apresentaram maior concentração de enzimas antioxidantes, exercendo impacto positivo sobre a oxidação de espécies reativas, já em relação ao perfil hematológico apenas os neutrófilos foram influenciados pela adição dos extratos vegetais, sendo assim melhorias essas podem prevenir doenças, impactando positivamente na saúde dos animais, tendo em vista a fase de criação se tratar de um momento bastante desafiador à saúde dos animais.

No segundo artigo o fornecimento de extrato de chá verde ou orégano diretamente a bezerros durante o período de aleitamento resultou em efeitos benéficos menos pronunciados sobre o estado redox de bezerros leiteiros, com destaque apenas para a enzima GPx, a qual aumentou para grupo EO, e para a redução dos níveis de carbonilas no mesmo grupo de estudos, já em relação a oxidação de espécies reativas no estudo I observou-se uma menor oxidação de espécies reativas no plasma em relação ao controle, dessa forma como nosso estudo, observou-se que ambos os extratos mostram-se promissores na atuação no sistema antioxidante em bezerros leiteiros no nascimento aos 60 dias de vida.

Estudos sobre o estado redox para bezerros em aleitamento ainda encontra-se nas fases iniciais de desenvolvimento. Pesquisas têm comprovado a participação de radicais livres aumentadas em casos de enfermidades e diarreias em bezerros. No entanto, há muito a ser descoberto sobre o seu papel na sanidade e na produção de ruminantes. A clareza da compreensão sobre a ação dos radicais livres nos animais permitirá a concepção de terapias antioxidativas específicas. Assim como delinear padronização de técnicas, metodologias e valores de referência para os biomarcadores de ocorrência do desequilíbrio o estado redox.

Para que o futuro do uso de extratos vegetais se ainda mais promissor, é necessário, inicialmente, mais trabalhos voltados à qualidade dos extratos vegetais utilizados. É necessário encontrar uma forma de padronizar a composição química dos mesmos, e garantir ao produtor que, independente da época do ano ou do local onde for produzido este extrato, ele terá repetibilidade em seus resultados. Talvez trabalhos em conjunto com diversas áreas da ciência como agronomia, farmácia, biologia, por exemplo, seja a chave para buscar um resultado final que agrade produtor, consumidor e população.

REFERÊNCIAS

ABBAS, A. K. *et al*. **Imunologia celular e molecular.** 7. ed. Rio de Janeiro: Elsevier, 2011. 529 p.

ABRAHAMSE, S. L. *et al.* Absorption, distribution, and secretion of epicatechin and quercetin in the rat. **Nutrition Research**, New York, v. 25, n. 3, p. 305-317, 2005.

AESCHBACH, R. *et al.* Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. **Food and Chemical Toxicology**, Oxford, v. 32, n. 1, p. 31-36, 1994.

ALEXANDER, M. Aromatherapy and immunity: how the use of essential oils aids immune potentiality. **International Journal of Aromatherapy**, London, v. 12, n. 1, p. 49- 56, 2002.

ALMEIDA, L. M. V. *et al.* Protective effects of resveratrol on hydrogen peroxide induced toxicity in primary cortical astrocyte cultures. **Neurochemical Research**, New York, v. 33, n. 1, p. 8-15. 2008.

BALDWIN, R. L. *et al.* Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. **Journal of Dairy Science**, Champaign, v. 87, n. 9, p. 55-65, 2004.

BALLATORI, N. Glutathione dysregulation and the etiology and progression of humans diseases. **Journal of Biological Chemistry**, Balimore, v. 390, n. 3, p. 191-214, 2009.

BANIAS, C. *et al.* The effect of primary antioxidants and synergists on the activity of plant extracts in lard. **Journal of the American Oil Chemists Society**, Champaign, v. 69, n. 6, p. 520-524, 1992.

BASILICO, M. Z. *et al.* Inhibitory effects of some spice essential oils on Aspergillus ochraceus NRRL 3174 growth and ochratoxin a production. **Letters in Applied Microbiology**, Oxford, v. 29, n. 4, p. 238-241, 1999.

BENCHAAR, C. *et al.* A review of plant-derived essential oils in ruminant nutrition and production. **Animal Feed Science and Technology**, Amsterdam, v. 145, n. 1-4, p. 209-228, 2008.

BITTAR, C.M. *et al.* Desempenho e desenvolvimento do trato digestório superior de bezerros leiteiros alimentados com concentrado de diferentes formas físicas. **Revista Brasileira de Zootecnia,** Viçosa v. 38, n. 8, p.1561-1567, 2009.

BLACK, P. H. The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes

and metabolic syndrome X. **Brain, Behavior, and Immunity**, San Diego CA, v. 17, n. 5, p. 350-364, 2003.

BLACK, P. H.; GARBUTT, L. D. Stress, inflammation and cardiovascular disease. **Journal of Psychosomatic Research**, Oxford, v. 52, n.1, p. 1-23, 2002.

BUNEŠOVÁ, V. *et al.* Effect of rearing systems and diets composition on the survival of probiotic bifidobacteria in the digestive tract of calves. **Livestock Science**, Amsterdam, v. 178, p. 317-321, 2015.

BURT, S. Essential oils: their antibacterial properties and potential applications in foods: a review. **International Journal of Food Microbiology**, Amsterdam, v. 94, n. 3, p. 223-253, 2004.

BUSQUET, M. *et al.* Plant extracts affect in vitro rumen microbial fermentation. **Journal of Dairy Science**, Champaign, v. 89, n. 2, p. 761-771, 2006.

CALSAMIGLIA, S. *et al.* Invited review: essential oils as modifiers of rumen microbial fermentation. **Journal of Dairy Science**, Champaign, v. 90, n. 6, p. 2580–2595, 2007.

CERVATO, G. *et al.* Antioxbdant properties of oregano (Origanum vulgare) Leaf extracts. **Journal of Food Biochemistry**, Westport, v. 24, n. 6, p. 453-465, 2000.

CHAVES, A. V. *et al.* Dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. **Livestock Science**, Amsterdam, v. 141, n. 2-3, p. 213-220, 2011.

CHAVES, A. V. *et al.* Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. **Animal Feed Science and Technology**, Amsterdam, v. 145, n. 1-4, p. 396-408, 2008.

CHRISTAKI, E. *et al.* Aromatic plants as a source of bioactive compounds. **Agriculture**, Basel, v. 2, n. 3, p. 228-243, 2012.

CHRISTAKI, E. *et al.* Aromatic plants as a source of bioactive compounds. **Agriculture**, Basel, v. 2, n. 3, p. 228-243, 2012.

CHURCH, D. C. **Fisiología digestiva y nutrición de los rumiantes**: fisiología digestiva. Zaragoza: Acribia, 1974. 1 v.

CHURCH, D. C. **Fisiología digestiva y nutrición de los rumiantes**: nutrición prática. Zaragoza: Acribia, 1974. 3 v.

COBELLIS, G. *et al.* Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. **Science of the Total Environment**, Amsterdam, v. 545-546, n. 12, p. 556-568, 2016.

COELHO, S. G. Desafios na criação e saúde de bezerros. **Ciência Animal Brasileira**, Goiânia, 2009. Supl. 1. Palestra apresentada durante o VIII Congresso Brasileiro de Buiatria, realizado em Belo Horizonte, MG em outubro de 2009.

COMHAIR, S. A. A.; ERZURUM, S. C. Antioxidant responses to oxidant-mediated lung diseases. **American Journal of Physiology**: lung cellular and molecular physiology, Bethesda, v. 283, p. 246-255, 2002.

COWAN, M. M. Plant products as antimicrobial agents. **Clinical Microbiology Reviews**, Washington, v. 12, n. 4, p. 564-582, 1999.

CRUVINEL, W. M. *et al.* Sistema imunitário: parte I: fundamentos da imunidade inata com ênfase nos mecanismos moleculares e celulares da resposta inflamatória. **Revista Brasileira de Reumatologia**, Campinas, v. 50, p. 434-461, 2010.

CULOTTA, V. C. Superoxide dismutase, oxidative stress, and cell metabolism. **Current Topics in Cellular Regulation**, New York, v. 36, p. 117-132, 2000.

CYBORAN, S. *et al.* Concentrated green tea supplement: biological activity and molecular mechanisms. **Life Sciences**, Oxford, v. 126, p. 1-9, 2015.

DAMASCENO, D. C. *et al.* Radicais livres, estresse oxidativo e diabete. **Diabetes Clínica**, São Paulo, v. 5, p. 355-361, 2002.

DIDRY, N.; DUBREUIL, L.; PINKAS, M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. **Pharmaceutica Acta Helvetiae**, Zurich v. 69, n. 1, p. 25-28, 1994.

DORMAN, H. J. D.; DEANS, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **Journal of Applied Microbiology**, Oxford v. 88, n. 2, p. 308-316, 2000.

ELGAYYAR, M. *et al.* Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. **Journal of Food Protection**, Ames, Iowa, v. 64, n. 7, p. 1019-24, 2001.

ELSHAHAWY, I. I. Oxidantantioxidant status in calves supplemented with green tea extract. **International Scholarly and Scientific Research & Innovation**, United Kingdom, v. 12, n. 4, p. 108-111, 2018.

FANG, Y. Z. *et al.* Free radicals, antioxidants and nutrition. **Nutrition**, Tarrytown, v. 18, n. 10, p. 872-879, 2002.

FARKAS, O. *et al.* Quantitative structure: antioxidant activity relationships of flavonoid compounds. **Molecules**, Basel, v. 9, n. 12, p. 1079–1088, 2004.

FASSEAS, M. K. *et al.* Antioxidant activity in meat treated with oregano and sage essential oils. **Food Chemistry**, Barking, v. 106, n. 3, p. 1188-1194, 2008.

FORMAN, H. J. *et al.* Glutatione: overview of its protective roles, mensurement and biosynthesis. **Molecular Aspects of Medicine**, Elmsford NY, v. 30, n. 2, p. 1-12, 2009.

FRANDSON, R. D. *et al.* **Anatomia e fisiologia dos animais de fazenda**. 7. ed. Rio de Janeiro: Guanabara Koogan, 2011.

FROEHLICH, K. A. *et al*. Evaluation of essential oils and prebiotics for newborn dairy calves. **Journal of Animal Science,** Champaing v. 95, n. 8, p. 3772-2782, 2017.

FUJIWARA, R. *et al.* Psychoneuroimmunological benefits of aromatherapy. **International Journal of Aromatherapy,** França, v. 12, n. 2, p. 77-82, 2002.

GABBI, A. M.; VIÉGAS, J.; MORAES, R. S. Hematological parameters of dairy heifers submitted to diets with phytogenic additives. **Brazilian Journal of Health and Animal Production**, Ondina, v. 10, n. 4, p. 917-928, 2009a.

GABBI, A. M. et al. Productive performance and behavior of dairy heifers submitted to diets with phytogenic additive. **Brazilian Journal of Health and Animal Production**, Ondina, v. 10, n. 4, p. 949-962, 2009b.

GLADINE, C. *et al.* Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. **British Journal of Nutrition**, London, v. 98, n. 4, p. 691-701, 2007.

GONÇALVES, G. *et al.* Green tea extract improves the oxidative state of the liver and brain in rats with adjuvant-induced arthritis. **Food and Function**, Cambridge, v. 6, n. 8, p. 2701-2711, 2015.

GOODSELL, D. S. **Superoxide dismutase**. *In:* RCSB Protein Data Bank. United States, 2007.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radical Biology & Medicine**. 3rd ed. New York: Oxford University Press, 1999.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radical Biology & Medicine***.* 4th ed. Oxford, UK: Clarendon Press, 2007.

ISHIHARA, N. *et al.* Improvement of intestinal microflora balance and prevention of digestive and respiratory organ diseases in calves by green tea extracts. **Livestock Production Science**, Amsterdam, v. 68, n. 2, p. 217-229, 2001.

JONES, M. L.; ALLISON, R. W. Evaluation of the ruminant complete blood cell count. **Veterinary Clinics of North America: Food Animal Practice**, Philadelphia, v. 23, n. 3, p. 377-402, 2007.

KAMPEN, A.H. *et al.* Lymphocyte subpopulations and neutrophil function in calves during the first 6 months of life. **Veterinary Immunology and Immunopathology**, Amsterdam, v. 113, n. 3, p. 53-63, 2006.

KATSOULOS, P. D. *et al.* Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves. **Research in Veterinary Science,** London, v. 115, p. 478-483, 2017.

KERTZ, A. F. *et al.* A 100-year review: calf nutrition and management. **Journal of Dairy Science**, Champaing, v. 100, n. 12, p. 10151-10172, 2017.

KHAN, N.; MUKHTAR, H. Tea polyphenols for health promotion. **Life Sciences**, Oxford, v. 81, n. 7, p. 519-533, 2007.

KILMARTIN, P. A.; HSU, C. F. Characterisation of polyphenols in green, oolong, and black teas, and in coffee, using cyclic voltammetry. **Food Chemistry**, Barking, v. 82, n. 4, p. 501-512, 2003.

KOLLING, G. J. *et al*. Oregano extract added into the diet of dairy heifers changes feeding behavior and concentrate intake. **The Scientific World Journal**, New York, v. 2016, p. 6, 2016.

KOLLING, G. J. *et al.* Performance and methane emissions in dairy cows fed oregano and green tea extracts as feed additives. **Journal of Dairy Science**, Champaign, v. 101, n. 5, p. 4221-4234, 2018.

KOURY, J. C; DONANGELO, C. M. Zinco, estresse oxidativo e atividade física. **Revista Nutrição,** Campinas, v. 16, n. 4, p. 433-441, 2003.

LAGOURI, V. *et al*. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. **European Food Research and Technology**, Berlin, v. 197, n. 1, p. 20-23, 1993.

LAMBERT, J. D.; YANG, C. S. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis**, Amsterdam, v. 523, p. 201-208, 2003.

LAMBERT, R. J. W. *et al.* A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. **Journal of Applied Microbiology**, Oxford, v. 91, n. 3, p. 453-462, 2001.

LAMOTHE, S. *et al.* Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. **Food and Function**, Cambridge, v. 5, n. 10, p. 2621-2631, 2014.

LAUZON, K. *et al.* Antioxidants to prevent bovine neutrophil-induced mammary epithelial cell damage. **Journal of Dairy Science**, Champaign, v. 88, n. 12, p. 4295-4303, 2005.

LEJONKLEV, J. *et al.* Effect of oregano and caraway essential oils on the production and flavor of cow milk. **Journal of Dairy Science**, Champaign, v. 99, n. 10, p. 7898-7903, 2016.

LI, S. P. *et al.* Synergy of Astragalus polysaccharides and probiotics (Lactobacillus and Bacillus cereus) on immunity and intestinal microbiota in chicks. **Poultry Science**, Edinburgh, v. 88, p. 519-525, 2009.

LOGAN, E. F. Colostral immunity to colibacillosis in the neonatal calf. **The British Veterinary Journal***,* Inglaterra, v. 5, p. 405-412, 1974.

LOGAN, E. F. *et al*. Changes in the serum immunoglobulin levels of colostrum-fed calves during the first 12 weeks postpartum. **Research in Veterinary Science**, London, v. 14, p. 394-397, 1973.

LONGENBACH, J. I.; HEINRICHS, A. J. A review of the importance and physiological role of curd formation in the abomasum of young calves. **Animal Feed Science and Technology**, Amsterdam, v. 73, n.1, p. 85-97, 1998.

MACIEJ, J. *et al.* Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves. **Journal of Dairy Science**, Champaign, v. 99, n. 1, p. 805-811, 2016.

MANACH, C. *et al.* Comparison of the bioavailability of quercetin and catechin in rats. **Free Radical Biology and Medicine**, New York, v. 27, n. 11-12, p. 1259-1266, 1999.

MANACH, C. *et al.* Polyphenols: food sources and bioavailability. **The American Journal of Clinical Nutrition**, Bethesda, v. 79, n. 5, p. 727-747, 2004.

MCGUIRE, T. C. *et al.* Failure of colostral immunoglobulin transfer in calves dying from infectious disease. **Journal of the American Veterinary Medical Association**, Chicago, v. 169, n.7, p. 713-718, 1976.

McKay, D. L.; Blumberg, J. B. The role of tea in human health: an update. **Journal of the American College of Nutrition**, New York, v. 21, n. 1, p. 1-13, 2002.

MEALE, S. J. *et al.* Development of ruminal and fecal microbiomes are affected by weaning but not weaning strategy in dairy calves. **Frontiers in Microbiology**, v. 7, n. 582, p. 1-16, 2016.

NAKAGAWA, K. *et al.* Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human

plasma. **Bioscience, Biotechnology and Biochemistry**, Tokyo, v. 61, n. 12, p. 1981-1985, 1997.

NISHIDA, T. *et al.* Effects of Green Tea (Camellia sinensis) Waste Silage and Polyethylene Glycol on Ruminal Fermentation and Blood Components in Cattle. **Asian-Austalasian Journal Animal Science**, Seoul, v. 19, n. 12, p. 1728–1736, 2006.

OLIVEIRA, J. S. *et al.* Fisiologia, manejo e alimentação de bezerros de corte. **Arquivos de Ciências Veterinárias e Zoologia da UNIPAR**, Umuarama, v. 10, n.1, p.39-48, 2007.

PARASKEVAKIS, N. Effects of dietary dried Greek Oregano (Origanum vulgare ssp. hirtum) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats. **Animal Feed Science and Technology**, Amsterdam, v. 209, p. 90-97, 2015.

PEIXOTO-NEVES, D. *et al.* Vasorelaxant effects of the monoterpenic phenol isomers, carvacrol and thymol, on rat isolated aorta**. Fundamental and Clinical Pharmacology**, Paris, v. 24, n. 3, p. 341-350, 2009.

POKORNY, J.; YANISHLIEVA, N.; GORDON. M. (ed.). **Antioxidants in foods**: practical applications. Boca Raton: CRC ; Cambridge: Woodhead, 2001. 288 p.

ROITT, I. *et al.* **Imunologia**. 5. ed. São Paulo: Helvética Editorial, 1999.

ROSENFELDT, F. *et al.* Oxidative stress in surgery in an ageing population: Pathophysiology and the therapy. **Experimental Gerontology**, Oxford, v. 48, n.1, p. 45-54, 2013.

RUSAK, G. *et al.* Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. **Food Chemistry**. Barking, v.1, n. 10, p. 852–858, 2008.

SENANAYAKE, S. N. Green tea extract: Chemistry, antioxidant properties and food applications–A review. **Journal of Functional Foods**, London, v. 5, n. 4, p. 1529-1541, 2013.

SIES, H. Oxidative stress: oxidants and antioxidants. **Experimental Physiology.** Oxford, v. 82, n. 2, p. 291-295, 1997.

SOTO, L. P. *et al.* Design of macrocapsules to improve bacterial viability and supplementation with a probiotic for young calves. **Animal Feed Science and Technology**, Amsterdam, v. 165, n. 4, p. 176-183, 2011.

SUÁREZ, B. J. *et al.* Effects of supplementing concentrates diffreng in carbohydrate composition in veal calf diets: II. Rumen development. **Journal of Dairy Science,** Champaing, v. 89, n. 11, p. 4376-4386, 2006.

TIZARD, I. R. **Imunologia veterinária**: uma introdução. 8. ed. Rio de Janeiro: Elsevier, 2008.

ULTEE, A. *et al.* Adaptation of the food-borne pathogen Bacillus cereus to carvacrol. **Archives of Microbiology**, Berlin, v. 174, n. 4, p. 233-238, 2000.

USDA- UNITED STATES DEPARTMENT OF AGRICULTURE. **Colostrum feeding and management on U.S. dairy operations 1991-2007**. Washington, mar. 2008. Disponível em: https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_is_Colostrum.pdf. Acesso em: 2 jan. 2018.

VANNUCCHI, H. *et al.* Papel dos nutrientes na peroxidação lipídica e no sistema de defesa antioxidante. **Medicina,** Ribeirão Preto, v. 31, n. 1, p. 31-44,1998.

WEAVER, D. M. *et al.* Passive transfer of colostral Immunoglobulins in calves**. Journal of Veterinary Internal Medicine,** Philadelphia, v. 14, n. 6, p. 569-577, 2000.

WEIN, S. *et al.* Systemic absorption of catechins after intraruminal or intraduodenal application of a green tea extract in cows. **PloS ONE**, San Francisco, v. 11, n. 7, [art.]. e0159428, 2016.

YANG, M. *et al.* Colostrum quality affects immune system establishment and intestinal development of neonatal calves. **Journal of Dairy Science**, Champaign, v. 98, p. 7153-7163, 2015.

YOSHINO, K. *et al.* Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. **The Journal of Nutritional Biochemistry**, New York, v. 10, n. 4, p. 223-229, 1999.

ZHU, Q. Y. *et al.* Stability of green tea catechins. **Journal of Agricultural and Food Chemistry**, Washington, v. 45, n. 12, p. 4624–4628, 1997.

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