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DISSERTAÇÃO DE MESTRADO

PROTOZOÁRIOS GASTROINTESTINAIS EM BEZERROS LEITEIROS NO OESTE CATARINENSE: EPIDEMIOLOGIA, PROFILAXIA E CONTROLE

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, Área de Concentração Ciência e Produção Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de **Mestre em Zootecnia**.

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Co-orientador: Ana Luiza Bachmann Schogor

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PROTOZOÁRIOS GASTROINTESTINAIS EM BEZERROS LEITEIROS NO OESTE CATARINENSE: EPIDEMIOLOGIA, PROFILAXIA E CONTROLE

Elaborada por
Andreia Volpato

como requisito para obtenção do grau de
Mestre em Zootecnia

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
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PROTOZOÁRIOS GASTROINTESTINAIS EM BEZERROS LEITEIROS NO OESTE CATARINENSE: EPIDEMIOLOGIA, PROFILAXIA E CONTROLE

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Chapecó, 06 de abril de 2017

O objetivo desse estudo foi identificar os principais protozoários que acometem bezerras leiteiras no oeste catarinense e verificar se protocolos profiláticos e terapêuticos são eficientes no controle de agentes parasitários e, consequentemente, da diarreia. Ao todo, quatro experimentos foram realizados para o desenvolvimento deste trabalho. Para o experimento I foram coletadas amostras fecais de 243 bezerros de até 60 dias de idade em 43 propriedades da região Oeste de Santa Catarina, Brasil. O gênero *Giardia* apresentou maior prevalência, em 26,75% (65/243) das amostras, seguido de *Eimeria* em 21,81% (53/243) e *Cryptosporidium* em 20,99% (51/243). Foi identificado que o manejo de alimentação, período de tempo que os bezerros permaneceram com suas mães e contato com cães, são fatores de risco para infecções por *Cryptosporidium*. O risco de contrair *Giardia* aumentou de acordo com a fonte de leite, enquanto o tipo de superfície e a idade foram apontadas como fatores de risco para *Eimeria*. No experimento II, foi administrado secnidazol via oral, em forma de cápsula e dose única, para 12 animais. Cinco dias após o tratamento, os animais não estavam excretando cistos de *Giardia*, enquanto os bezerros que não receberam o tratamento ainda excretavam cistos do parasito. Após 30 dias de tratamento, 83,3% dos animais permaneceram sem excretar cistos de *G. duodenalis*. No experimento III, foi aplicado selenito de sódio e vitaminas A e E em 16 bezerras com um dia de vida. A segunda dose foi aplicada com 10 dias de idade juntamente com o fornecimento de uma cápsula de secnidazol. As bezerras tratadas apresentaram maiores valores de hematócrito (60 dias de idade), proteínas totais (dias 15 e 30), ceruloplasmina (dias 15, 30 e 60), IgG de cadeia pesada (dias 15, 30, 45 e 60), IgG de cadeia leve (dias 45 e 60) e haptoglobinas (dias 15, 30, 45 e 60). O grupo tratado não apresentou excreção de cistos de *Giardia* até os 30 dias de idade. Foi observado maior ganho de peso nos animais tratados aos 210 dias de idade. Para o experimento IV, 15 bezerras consumiram um produto comercial a base de componentes de óleos essências do nascimento até 30 dias de idade (carvacrol e cinamaldeido). As bezerras tratadas apresentaram maiores valores de hematócrito (dias 45 e 60) e atividade de glutationa S-transferase (60). A contagem bacteriana foi reduzida no grupo tratado (dias 30 e 60). O ganho de peso foi superior nas bezerras tratadas com mix de óleos essenciais aos 60 e 210 dias de idade. Com base nesses resultados concluímos que o gênero *Giardia* é um protozoário prevalente em bezerras na região Oeste catarinense, porém o secnidazol é eficaz na prevenção e tratamento contra infecções causadas por esse parasito. O protocolo profilático, constituído de selênio, vitaminas e secnidazol têm efeitos benéficos à saúde dos animais, pois favorece a melhora do sistema imunológico e, consequentemente, contribui para o desempenho dos animais. Assim como, a adição de fitoterápico, à base de cravacrol e cinamaldeído, pode minimizar infecções bacterianas e promover maior ganho de peso corporal nos bezerros. De modo geral, essa dissertação reúne diferentes protocolos alternativos para tratamento ou prevenção de doenças parasitarias em bezerras.
Palavras-chave: Doenças parasitárias e bacterianas; controle alternativo; efeito metafilático; efeito nutracêutico; tratamento.

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

GASTROINTESTINAL PROTOZOAL IN DAIRY CALVES IN WESTERN CATARINENSE: EPIDEMIOLOGY, PROPHYLAXY AND CONTROL

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Chapecó, April, 06th, 2017

The objective of this study was to identify the main protozoa that affect dairy calves in western Santa Catarina and to verify if prophylactic and therapeutic protocols are efficient in the control of parasitic agents and consequently of diarrhea. In all, four experiments carried out to develop this work. For the experiment one was collected fecal samples of 243 calves up to 60 days old in 43 farms of the western region of Santa Catarina, Brazil. The genus *Giardia* showed a higher prevalence in 26.75% (65/243) of the samples, followed by *Eimeria* in 21.81% and *Cryptosporidium* in 20.99% (51/243). Feeding management, the length of time that calves stayed with their mothers and contact with dogs were identified as risk factors for *Cryptosporidium* infections. The risk of contracting *Giardia* increased according to the source of milk, while surface type and age were identified as risk factors for *Eimeria*. In experiment II, secnidazole was administered orally, in capsule form and single dose, to 12 animals. Five days after treatment, the animals were not excreting *Giardia* cysts, whereas calves that did not receive the treatment still excreted cysts from the parasite. After 30 days of treatment, 83.3 of the animals remained without excreting *G. duodenalis* cysts. In experiment III, sodium selenite and vitamins A and E were applied to 16 calves with one day of life. The second dose was applied at 10 days of age along with the delivery of a secnidazole capsule. The treated calves presented higher values of hematocrit (60 days of age), total proteins (days 15 and 30), ceruloplasmin (days 15, 30 and 60), heavy chain IgG (days 15, 30, 45 and 60), IgG (days 45 and 60) and haptoglobins (days 15, 30, 45 and 60). The treated group did not present excretion of *Giardia* cysts until 30 days of age. Greater weight gain was observed in animals treated at 210 days of age. For the experiment IV, 15 calves consumed a commercial product based on components of essential oils from birth to 30 days of age (carvacrol and cinnamaldehyde). Treated calves showed higher values of hematocrit (days 45 and 60) and activity of glutathione S-transferase (60). The bacterial count was reduced in the treated group (days 30 and 60). The weight gain was higher in calves treated with a blend of essential oils at 60 and 210 days of age. Based on these results we conclude that the genus *Giardia* is a protozoan prevalent in heifers of the western region of Santa Catarina, but secnidazole is effective in the prevention and treatment against infections caused by this parasite. The prophylactic protocol, consisting of selenium, vitamins and secnidazole, has beneficial effects to the health of the animals, as it favors the improvement of the immune system and consequently contributes to the performance of the animals. As well as, the addition of phytomedicine based on carvacrol and cinnamaldehyde can minimize bacterial and promote greater body weight gain in calves. In general, this dissertation brings together different alternative protocols for the treatment or prevention of parasitic diseases in heifers.

Keywords: Parasitic and bacterial diseases; alternative control; metaphylactic effect; nutraceutical effect; treatment.

SUMÁRIO

CAPÍTULO I

1. REVISÃO DE LITERATURA

1.1 BOVINOCULTURA DE LEITE

O brasileiro consome em média 173 kg de lácteos por ano, segundo dados do IFCN (International Farm Comparison Network), valor que está abaixo das recomendações da FAO (Food and Agriculture Organization of the United Nations) que recomenda o consumo de 200 kg/pessoa/ano (FAO, 2018; IFCN, 2016). No entanto, com a aplicação de campanhas de incentivo ao consumo de leite e derivados, a média brasileira poderá aumentar e, consequentemente, aumentará a demanda interna, que já está acima da produção nacional.

Segundo dados do último levantamento, realizado pelo Instituto Brasileiro de Geografia e Estatística (IBGE), em 2016 no Brasil foram produzidos 33,62 bilhões de litros de leite (IBGE, 2016). No mesmo ano, a população brasileira era de 207,7 milhões de pessoas, com isso, foram produzidos 162 kg de leite/pessoa/ano, quantidade insuficiente para atender a população. Desta forma, apesar do Brasil ser o quinto maior produtor mundial, o país importa para suprir as demandas atuais do mercado interno (EMBRAPA, 2018). Além disso, para 2028, estima-se que a população brasileira seja de 221,4 milhões de pessoas (IBGE, 2013). Em vista disso, a produção nacional de leite tem muito a crescer para atender a demanda interna.

Ainda em 2016, o estado de Santa Catarina conquistou o quarto lugar no ranking da produção nacional de leite, com 3,1 milhões litros de leite produzidos. Essa posição foi alcançada, pois Santa Catarina foi o único dos cinco principais estados produtores a apresentar crescimento de produção (3,8%) (IBGE, 2016). Crescimento esse que vem acontecendo no decorrer dos anos, por exemplo, de 2000 a 2013, o crescimento da produção catarinense foi de 190% (JOCHIMS et al., 2016).

Contudo, a mesorregião Oeste catarinense é a maior responsável pela produção do estado e a terceira maior bacia leiteira do Brasil (CEPEA, 2016). O Oeste catarinense é considerado uma das bacias leiteiras nacionais mais promissoras, em vista do seu crescimento médio anual para produção de leite, que passou de 274,7 milhões de litros em 1990, para 2,2 bilhões litros em 2014 (JOCHIMS et al., 2016). Bacia leiteira essa caracteriza por pequenas propriedades rurais, pois 70 % dos estabelecimentos agropecuários possuem até 20 hectares e respondem por 72,1% da produção de leite na região (FISCHER et al., 2011). De acordo com dados de 2007, o Oeste abrigava 82,1 mil estabelecimentos agropecuários, dos quais 89,4%

eram compostos por agricultores familiares, percentual acima do observado no Estado, na Região Sul e no Brasil (JOCHIMS et al., 2016). A escolha pela bovinocultura de leite leva em consideração a garantia aos produtores de uma renda mensal, diferente de outras atividades como a suinocultura e a avicultura (MILKPOINT, 2018), ou agricultura.

O leite está entre os seis primeiros produtos mais importantes da agropecuária brasileira, à frente de produtos tradicionais como soja, milho, café beneficiado e arroz (EMBRAPA, 2018). Em 2002, o agronegócio participava com 26,16 % no PIB do Brasil, 20,26 % no ramo agrícola e 5,90 % no ramo pecuário. Já no ano passado, a participação do agronegócio no PIB do Brasil foi de 20 %, destes 13,94 % do ramo agrícola e 6,05 % do ramo pecuário (CEPEA, 2017). Contudo, o leite contribui com 22,4% do Valor Bruto da Produção Pecuária, superado apenas pelo Valor da Produção da carne bovina (EMBRAPA, 2018). Desta forma, é notável a importância da pecuária, com destaque para o leite, no PIB Agropecuário e do Brasil.

Sobre tudo, a bovinocultura de leite tornou-se uma atividade importante para a economia brasileira e fonte de renda para a agricultura familiar e pequenas propriedades, principalmente no Sul do país. A criação de bezerras sadias contribuiu diretamente para o crescimento do setor e produção de leite. No entanto, durante a fase de cria os produtores enfrentam alguns desafios como as elevadas taxas de morbidade e mortalidade por diversas causas. Sendo a diarreia infecciosa a principal causa da morte precoce de bezerras, como será destacado a seguir.

1.2 CRIAÇÃO DE BEZERRAS

A criação de bezerras é importante na reposição do rebanho, uma vez que, as taxas de vacas descartadas nas propriedades leiteiras podem chegar até 25% ao ano (SANTOS et al., 2010). No entanto, esses animais não possuem um sistema imunológico estabelecido e são susceptíveis a contrair infecções que podem ocasionar até mesmo a morte. Em ruminantes, ocorre pouca transferência de imunoglobulinas maternas para o feto em razão da placenta ser do tipo sinepiteliocorial, isto é, o epitélio coriônico está em contato direto com os tecidos uterinos. Portanto, os recém-nascidos devem receber anticorpos através do colostro (CHAPPUIS, 1998). A transferência passiva de imunoglobulinas presentes no colostro materno para os bezerros é a maneira mais importante de fornecer proteção imunológica imediata. A proteólise no estômago dos recém-nascidos é inibida pela ação da tripsina presente no colostro e o intestino delgado é capaz de absorver macromoléculas como as imunoglobulinas porque é revestido por células epiteliais imaturas altamente vacuolizadas (KRUSE, 1983).

Contudo, a permeabilidade é elevada imediatamente após o nascimento e diminui

rapidamente depois das 24 horas, em decorrência da maturação das células intestinais e estabelecimento de flora intestinal que degrada as imunoglobulinas (CHAPPUIS, 1998). O intervalo entre o nascimento e a ingestão do colostro, o consumo de leite antes do colostro, quantidade de colostro ingerido, concentração de imunoglobulinas no colostro, exposição ao estresse e alterações da permeabilidade no intestino são os principais fatores que influenciam a absorção de imunoglobulinas (KRUSE, 1983).

Portanto, a má colostragem compromete a imunidade dos bezerros e favorece o surgimento de infecções. A diarreia é o principal sinal clinico decorrente de infecções intestinais, pois está associado a altas taxas de morbidade e mortalidade desses animais (MEGANCK et al., 2014). Na literatura, as taxas de mortalidade variam de 4,6 % até 22,0 % (GUILLIKSEN, 2009; TREFZ, 2017). A maioria dos casos de mortalidade associados a diarreia ocorrem nas primeiras duas semanas de vida quando as bezerras são expostas pela primeira vez a diversos agentes infecciosos e o sistema imunológico é imaturo e não consegue muitas vezes responder fortemente ao agente agressor (WATTIAUX, 2002). A diarreia provoca perda de fluidos corporais e pode levar os animais a um quadro de desidratação severa em menos de 24 horas. Quando os bezerros sofrerem diminuição de 12-14% de fluidos corporais devido à desidratação pode ser fatal. No entanto, as perdas financeiras vão além da mortalidade de bezerros, mas também o custo da medicação e o trabalho necessário para tratar bezerros acometidos (WALKER, 1997). Portanto, a ocorrência de diarreia necessita de atenção, pois promove perdas econômicas aos produtores e prejudica a reposição do rebanho.

De acordo com a literatura, a diarreia infecciosa pode ser causada por diferentes patógenos, como as bactérias *Escherichia coli*, *Salmonella* spp. e *Clostridium perfingens*; vírus como *Rotavirus*, *Coronavirus* e *Adenovius*; e protozoários do gênero *Cryptosporidium* spp., *Giardia* spp. e *Eimeria* spp. (MILLEMAN, 2009; WATTIAUX 2002).

Entre os agentes patogênicos que infectam o trato intestinal estão os protozoários, com destaque para o gênero *Eimeria*. Este gênero causa a coccídiose principalmente em animais jovens, provocando diarreia em elevadas infecções. Consequentemente, perdas econômicas significativas são registradas por afetar o crescimento, assim como aumentar a ocorrência de mortalidade elevadas e custos com prevenção e tratamento (GIARETTA et al., 2014). São parasitos intracelulares obrigatórios da mucosa intestinal, transmitido por oocistos liberados no ambiente (MEIRELES et al., 2012; MONTEIRO, 2011). A susceptibilidade dos animais pode estar relacionada, principalmente a idade, a genética, a imunidade, a fatores climáticos e ambientais, entre outros complicadores (MEIRELES et al. 2012). Geralmente afeta animais em confinamento, durante o verão, e a limpeza e higienização precária das instalações são alguns

fatores associados à infecção por *Eimeria* spp. (GIARETTA et al., 2014).

Outro gênero que também acomete os animais jovens e apresenta patogenia semelhante a eimeriose é causada pelo *Cryptosporidium* spp, agente causador da cryptosporidiose bovina. Os protozoários desse gênero infectam os animais independente da categoria, sexo ou idade, podem ou não demostrar sinais clínicos e em geral causa perdas econômicas (COUTO e BOMFIM, 2012). Os hospedeiros eliminam a forma infectante, isto é, o oocisto nas fezes que contaminam o ambiente, os alimentos e/ou a água, com isso, os animais susceptíveis adquirem a infecção via ingestão ou inalação (MARTINS-VIEIRA et al., 2009). Animais jovens são mais suscetíveis à infecção e apresentam diarreia como principal sinal clínico e, quando intensa, pode provocar a morte de bezerros de até 3 meses de idade (COUTO e BOMFIM, 2012). A prevalência da infecção por esse parasito é considerada alta em bovinos de leite jovens e em bovinos de corte mantidos em confinamento (MARTINS-VIEIRA et al., 2009).

Além dos dois gêneros já mencionados, a *Giardia* spp. também é um protozoário importante para a saúde de animais jovens. As semelhanças entre os protozoários intestinais já citados são um dos fatores que possibilita os três gêneros parasitarem o mesmo hospedeiro no mesmo período de tempo. Considerada um dos principais e mais importantes parasitos intestinais de humanos e de animais, a *Giardia* spp. também está associada com a ocorrência de diarreia. Este protozoário pode estar envolvido no aparecimento de diarreia neonatal, de forma dependente ou associado com outros endoparasitas (GUIMARÃES et al., 2001). A transmissão ocorre geralmente através de água contaminada com cistos e, além da participação na morbidade animal, é estudada como provável potencial zoonótico (MARQUI et al., 2011). A alta prevalência deste protozoário em bezerros está relacionada à idade, assim como a dose infectante, a susceptibilidade do hospedeiro e a virulência dos cistos são alguns fatores ligados à doença (SILVA JUNIOR et al., 2011).

Os protozoários são importantes causadores de diarreia conforme já descrito, porém é reconhecido que as bactérias são mais agressivas em termos de mortalidade de animais. Entre os agentes bacterianos, a principal bactéria envolvida na enterite neonatal é a *Escherichia coli*. A colibacilose acomete, principalmente, os bezerros nas três primeiras semanas de vida. Apesar de fazer parte da microbiota comum residente do trato intestinal de bezerros, existem certas cepas de *E. coli* que são altamente patogênicas (JESSE et al., 2016). A *E. coli* causa diarreia severa, desidratação, febre e fadiga, o que resulta em perdas econômicas para os produtores de leite. A transmissão é mais frequentemente através da via oral-fecal por ingestão de alimentos e água contaminados (JESSE et al., 2016).

Todos esses agentes patogênicos citados são responsáveis por infectar o trato intestinal

dos animais e causar diversos sinais clínicos como diarreia, perda de apetite, má absorção de nutrientes, desidratação, perda de peso e lesões na mucosa intestinal. Importante ressaltar, que se o animal não morrer, esses sinais clínicos retardam o crescimento. Por tanto, o combate destes patógenos é realizado, habitualmente, por meio de medicamentos à base de antibióticos e antiparasitários. As bactérias patogênicas são responsáveis pela ampla utilização de antibióticos. Porém o uso excessivo destes compostos químico pode ser prejudicial para os animais, além de atuarem após a infecção já se instalar. Além disso, o uso exacerbado desses antibióticos já apresenta casos de resistência em alguns rebanhos brasileiros, como foi relatado em pesquisa realizada no estado do Rio Grande do Sul, onde 21 rebanhos participaram da pesquisa, e 93 dos 159 animais (58,5%) apresentaram resistência a pelo menos um antimicrobiano (SANTIAGO-NETO et al., 2014).

Em razão disso, as buscas por alternativas intensificaram, como a utilização de aditivos que beneficiem os animais e justifiquem seu uso, como extratos vegetais, fitoterápicos e óleos essências. Os aditivos que contêm na sua fórmula componentes de origem natural tem a possibilidade de diminuir infecções que causam diarreia e, consequentemente, o uso de antibióticos, sem comprometer o crescimento dos animais.

1.3 ADITIVO A BASE DE ÓLEOS ESSÊNCIAS

Os óleos essências e seus compostos possuem ação antibacteriana e, com isso, reduzem os gastos com antibióticos e melhoram a saúde dos bezerros. Além da ação antibacteriana, os óleos essenciais possuem efeitos antifúngicos, anticcocidianos, antioxidantes, entre outros (KALEMBA e KUNICKA, 2003; LEE, 2002; MIGUEL, 2010). Em razão disso, são amplamente estudados e explorados, inclusive na produção animal (BENCHAAR et al., 2008; CHOUHAN et al., 2017). Os óleos essenciais são compostos por várias moléculas diferentes derivadas dos fenilpropanóides ou de terpenóides (SIMÕES, 1999). O óleo de orégano, por exemplo, possui a maior concentração natural de carvacrol (80%), um fenol monoterpenico que está presente em muitos óleos essenciais da família Labiatae (JAYAKUMAR et al., 2012). O carvacrol é uma molécula responsável por diversas ações como resposta antioxidante e antibacteriana (BARNWAL et al., 2017; HELANDER et al., 1998).

Após confirmar a atividade inibitória do óleo essencial de orégano sobre *Pseudomonas aeruginosa*, os pesquisadores comprovaram que 96 % da inibição pode ser atribuída ao efeito aditivo dos seus componentes timol e carvacrol, com os restantes 4% aos demais componentes (LAMBERT, 2001). O mecanismo de ação do óleo de orégano, bem como seus constituintes

timol e carvacrol, contra esses agentes patogênicos envolve dano na integridade da membrana celular, aumento da permeabilidade, provocam vazamento de íons, como potássio e fosfato, e alterações do pH interno das células (LAMBERT, 2001).

O cinamaldeído é um composto presente no óleo de canela e possuí ação antibacteriana, como por exemplo, inibição do crescimento de *Mycobacterium avium* subsp. *paratuberculosis* (Map) (WONG et al., 2008). O modo de ação do cinamaldeido é semelhante ao carvacrol, pois quando as células entram em contato com o composto apresentam um aumento linear no nível de fosfato extracelular dependente da concentração e do tempo de exposição, sugerindo aumento da permeabilidade da membrana (NOWOTARSKA et al., 2017).

A partir dos conhecimentos adquiridos com testes em laboratórios sobre microrganismos, pesquisadores buscaram testar se os efeitos dos óleos essências ocorrem quando adicionados na dieta dos animais e quais benéficos poderiam oferecer. Em leitões, a utilização do óleo orégano e óleo de canela como aditivos na alimentação melhora o ganho de peso (FRANZ et al., 2009; LI et al., 2012). A inclusão de cinamaldeído na dieta de gado de corte, particularmente no início do confinamento, ajudou a promover maior ingestão de alimentos e reduzir os efeitos do estresse (YANG et al., 2010). No entanto, os efeitos desses óleos parecem depender da espécie animal e dose adicionada a dieta, pois trabalhos mostram que o carvacrol e cinamaldeido não afetam o desemprenho de cordeiros e ovelhas (CHAVES et al., 2008; KOYUNCU e CANBOLAT, 2010; ÜNAL et al., 2013).

Em estudo realizado por Santos et al. (2015), a adição de uma mistura comercial de carvacrol e cinamaldeído oriundo de óleos essenciais na dieta não afetou o desempenho, escore fecal e parâmetros sanguíneos dos bezerros, com exceção da concentração de amônia-N ruminal. No entanto, os autores sugeriram a realização de novos estudos com a utilização desses componentes dos óleos essências, em relação as doses e vias de administração, para avaliar se estes seriam substitutos promissores para os antibióticos. Além dos produtos naturais, as suplementações minerais e vitamínicas possuem potenciais para melhorar as defesas imunológicas e estabelecer o equilíbrio entre oxidantes- antioxidantes, fatores que podem impedir o aparecimento de infecções intestinais e melhorar a saúde dos animais.

1.4 SUPLEMENTAÇÃO MINERAL E VITAMÍNICA

Os minerais e as vitaminas são indispensáveis para o bom funcionamento do organismo, porém este não é capaz de produzir alguns elementos. Por esta razão, o organismo depende da ingestão via dieta de alguns minerais e vitaminas, além disso, algumas vitaminas são

armazenadas no animal e podem ser utilizadas de acordo com suas necessidades, sem que os animais apresentem sinais clínicos de intoxicação e deficiências. Entre os minerais importantes para os animais, o selênio atua principalmente como um antioxidante para proteger as membranas da célula e prevenir a geração de radicais livres, diminuindo assim o risco de doenças (LIMA e DOMINGUES, 2007). O selênio e a vitamina E atuam contra os peróxidos no organismo animal (ZANETTI et al., 1998). A vitamina E previne a geração dos radicas livres, enquanto o selênio é responsável pela destruição através da enzima glutationa peroxidase. O peróxido de hidrogênio (H_2O_2) é uma espécie reativa formado a partir do oxigênio presente nos tecidos, que apesar da sua importância é tóxico para as células, portanto dependem da glutationa peroxidase, constituída de selênio, para sua degradação (ZANETTI et al., 1998).

Em bezerros, a exigência de selênio é de 100 μg/kg MS por dia (NRC, 2001). Para vitamina E, o NRC (2001) indica a necessidade nutricional diária de 40 a 60 unidades internacionais (UI). Pesquisas mostraram que a deficiência e baixos níveis de selênio das vacas aumentou o risco de doenças em bezerros relacionadas à baixa imunidade (ENJALBERT et a., 2006). O leite, por sua vez, não garante a manutenção dos níveis de selênio exigidos pelos bezerros, justamente porque o selênio não é devidamente transferido para o mesmo (PEHRSON et al., 1999). A suplementação com selênio, em geral, não influencia o crescimento, ganho de peso e taxa de mortalidade em bezerros, mas possuem efeitos benéficos ao sistema imunológico (MEHDI e DUFRASNE, 2016).

Pesquisadores observaram que vacas suplementadas com selênio e novilhas suplementadas também com DL-alfa-tocoferol antes do parto promoveram maiores concentrações de imunoglobulinas e contagem de leucócitos nos bezerros recém-nascidos, respectivamente (GUYOT et al., 2007; MOEINI et al., 2011; ROWNTREE et al., 2004). O selênio promove aumento da produção de linfócitos, pois se encontra nos principais órgãos do sistema imunológico, como medula e no timo (HOFFMANN e BERRY, 2008; SPALLHOLZ et al., 1990). No entanto, a vitamina E impede a supressão da função dos neutrófilos e dos macrófagos no sangue e estimula a atividade dos linfócitos T auxiliares (POLITIS et al., 1995; TANAKA et al., 1979).

É reconhecido o papel e importância dos minerais e das vitaminas, em geral, para o funcionamento de alguns sistemas biológicos. No entanto, por mais bem estruturado que o sistema imune dos animais esteja, alguns agentes patogênicos possuem a capacidade de infectar e causar doenças. Em virtude disso, a utilização combinada de suplementação mineral e de vitaminas com substâncias químicas com propriedades farmacológicas poderia atuar

conjuntamente e de forma mais eficiente na prevenção de doenças, como a diarreia infecciosa.

1.5 CONTROLE DE GIARDIASE

A mais de 20 anos foram realizados experimentos para testar substâncias químicas capazes de tratar infecções causadas por *Giardia*. O febendazol e o albendazol foram eficazes na supressão da excreção de cistos de *Giardia* por bezerros infectados (XIAO et al., 1996). Alguns anos depois essas substâncias começaram a ser comercializadas e utilizadas amplamente a campo. O tratamento com albendazol é fornecido uma vez ao dia durante três dias e o febendazol é fornecido duas vezes ao dia com duração de três dias (XIAO et al., 1996). Esse tipo de tratamento com aplicações consecutivas dificulta o manejo da propriedade e estressa os animais. O metronidazole não é usado em animais de produção, sendo seu uso mais frequentes em animais de companhia.

O tratamento para giardíase em humanos é realizado de forma eficaz com a utilização da substancia química denominada secnidazole. No entanto, esta substância não é utilizada em animais. Seu comportamento no organismo animal e em especiais de *Giardia* que os acomete necessita ser estudada. Quando testado em camundongos swiss, o secnidazole apresentou 87,5% de eficiência na cura da giardíase (FRANCO et al., 2015). A utilização em cordeiros naturalmente infectados por *Giardia* reduziu significativamente a excreção de cistos (99,98%), tornando-o uma opção de tratamento (URAL et al., 2014). Como tratamento, o secnidazol tem capacidade de combater este parasito, porém não há estudos sobre a utilização profilática em bezerros.

O secnidazole é uma molécula da classe dos nitroimidazoles, derivado do 5-nitroimidazole e, assim como outras drogas dessa classe, incorporam microrganismos por meio de difusão passiva e são ativados por redução do grupo de nitro (GARDNER e HILL, 2001). Na Europa, a classe nitroimidazoles está na lista de medicamentos veterinários com efeito residual nos alimentos de origem animal e, portanto, seu uso é proibido para animais de produção (EUROPEAN COMMISSION, 2015). No entanto a normativa publicada em 2015 cita apenas a classe de modo geral e o secnidazol não havia sido estudado para animais de produção. Acreditamos que em uma eventual investigação não será detectado nenhum resíduo no leite, uma vez que bezerras tornam-se produtores apenas 24 meses de idade. Além disso o secnidazole é uma substância farmacológica cuja absorção é rápida e completamente, com uma meia-vida de 17 a 29 horas e metabolizado por oxidação no fígado (GILLIS e WISEMAN, 1996). É importante ressaltar que o uso de fármacos da classe dos nitroimidazoles na Europa e

Estados Unidos em animais em fase de produção tem restrição.

1.6 OBJETIVOS

1.6.1 OBJETIVO GERAL

Identificar os principais protozoários que acometem bezerras leiteiras no oeste catarinense e verificar se protocolos profiláticos e terapêuticos são eficientes no controle de agentes parasitários e consequentemente na redução dos casos de diarreia.

1.6.2 OBJETIVOS ESPECÍFICOS

- Identificar os protozoários que acometem bezerros leiteiros e fatores de risco que favorecem a infecção;
- Verificar se o tratamento com secnidazole é eficaz no controle de giardíase em bezerros;
- Testar se um protocolo profilático baseado na suplementação mineral e vitamínica associado ao secnidazole é capaz de ativar resposta imunologia e antioxidante e prevenir infecção por *Giardia* spp. e consequentemente a diarreia.
- Testar se a adição de produto comercial a base de óleos essenciais tem efeito benéfico à saúde animal, assim como favorece o desempenho.

CAPÍTULO II

2. ARTIGO E MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de dois artigos e dois manuscritos com suas formatações de acordo com as orientações das revistas as quais foram submetidos. Os projetos foram aprovados pelo CEUA da UDESC (Anexo I e II).

Artigo I - **Gastrointestinal protozoa in dairy calves: identification of risk factors for infection**
Publicado: Revista MZV Cordoba (Anexo III)

Artigo II - **Secnidazole for control of giardiasis in dairy calves**
Publicado: Experimental Parasitology

Manuscrito I - **A prophylactic protocol to stimulate the immune response also control infectious disease and, consequently, minimizes diarrhea in newborn heifers**
Submetido: Microbial Pathogenesis

Manuscrito II - **A mix of essential oils as feed additive for dairy calves reduce fecal bacterial counts and enhance growth**
Submetido: Journal of Animal Physiology and Animal Nutrition

2.1 ARTIGO I

Gastrointestinal protozoa in dairy calves: identification of risk factors for infection

Protozoos gastrointestinales en terneros lecheros: identificación de factores de riesgo para la infección

Gastrointestinal protozoa in dairy calves

ABSTRACT

Objective. This study aimed to evaluate the occurrence of gastrointestinal protozoa in dairy calves and to identify potential risk factors for this type of infection. **Materials and methods.** For this purpose, 243 fecal samples were collected from calves up to 60 days of age in 43 dairy farms located in the West region of Santa Catarina state, Brazil. Samples were examined by centrifugal-flotation technique. **Results.** As a result, *Giardia* was present in 26.75% (65/243) of all samples, *Eimeria* in 21.81% (53/243), and *Cryptosporidium* in 20.99% (51/243). Additionally, 46.50% (113/243) of the samples were negative for any protozoa, while 39.10% (95/243) and 14.40% (35/208) showed single and mixed infections, respectively. There was a higher association between *Cryptosporidium* and *Giardia* (6.99%) in cases of mixed infections. However, the triple protozoa association had the lowest prevalence in mixed infections (2.06%). Epidemiologically, a questionnaire was applied to determine risk factors for these parasitic infections. Based on the statistical model applied, some risk factors for *Cryptosporidium* infections were identified, highlighting feeding management, period of time that calves stayed with their mothers (cows), and contact with dogs; the risk of contracting *Giardia* increased according to the milk source, while the floor type bedding, and age were appointed as risks factors for *Eimeria*. **Conclusions.** Therefore, it is possible to confirm that *Giardia*, *Cryptosporidium* and *Eimeria* may infect dairy calves, and the knowledge of some risk factors associated to their infection in calves.

Key words: dairy cattle, parasitic diseases, *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp. (*Source: CAB, MeSH*).

RESUMEN

Objetivo. El objetivo evaluar la ocurrencia de protozoos gastrointestinales en terneros lecheros y de identificar posibles factores de riesgo para infección. **Materiales y métodos.** Se recogieron 243 muestras de heces de terneros de hasta 60 días de edad en 43 granjas lecheras ubicadas en la región del oeste del estado de Santa Catarina, Brasil. Las muestras fueron examinadas por la técnica de centrifugación-flotación. **Resultados.** Como resultado, *Giardia* estaba presente en 26.75% (65/243) de todas las muestras, *Eimeria* en 21.81% (53/243), y *Cryptosporidium* en 20.99% (51/243). Además, 46.50% (113/243) de las muestras fueron negativas para cualquier protozoos, mientras que 39.10% (95/243) y 14.40% (35/208) mostraron infecciones simples y mixtas, respectivamente. Hubo una mayor asociación entre *Cryptosporidium* y *Giardia* (6.99%) en los casos de infecciones mixtas. Sin embargo, la asociación de triple protozoos tenía la menor prevalencia de infecciones mixtas (2.06%). Epidemiológicamente, se aplicó un cuestionario para determinar los factores de riesgo para estas infecciones parasitarias. Se han identificado algunos factores de riesgo de infecciones por *Cryptosporidium*, destacando manejo de la alimentación, periodo de tiempo que los terneros permanecieron con sus madres (vacas), y el contacto con los perros; el riesgo de contraer *Giardia* incrementado en función de la fuente de la leche, mientras que el tipo de suelo de las camas, y la edad fueron nombrados como los riesgos de factores de *Eimeria*. **Conclusiones.** Es posible confirmar que *Giardia*, *Cryptosporidium* y *Eimeria* pueden infectar a terneros lecheros, y el conocimiento de algunos factores de riesgo asociados a la infección en terneros.

Palabras clave: ganado lechero, enfermedades parasitarias, *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp. (Fuente: CAB, MeSH).

INTRODUCTION

Dairy cattle production is an important livestock activity, especially because milk has high nutritional value and it is consumed by most of the world's population. This activity generates income and has social impact for many segments of the production chain, such as producers, processors, equipment suppliers, traders, among others. In Brazil, dairy cattle production has a variety of profiles, that goes from properties with low technological level to those with high standards of production throughout this continental country. According to FAO (1), The United States of America is the largest milk producer (87,461,300 tons per year), and Brazil is the fifth

largest with an annual production of 31,667,600 tons per year, corresponding to 5.3% of the world production.

Dairy cattle business is growing in Brazil and milk production increased 350% between 1974 and 2011 (2). This growth lead to a solid system for raising heifers, aiming herd replacement; however, producers are still facing some challenges, such as high neonatal mortality. The main cause of early death of calves is diarrhea, mainly caused by infective agents (3-5). Some authors have described diarrhea as the leading cause of death in calves due to dehydration (3,5) during the first two weeks of life up to 3-4 months of age (5). Environmental factors, nutritional issues and infectious agents are usually involved in cases of diarrhea as the protozoa *Cryptosporidium* spp., *Giardia* spp. and *Eimeria* spp. (3-5).

Protozoa of the genus *Eimeria* Schneider, 1875, may cause coccidiosis in cattle, more often in young animals, and this disease is characterized by diarrhea or dysentery, related to significant economic losses due to the occurrence of high morbidity and mortality, as well as increased costs for prevention and treatment (6). *Eimeria* is an obligate intracellular parasite of the intestinal mucosa, leading (in some cases) to massive cell destruction of the large intestine, causing the malabsorption syndrome (7,8). The bovine cryptosporidiosis is a protozoal disease caused by *Cryptosporidium,* Tyzzer 1907, and it infects animals regardless of sex, age, or type that may or may not demonstrate clinical signs, but often causing great economic losses (9). Infected hosts eliminate the infective form of the parasite, called oocysts, in their feces, contaminating the environment, food and/or water. Thus, susceptible animals become infected through oocyst ingestion or inhalation (10). *Giardia* spp., Kunstler 1882, is considered one of the most important intestinal parasite of humans and animals, usually associated to the occurrence of diarrhea. This protozoan may be involved in the onset of neonatal diarrhea, independently or associated with other endoparasites (11).

Therefore, as noted above, protozoal infections in domestic animals lead to severe health problems, in addition to economic losses to producers. Thus, the aim of this study was to evaluate the occurrence of infections caused by *Cryptosporidium, Giardia* and *Eimeria* in calves with or without diarrhea, as well as identify potential risk factors for infection.

MATERIALS AND METHDS

Local and animals. This study was carried out in 43 dairy farms located in the Western region of Santa Catarina State, Southern Brazil. Fecal samples (n=243) of female calves, aging from one to 60 days were randomly collected regardless of race, type of environment, or animal management. On average, five grams of feces were collected aseptically (using latex gloves) from each calf with or without diarrhea, properly stored on ice, transported to the laboratory, and kept at 5°C until analyses.

Parasitological examination. In order to visualize the presence of parasites in fecal samples, the centrifugal flotation technique was used. Briefly, saturated sugar solution was mixed with 2 grams of feces, in order to cause cysts and oocysts flotation (7). Once homogenized, filtration was performed using a plastic sieve, and the material filtered was allocated in Falcon tubes. Then, the tube was filled up with sugar solution to form a meniscus, covered by a glass coverslip, and centrifugated at 2000 rpm by 5 minutes. The coverslip was removed and placed on a glass slide, with a drop of lugol diluted in distilled water (1v/v) for microscope observation (7). Although it is a qualitative technique, some authors have used this methodology to determine the degree of parasitic infection as follow: mild (from 1 to 100 cysts/oocysts), moderate (from 101 to 300 cysts/oocysts) and severe (more than 301 cysts/oocysts) (12). In fecal samples with the presence of helminth´s eggs, it was performed culture for individual larvae assessment for genus identification.

Epidemiological survey. Farm and animal information were obtained throughout the application of a questionnaire to the farmers. This information was crosschecked with the results on fecal examination, in order to identify the risk factors for cryptosporidiosis, giardiasis and eimeriosis. The following questions were asked: type of feeding (cow's milk, artificial milk, concentrate, milk and concentrate, milk, concentrate and hay); housing (individual or collective); type of bedding (concrete, slatted, soil or concrete/slatted); cleaning interval (1-7, 8-15 or >15 days); milk supply (bottle, bucket, both, or milk was not provided); contact time with the cow (<1, 1-5 or >5 hours); water source (well or fountain); contact with rats (yes or no); contact with dogs (yes or no); contact with cats (yes or no); contact with chickens (yes or no); presence of flies (yes or no); animal age (1-15, 16-30, 31-45 or ≥46 days); race (Holstein, Jersey, or crossbreed) and fecal consistency (normal or *diarrhearic*).

Statistical analysis. The data generated from the interviews (independent variables) and parasite identification – *Giardia, Cryptosporidium* and *Eimeria* (response variable) were recorded and analyzed by R-language, v.3.1.1 (R Development Core Team, 2012). Cross tabulation and descriptive statistics, such as frequency and percentage, were performed on all independent variables. Independent variables (Table 1, 2 and 3) were first screened based on all the response variable (*Giardia, Cryptosporidium* and *Eimeria*). Variables with large amounts of missing data (>10%) and limited variability (<20%) were not included in the multivariable model. The remaining variables were individually accounted into an unvariable logistic regression model (chi-square test). Under the assumption that each animal is clustered in a herd, a mixed model was performed using the farm random-effect, due to the lack of independency among samples from animals in the same farm. Univariate analysis was first conducted using all the fourteen pre-selected variables. Subsequently, each variable with $P \leq 0.15$ was selected for inclusion in the multivariable analysis. It was build one final model for each outcome of interest individually for *Giardia, Cryptosporidium* and one for *Eimeria.* Variance inflation factor (VIF) was estimated to verify the relation between all selected independent variables to check for potential collinearity, in which coefficient >2.50 was considered high. If a high VIF was found, the variable with lower p-value was considered for the multivariable model. A crude relative risk (RR) was applied to assess the impact of individual factors on the outcomes. Furthermore, selected variables, when considering *Cryptosporidium* as an outcome (n=8), were included in the multivariable model (feeding, housing, time with the cow, water source, contact with rats, contact with dogs, presence of flies, and age). When the outcome was *Giardia* (n=6), it was included in the multivariable model (feed, milk supply, time with the cow, water source, contact with dogs, and age) and finally when the outcome was *Eimeria* (n=2), it was included in the multivariable model (bedding type and age).

A second univariate model was built in order to verify the presence of an effect-cause situation. As outcome variable, it was considered the presence of any protozoan (*Giardia, Cryptosporidium* or *Eimeria*) and fecal consistence (regular or presence of diarrhea) as the predicted variable. For this model a $P \leq 0.05$ was considered significant associated.

Multivariate models were built in a manual forward method, where each remaining variable was added to the best previous model selected by the Akaike Information Criterion (AIC). A backward elimination step was used, resulting on a final model in which only variables with

$p \leq 0.05$ were retained. Confounding effects were investigated by checking changes in the point estimate of each variable that remained in the model. Changes in parameter estimated as >25% were considered a confounder factor, and it was kept in the model until the final model, and finally two-way interaction term between variables with biological plausibility were investigated. We used deviance perform as a goodness of fit test for overall model.

RESULTS

Among all fecal samples examinated, 46.50% (113/243) were protozoa negative, while the other samples (53.50%; 130/243) were positives for one or more parasites. The percentage of calves infected by *Cryptosporidium* spp. was 20.99% (51/243); by *Giardia* spp. 26.75% (65/243); and by *Eimeria* spp. 21.81% (53/243) (Figure 1-A). Out of 243 samples, 95 (46.50%) showed a single protozoan infection (Figure 1-B), while 35 (14.40%) had mixed infection caused by two or three protozoa. Among the mixed infections, the association of *Cryptosporidium* spp and *Giardia* spp was the most common (6.99%, or 17/243 calves), followed by *Eimeria* spp and *Cryptosporidium* spp (2.88%, or 7/243 calves), and *Giardia* spp associated with *Eimeria* spp (2.06%, 6/243 calves). The mixed infection caused by all three protozoa represented 2.40% (5/243 calves).

The degree of infection by *Cryptosporidium* spp, *Giardia* spp, and *Eimeria* spp was rated as mild, moderate and high, respectively (Figure 1-C). We found all three levels of infection caused by *Eimeria* spp and *Giardia* spp, differently to *Cryptosporidium* spp that did not cause severe infection. The mild type of infection was the most frequently observed type for the three protozoa (Figure 1-C).

Diarrhea was observed on 25.51% of the calves, where only 13.17% were positive for at least one protozoa. Thus, 74.49% did not have diarrhea, though 40.33% of calves were affected by one of the investigated protozoa. Moreover, the cause-effect analyses did not find significant association between the presence of *Cryptosporidium* spp, *Giardia* spp and *Eimeria* spp and fecal consistency ($p>0.05$). It was found that 4.12% (10/243) of the fecal samples also had eggs of parasites from the Trichostrongylidae family, corresponding to *Haemonchus* spp. and *Trichostrongylus* spp.

The results of univariate analysis for risk factors for infection by *Cryptosporidium* spp, *Giardia*

spp. and *Eimeria* spp. are shown in Table 1, 2 and 3, respectively. For the *Cryptosporidium* genus, it was found that collective housing, along with longer periods of time with its mother after birth, and direct contact with dogs and cats, increases the chance of infection to calves. For *Giardia* spp, the main factors for infection were: not supplying milk, sources of water intake, contact with dogs, and animals age between 31 and 45 days old. For *Eimeria* spp, two risk factors were observed in the univariate analysis: age and pens with concrete and slatted floors.

The results of risk factors for *Cryptosporidium* spp, *Giardia* spp and *Eimeria* spp infection on the multivariate analysis are shown in Table 4. We found that calves fed with concentrate had, on average, 37 times higher risk to be infected compared to those fed with cow's milk, contrasting to artificial milk that showed 9.91 times more risk (Table 4). Similarly, calves on a diet of milk/concentrate and milk/concentrate/hay were 9.80 and 4.79 times more likely to be infected by these protozoans, respectively. However, the period of time that calves had direct contact with their mothers, immediately after birth (1 to 5 hours, and more than 5 hours) increased the risk of infection by *Cryptosporidium* spp in 0.15 and 0.30 times, respectively. Contact with dogs increased the relative risk of infection by *Cryptosporidium* spp in 3.57 times. For the presence of *Giardia* spp., a single variable was identified as a risk factor by the multivariate analysis: milk source. Both forms of supply (bottle and bucket) increased their risk on 5.40 times. Regarding the risk factors for *Eimeria* spp infection, we noticed that concrete and slatted floors were three times more risky for infections compared to concrete floors. The animal age was also significantly associated with the occurrence of *Eimeria* spp, since we noticed that animals with more than 15 days of age had more chances of infection.

DISCUSSION

In this study, a higher number of calves up to 60 days of age were infected by *Giardia* spp. However, on a similar study conducted in Brazil, observed lower prevalence (26.75%). According to the literature, *Giardia* spp. cysts were observed in 9% of 120 fecal samples from calves aged 1 to 90 days (11). Additionally, in another study with 560 calves showed 17% (95) positivity for *Giardia* spp. (13). The index of positive animals in this study was similar to the global average rate (25.56%) of calves shedding *Giardia duodenale* (14). According to the multivariate analysis, bovine milk supplied by bottle and bucket increased the chances of infection by *Giardia* spp. Furthermore, the calves age may influence this high percentage of

positive samples for *Giardia* spp, since, in its majority, the prevalence was observed in animals up to four weeks of age (13). Corroborating to our findings, age was also found to be a highly significant risk factor for *Giardia* infection when Geurden et al (15) showed that calves had higher risk of being infected before reaching 8 weeks. This result is a significant finding, since it represents a risk to animal health, considering that *Giardia* spp is a worldwide important zoonotic agent (16).

Our results show that the prevalence of protozoal infection caused by *Eimeria* was 21.81%, a reduced index compared to those reported by researchers (17), while analyzing 720 samples of feces observed the presence of oocysts of *Eimeria* spp. in 43.60% of the samples. The eimeriosis is a disease that can cause severe morbidity, affecting animal growth and performance. Calves with positive diagnosis showed lower body weight gain than those *Eimeria* spp negative (18). Statistical analysis revealed that infection by *Eimeria* is easier to happen in concrete floors, depending on animal age. According to the literature, individual housing may reduce the risk for coccidiosis, and on the contrary risk of infection increases with the size of the herd, in addition, the most affected calves were between 3 to 13 months old (19).

Cryptosporidium spp. was detected in 20.99% of the samples in current study, similarly to the result observed in calves aged between 7 to 21 days, since they showed 21.62% of positivity for oocysts (13). Similarly, another study conducted in Nellore calves, showed that 25.38% (n=130) of the animals shed oocysts of this parasite in their feces (4). There is a report on a cryptosporidiosis outbreak in 2012 in a herd of 400 calves, from which 35 (8.75%) got sick and 16 (4%) died. This farm had high mortality (about 70 calves) in 2011 due to cryptosporidiosis, but the attempts to diagnosis the disease by parasitological tests and fecal cultures failed (20). In a study conducted in Argentina, a prevalence of 19.35% for *Cryptosporidium* spp. was found, similarly to the results observed in our study (21). In a work developed by Carvalho et al (22), infection by *Cryptosporidium* spp. was observed in all evaluated moments. In the current study, the multivariate analysis showed several ways on how calves could become infected by *Cryptosporidium* spp. The main reasons for infection may have been the food, especially by concentrate, followed by the length of time with cow postpartum, and contact dogs. These last two are risk factors easily understood, since adult animals usually are carriers of cryptosporidiosis, eliminating the agent in the feces, contaminating the environment. By analyzing infection by *Cryptosporidium parvum*, researchers have identified different risk factors, such as the use of buckets to feed the calves and supplementation with fermented milk

(23).

The occurrence of diarrhea recorded in our study (25.51%) confirms the relevance of this clinical sign for calves, and it revealed that approximately 50% of calves with diarrhea were positives for at least one protozoan. In another study evaluating 1,974 calves, researchers found that 19.75% showed feces soft to liquid in consistency, a diarrhearic characteristic (3). Souza et al. (24) reported that diarrhea is a relatively common problem in calves (28.04%) and mainly caused by viral and/or bacterial (54.93%) agents. Although this work did not investigate viruses and bacteria, the percentage of infected samples points to protozoa. However, diarrhea can be triggered by the interaction of several factors such as bacteria, viruses, protozoa, immune deficiencies and environmental characteristics. Carvalho et al (22) reinforce this possibility because in their study they showed that the association between pathogens occurs from the first day of the diarrhea onset. It is important to emphasize that in this study the percentage of calves with normal feces represented 74.49%; however, more than 50% were positives for at least one of the protozoa investigated. Some infected calves had normal fecal consistency, probably linked them to mild degree of infection. Again, this can be a complicating factor because, according to this study, asymptomatic animals are potential carriers of cysts/oocysts and considered important sources of transmission to healthy calves, acting as environmental contaminants (25).

Helminth´s eggs of the Trichostrongylidae family were found in a very low number of samples, probably because a small portion of the animals had more than 30 days of life and limited contact with pastures. The provision of the roughage to the calves usually occurs after 30 days of age, and this factor favors the gastrointestinal helminth infection, since the animal is likely to ingest the larvae present in pastures (2, 7).

Based on these results, we concluded that infection by *Giardia* spp, *Cryptosporidium* spp, and *Eimeria* spp is substantially higher in calves, and might be interfering with animal growth leading to poor performance as a result of the intestinal damage cause by them. There was no a relation between the occurrence of diarrhea and parasitism in the statistical analysis of cause-effect. This study identified risk factors for infection such as food, time spent with the cow during postpartum and contact with dogs. These are risk factors for *Cryptosporidium* spp, while the milk supply was a risk factor for *Giardia* spp, and the floor type and age were risk factors for *Eimeria* spp. infection.

Ethics Committee. The experiment was approved by the Ethics Committee in Research with Animals of the *Universidade do Estado de Santa Catarina* (UDESC), under protocol number 4964301116.

REFERENCES

1. FAO - Food and Agriculture Organization of the United Nations. Roma: FAO Statistical Yearbook, 2013.

2. Maia GBS, Pinto AR, Marques CYT, Roitman FB, Lyra DD. Produção Leiteira no Brasil. BNDES Setorial 2013; 37(1):371-398.

3. Botteon RCCM, Botteon PTL, Júnior Santos JCB, Pinna MH, Lóss ZG. Frequência de diarreia em bezerros mestiços sob diferentes condições de manejo na região da média Paraíba – Rio de Janeiro e Minas Gerais. Braz J Vet Res Anim Sci 2008; 45(2):153-160.

4. Oliveira Filho JP, Silva DPG, Pacheco MD, Mascarini LM, Ribeiro MG, Alfieri AA, Alfieri AF, Stipp DT, Barros BJP, Borges AS. Diarreia em bezerros da raça Nelore criados extensivamente: estudo clínico e etiológico. Pesq Vet Bras 2007; 27:419-424.

5. Wattiaux MA. Essenciais em Gado de Leite—Criação de Novilhas. The Babcock Institute: University of Wisconsin-Madison. 2012; 121-124.

6. Sharma S, Vijayachari P, Sugunan AP. Histopathological studies of *Eimeria bovis* infection in Calves. J Veterinar Sci Technol 2015; 6(1): 235.

7. Bastiani FT, Da Silva AS, Dück MRK, Tonin AA, Monteiro SG. Outbreak of eimeriosis and giardiasis associated to mortality of lambs in southern Brazil. Comp Clin Pathol 2012; 21(3): 371-373.

8. Meireles GS, Silva NMP, Galvão GS, Almeida CRR, Flausino W, Lopes CWG. Surto de coccidiose em bezerros búfalos (*Bubalus bubalis*) por *Eimeria bareillyi* GIL et al., 1963 (Apicomplexa: Eimeriidae) - Relato de casos. Rev Bras Med Vet 2012; 34(2):116-120.

9. Couto MCM, Bomfim TCB. Espécies de *Cryptosporidium* que infectam bovinos: características etiológicas e epidemiológicas. Vet Not 2012; 18(2):94-109.

10. Martins-Vieira MBC, Brito LAL, Heller L. Oocistos de *Cryptosporidium parvumem* fezes de bezerros infectados experimentalmente. Arq Bras Med Vet Zootec 2009; 61(6):1454-1458.

11. Guimarães AM, Guedes E, Carvalho RA. Ocorrência de *Giardia* spp. em bezerros leiteiros no Brasil. Arq Bras Med Vet Zootec 2011; 53(6):652-653.

12. Gressler LT, Silva AS, Silva MK, Tonin AA, Monteiro SG. Gastrointestinal parasites of cavy (*Cavia aperea aperea*) in southern Brazil. Res Vet Sci 2010; 89(2):206-208.

13. Gow S, Waldner C. An examination of the prevalence of and risk factors for shedding of Cryptosporidium spp. and Giardia spp. in cows and calves from western Canadian cow-calf herds. Vet Parasitol 2006;137(1-2): 50-61.

14. Silva Júnior FA, Carvalho AHO, Rocha CMBM, Guimarães AM. Fatores de risco associados à infecção por *Cryptosporidium* spp. e *Giardia duodenalis* em bovinos leiteiros na fase de cria e recria na mesorregião do Campo das Vertentes de Minas Gerais. Pesq Vet Bras 2011; 31(8):690-696.

15. Geurden T, Vanderstichel R, Pohlec H, Ehsan A, von Samson-Himmelstjerna G, Morgan ER, et al. A multicenter prevalence study in Europe on *Giardia duodenalis* in calves, with molecular identification and rick factor analysis. Vet Parasitol 2012; 190: 383-390.

16. Hunter PR, Thompson ARC. The zoonotic transmission of *Giardia* and *Cryptosporidium.* Int J Parasitol 2005; 35(12):1181-1190.

17. Rebouças MM, Grasso LMPS, Filha Spósito E, Amaral V, Santos SM, Silva DM. Prevalência e distribuição de protozoários do gênero *Eimeria* (Aplicomplexa: Eimeriidae) em bovinos nos municípios de Altinópolis, Taquaritinga, São Carlos e Guaíra – Estado de São Paulo, Brasil. Rev Bras Parasitol Vet 1994; 3(2): 125-130.

18. Bangoura B, Daugschies A. Parasitological and clinical parameters of experimental *Eimeria zuernii* infection in calves and influence on weight gain and haemogram. Parasitol Res 2007; 100(6):1331–1340.

19. Tomczuk K, Grzybek M, Szczepaniak K, Studzinska M, Demkowska-Kutrzepa M, Roczen-Karczmarz M, Klockiewicz M. Analysis of intrinsic and extrinsic factors influencing the dynamics of bovine *Eimeira* spp. From centrl-eastern Poland. Vet Parasitol 2015; 214(1):22-28.

20. Vargas Jr SF, Marcolongo-Pereira C, Adrien ML, Fiss L, Molarinho KR, Soares MP, Schild AL, Sallis ESV. Surto de criptosporidiose em bezerros no Sul do Rio Grande do Sul. Pesq Vet Bras 2014; 34(8):749-752.

21. Tiranti K, Larriestra A, Vissio C, Picco N, Alustiza F, Degioanni A, Vivas A. Prevalence of *Cryptosporidium* spp. and *Giardia* spp., spatial clustering and patterns of shedding in dairy calves from Córdoba, Argentina. Rev Bras Parasitol Vet 2011; 20(2):140-147.

22. Carvalho JG, Carvalho AU, Heinemann MB, Coelho SG, Paes PRO, Moreira GHFA, Vespasiano LC, Filho Facury EJ. Estudo longitudinal da infecção por enteropatógenos em bezerros neonatos, com diarreia, sob diferentes estratégias de aleitamento. Pesq Vet Bras 2014; 34(6):529-536.

23. Delafosse A, Chartier C, Dupuy MC, Dumoulin M, Pors I, Paraud C. *Cryptosporidium parvum* infection and associated risk factors in dairy calves in western France. Prev Vet Med 2015; 118:406-412.

24. Sousa MV, Gonçalves RC, Lisbôa JAN, Almeida CT, Chiacchio SB. Aspectos clínicos e epidemiológicos da diarréia dos bezerros em Botucatu, SP. Rev Bras Ciênc Vet 2010; 79(2): 74-77.

25. Feitosa FLF, Shimamura GM, Roberto T, Mendes LCN, Peiró JR, Féres FC, Bovino F, Perri SHV, Meireles MV. Importância de *Cryptosporidium* spp. como causa de diarreia em bezerros. Pesq Vet Bras 2008; 28(10):452-456.

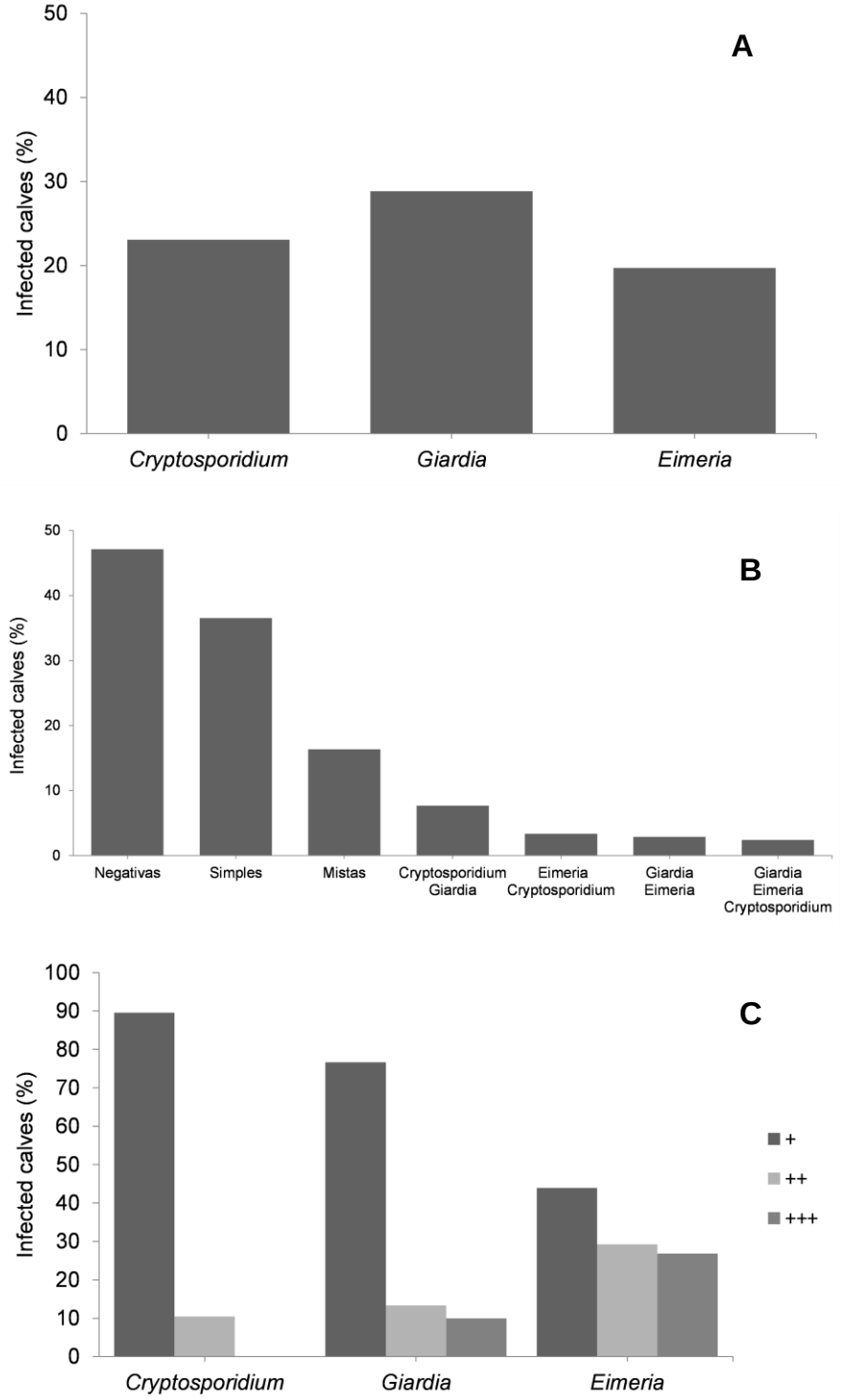

Figure 1. [A] Percentage of calves infected by *Cryptosporidium* spp. (51/243), *Giardia* spp. (65/243) and *Eimeria* spp. (53/243) in different cities of the Western Santa Catarina State. [B] Sample distribution – percentage of animals with negative parasitological results, animals with simple and mixed infections, in addition to the percentage of positive animals by each parasite type involved in mixed infections of calves (n=243) in several municipalities of Western Santa Catarina State. [C] Percentage of samples positives according to the degree of infection [mild (+), moderate (++) or high (+++)] caused by *Cryptosporidium* spp. (51/100%), *Giardia* spp. (65/100%), and *Eimeria* spp. (53/100%) in calves.

Table 1. Univariate analysis of risk factors for *Cryptosporidium* infection in calves.

Variables	No.	Frequency (%) Median	*P* - value	RR (CI: 95%)
Cryptosporidium				
Feed	239			
1. Cow's milk		12 (5)	-	-
2. Artificial milk		14 (6)	0.21	4.65 (0.39-54.27)
3. Concentrate		12 (5)	0.09	8.19 (0.70-94.59)
4. Milk + concentrate		85 (36)	0.19	4.32 (0.48-38.70)
5. Milk + concentrate + hay		116 (48)	0.51	2.06 (0.23-18.18)
Housing	239			
1. Individual		131 (55)	-	-
2. Grouped		108 (45)	<0.001	1.15 (1.01-3.01)
Floor	239			
1. Concrete		34 (14)	-	-
2. Slatted		109 (45)	0.23	0.54 (0.18-0.99)
3. Ground		56 (23)	0.74	0.83 (0.29-2.40)
4. Concrete + slatted		39 (16)	0.06	0.29 (0.08-1.09)
Cleaning interval	239			
1. 1-7 days		59 (33)	-	-
2. 8-15 days		6 (3)	0.88	0.20 (0.29-2.80)
4. >15 days		111 (64)	0.70	0.54 (0.01-0.89)
Supply milk	239			
1. Bottle		78 (52)	-	-
2. Bucket		6 (4)	0.54	0.54 (0.001-1.00)
3. Bottle and bucket		50 (33)	0.47	0.87 (0.30-2.58)
4. Without milk supply		16 (11)	0.57	0.94 (0.10-5.21)
Time with the cow	239			
1. < 1 hour		118 (50)	-	-
2. 1-5 hours		36 (15)	0.05	0.24 (0.05-1.00)
3. > 5 hours		85 (35)	0.57	0.78 (0.33-1.83)
Water source	239			
1. Artesian Well		109 (46)	-	-
2. Fount		130 (54)	0.13	1.94 (0.81-4.67)
Contact with rats	239			
No		38 (16)	-	-

Yes		201 (84)	0.66	1.44 (0.49-4.21)
Contact with dogs	239			
No		139 (58)	-	-
Yes		100 (42)	0.03	2.33 (1.04-5.22)
Contact with cats	239			
No		66 (28)	-	-
Yes		173 (72)	0.03	2.44 (1.05-4.21)
Contact with thicken	239			
No		185 (78)	-	-
Yes		54 (22)	0.49	1.27 (0.48-3.32)
Fly presence	239			
Low		86 (36)	-	-
Moderate		69 (30)	0.22	1.03 (0.18-1.49)
High		84 (35)	0.13	0.40 (0.12-1.33)
Age	239			
1. 1-15 days		54 (22)	-	-
2. 16-30 days		61 (25)	0.15	2.04 (0.76-5.47)
3. 31-45 days		43 (18)	0.48	1.50 (0.47-4.74)
4. > 46 days		84 (35)	0.24	1.80 (0.66-4.86)
Breed	239			
1. Holstein		195 (80)	-	-
2. Jersey		30 (12)	0.27	1.44 (0.53-3.85)
3. Crossbreed		17 (8)	0.78	2.19 (0.65-7.30)

Table 2. Univariate analysis of risk factors for *Giardia* infection in calves.

Variables	No.	Frequency (%) Median	*P* - value	RR (CI: 95%)
Giardia				
Feed	239			
1. Cow's milk		12 (5)	-	-
2. Artificial milk		14 (6)	0.49	0.46 (0.05-4.14)
3. Concentrate		12 (5)	0.06	6.42 (0.87-47.02)
4. Milk + concentrate		85 (36)	0.91	0.91 (0.19-4.42)
5. Milk + concentrate + hay		116 (48)	0.81	1.19 (0.25-5.58)
Housing	239			
1. Individual		131 (55)	-	-
2. Grouped		108 (45)	0.55	0.80 (0.33-1.66)
Floor	239			
1. Concrete		35 (14)	-	-
2. Slatted		109 (45)	0.78	1.16 (0.39-3.44)
3. Ground		56 (23)	0.26	0.49 (0.14-1.69)
4. Concrete + slatted		39 (16)	0.92	0.93 (0.25-3.44)
Cleaning interval	239			
1. 1-7 days		59 (33)	-	-
2. 8-15 days		6 (3)	0.47	2.09 (0.28-15.67)
4. > 15 days		111 (64)	0.55	0.20 (0.20-1.49)
Supply milk	239			
1. Bottle		78 (52)	-	-
2. Bucket		6 (4)	1	2.47 (0.001-65.00)
3. Bottle + bucket		50 (33)	0.36	1.47 (0.63-3.44)
4. Without milk supply		16 (11)	0.003	5.40 (1.73-16.83)
Time with the cow	239			
1. < 1 hour		118 (50)	-	-
2. 1-5 hours		36 (15)	0.07	2.54 (0.91-7.05)
3. > 5 hours		85 (35)	0.19	1.76 (0.75-4.11)
Water source	239			
1. Artesian Well		109 (46)	-	-
2. Fount		130 (54)	0.05	2.15 (0.97-4.78)
Contact with rats	239			
No		38 (16)	-	-

Yes		201 (84)	0.66	0.81 (0.31-2.09)
Contact with dogs	239			
No		139 (58)	-	-
Yes		100 (42)	0.05	2.10 (1.00-4.49)
Contact with cats	239			
No		66 (28)	-	-
Yes		173 (72)	0.24	1.71 (0.69-4.21)
Contact with chickens	239			
No		185 (78)	-	-
Yes		54 (22)	0.98	1.63 (0.61-4.37)
Presence of flies	239			
Low		86 (36)	-	-
Moderate		69 (30)	0.93	1.03 (0.43-2.47)
High		84 (35)	0.19	0.53 (0.20-1.36)
Age	239			
1. 1-15 days		54 (22)	-	-
2. 16-30 days		61 (25)	0.58	0.78 (0.33-1.84)
3. 31-45 days		43 (18)	0.04	0.35 (0.12-0.99)
4. > 46 days		84 (35)	0.19	0.56 (0.24-1.33)
Breed	239			
1. Holstein		195 (80)	-	-
2. Jersey		30 (12)	0.46	1.44 (0.53-3.85)
3. Crossbreed		17 (8)	0.20	2.19 (0.65-7.30)

Table 3. Univariate analysis of risk factors for *Eimeria* infection in calves.

Variables	No.	Frequency (%) Median	*P* - value	RR (CI: 95%)
Eimeria				
Feed	239			
1. Cow's milk		12 (5)	-	-
2. Artificial milk		14 (6)	0.75	1.66 (0.06-40.56)
3. Concentrate		12 (5)	0.44	3.70 (0.13-104.91)
4. Milk + concentrate		85 (36)	0.38	3.23 (0.23-45.82)
5. Milk + concentrate + hay		116 (48)	0.50	2.42 (0.17-33.80)
Housing	239			
1. Individual		131 (55)	-	-
2. Grouped		108 (45)	0.14	2.16 (0.76-6.14)
Floor	239			
1. Concrete		35 (14)	-	-
2. Slatted		109 (45)	0.20	0.42 (0.11-1.57)
3. Ground		56 (23)	0.58	1.44 (0.38-5.47)
4. Concrete + slatted		39 (16)	0.03	0.06 (0.004-0.86)
Cleaning interval	239			
1. 1-7 days		59 (33)	-	-
2. 8-15 days		6 (3)	0.85	0.75 (0.03-14.54)
4. >15 days		111 (64)	0.63	0.70 (0.16-2.97)
Supply milk	239			
1. Bottle		78 (52)	-	-
2. Bucket		6 (4)	0.19	7.75 (0.33-177.09)
3. Bottle + bucket		50 (33)	0.97	1.04 (0.07-14.71)
4. Without milk supply		16 (11)	0.85	0.74 (0.03-16.38)
Time with the cow	239			
1. < 1 hour		118 (50)	-	-
2. 1-5 hours		36 (15)	0.42	0.48 (0.08-2.86)
3. > 5 hours		85 (35)	0.78	0.82 (0.21-3.12)
Water source	239			
1. Artesian well		109 (46)	-	-
2. Fount		130 (54)	0.45	1.61 (0.45-5.69)
Contact with rats	239			
No		38 (16)	-	-

Yes		201 (84)	0.21	2.58 (0.56-11.78)
Contact with dogs	239			
No		139 (58)	-	-
Yes		100 (42)	0.56	1.41 (0.42-4.73)
Contact with cats	239			
No		66 (28)	-	-
Yes		173 (72)	0.70	0.70 (0.20-2.90)
Contact with chickens	239			
No		185 (78)	-	-
Yes		54 (22)	0.42	0.52 (0.10-2.53)
Presence of flies	239			
Low		86 (36)	-	-
Moderate		69 (30)	0.37	1.87 (0.46-7.51)
High		84 (35)	0.92	1.07 (0.23-4.92)
Age	239			
1. 1-15 days		54 (22)	-	-
2. 16-30 days		61 (25)	0.02	6.22 (1.33-29.08)
3. 31-45 days		43 (18)	0.001	10.63 (2.37-47.53)
4. > 46 days		84 (35)	0.001	11.94 (2.53-56.17)
Breed	239			
1. Holstein		195 (80)	-	-
2. Jersey		30 (12)	0.30	1.81 (0.57-5.69)
3. Crossbreed		17 (8)	0.55	1.59 (0.33-7.52)

Table 4. Multivariate analysis of risk factors for *Cryptosporidium, Giardia* and *Eimeria* infection in calves.

Variables	Estimate (β)	*P* - value	RR (CI: 95%)
Cryptosporidium			
Feed			
1. Cow's milk	-	-	-
2. Artificial milk	2.29	0.07	9.91 (7.76-12.65)
3. Concentrate	3.63	0.006	37.92 (17.28-83.20)
4. Milk + concentrate	2.28	0.04	9.80 (7.68-12.51)
5. Milk + concentrate + hay	1.56	0.016	4.79 (2.18-10.52)
Time with the its mother			
1. < 1 hour	-	-	-
2. 1-5 hours	1.84	0.006	0.15 (0.12-0.20)
3. > 5 hours	1.20	0.01	0.30 (0.13-0.66)
Contact with dogs			
No	-	-	-
Yes	1.27	0.001	3.57 (2.80-4.56)
Giardia			
Milk Supply			
1. Bottle	-	-	-
2. Bucket	-4.74	1	2.47 (1.93-3.15)
3. Bottle + bucket	1.68	0.003	5.40 (1.18-6.58)
4. Without milk supply	-1.87	0.87	3.87 (2.00-28.30)
Eimeria			
Floor			
1. Concrete	-	-	-
2. Slatted	-1.36	0.06	0.25 (0.20-0.32)
3. Ground	-0.04	0.95	0.95 (0.43-2.10)
4. Concrete + slatted	-3.48	0.01	0.03 (0.02-0.03)
Age			
1. 1-15 days	-	-	-
2. 16-30 days	2.05	0.007	7.81 (3.56-17.14)
3. 31-45 days	2.64	<0.001	14.02 (10.95-17.89)
4. ≥ 46 days	2.62	0.001	13.76 (6.27-30.20)

2.2 ARTIGO II

Secnidazole for control of giardiasis in dairy calves

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ABSTRACT

The aim of this study was to verify whether secnidazole, given in a single oral dose (10 mg/kg), decreases or eliminates the excretion of *Giardia duodenalis* cysts. Holstein calves were raised from birth to 15 ± 2 days of age in individual stalls. Subsequently, 12 calves were grouped and housed in collective stalls. After seven days (day of life 21), we collected stool samples directly from the rectal ampulla in order to determine the degree of parasitic infection. Fecal examination was performed by a centrifugal-flotation technique, which allows for visualization and quantification of *G. duodenalis* cysts. After division into control and treatment groups, six animals were treated with one 400 mg secnidazole capsule. The first stool collection following treatment was performed on day 5 and the second on day 30. This experiment was repeated at 15 days, with a total of 24 calves studied. Animals on the farm where the experiment was conducted often suffer from giardiasis, despite hygiene care (disinfection) and adequate facilities. All 24 calves were excreting *G. duodenalis* cysts prior to starting treatment. Five days after receiving the treatment, animals in the experiment group were *Giardia*-negative, i.e., they did not excrete parasite cysts, whereas calves in the control group continued to excrete cysts. After 30 days of treatment, the stool of most treated animals (83.3%) remained free of *G. duodenalis* cysts. Therefore, we believe that secnidazole was 100% effective in eliminating the excretion of *Giardia duodenalis* cysts.

Keywords: protozoan, diarrhea, nitroimidazole.

1. Introduction

The raising of heifers is very important to dairy cattle husbandry, as it ensures the replacement of cows, permits increases in herd size, and consequently, milk production. However, high rates of morbidity and mortality compromise production, although this phenomenon is not well-documented. Currently, the main cause of early death in heifers is infectious diarrhea, mainly caused by bacteria and protozoa. Infections caused by these pathogens cause malabsorption of nutrients, weight loss, decreased food intake, and slow growth. Infectious diarrhea creates substantial economic burdens for producers because of costs associated with treatment, and in many cases, the cost of replacing dead calves. It is estimated that diarrhea is directly associated with death of calves during the first week of life and at 8 to 31 days of age. The disease accounts for more than 50% of calf mortality (Gulliksen et al., 2009; USDA, 2007).

Among the agents capable of provoking intestinal infections leading to diarrhea in animals, most prominent is Giardia, the protozoan responsible for giardiasis. Infections caused

by *Giardia duodenalis* can be controlled with the use of paromomycin sulfate, an effective active ingredient when used for 5 consecutive days (Geurden et al., 2006). However, the most used and effective agent is the antiprotozoal fenbendazole, used for 3 consecutive days (Geurden et al., 2006; O'Handley et al., 2011). In other words, current treatments for giardiasis control in calves require continuous use, making management difficult for the animals and their caretakers.

Another potential treatment for giardiasis in calves is the antiprotozoal secnidazole. Single-dose therapy has been used in humans, but has never been studied in calves. Nevertheless, the drug showed promise in lambs (Ural et al., 2014), cats (Da Silva et al., 2011), and Swiss mice (Franco et al., 2015). The purpose of this study was to verify that secnidazole used in a single dose decreases or eliminates the excretion of *Giardia duodenalis* cysts in calves.

2. Material and methods

2.1. *Treatment*

The active ingredient secnidazole used in this study was purchased from Medley. Subsequently, 400 mg secnidazole capsules were produced in a compounding pharmacy.

2.2. *Animals and experimental design*

Newborn Holstein calves, weighing 38 ± 2.5 kg at birth, were housed in individual stalls and received two liters of colostrum within the first 2 hours after birth. After ingestion of colostrum, the animals received three liters of milk twice a day (40 ± 3.2 kg at 15 days of life), in addition to food and water *ad libitum*. Animals on the farm where the experiment was conducted often suffer from giardiasis, despite hygiene care (disinfection) and adequate facilities. This history of the disease was the reason for choosing this farm for the experiments.

After approximately 15 days, twelve calves were transferred and grouped in a collective stall, with automatic feeding (6 L/day), in addition to feed, water and hay *ad libitum*. The ration was produced on the same farm. It consisted of corn bran (47%), soybean meal (31%), soybean husk (17%), and mineral-vitamin commercial premix (5%), containing 22% protein. Hay was produced from Tifton 85, offered *ad libitum*.

At 15 days of life, the calves were divided into two groups: the treatment group received a capsule containing 400 mg of secnidazole orally, corresponding to approximately 10 mg/kg; and other group was used as a control, without receiving secnidazole. This experiment was performed in duplicate; 24 calves were divided into two stages (Step I and II), with a 15-day interval between the beginning of each stage.

2.3. *Sample collection*

The first fecal collection was performed seven days after grouping the animals in a collective stall and before the beginning of the experiment, which was designated as day 0. New fecal samples were collected on days 5 and 30 after secnidazole treatment. Fecal collections were performed directly from the rectal ampulla of the animals in order to verify the number of cysts of G. *duodenalis* per gram of feces.

2.4. *Parasitological examination of feces*

The parasitological examination of feces aimed to determine the presence and degree of infection of each animal with G. *duodenalis* cysts using a centrifugal-flotation technique (Faust et al., 1938), according to a methodology described in detail by Monteiro (2010). We used hypersaturated sugar solution, and counted cysts according to the methodology of Barbier et al., (1990).

2.5. *Statistical analysis*

Number of cysts were first analyzed descriptively. We computed measures of central tendency (median) and data dispersion (range-standing for the interval between the minimum and maximum values in the data). The variable was further subjected to Shapiro Wilk's W-test for normal distribution verification. Since most of the variable did not meet criteria for parametric testing, we used a nonparametric test (Kruskal-Wallis). We define statistical significance as P value < 0.05.

3. Results

For the 24 calves excreting *G. duodenalis* cysts before starting treatment, the results obtained in Step I were similar to those obtained in Step II. After administration of secnidazole, no *G. duodenalis* cysts were found in feces on day 5 (0%), and we saw a reduction of cysts at 30 days following treatment (83.3%, Fig. 1). Therefore, we observed a significant difference ($P < 0.05$) between groups with respect to cyst count on days 5 and 30 following treatment (Fig. 2).

Even after 30 days of secnidazole capsule intake, most calves remained *Giardia* negative, unlike those in the control group who continued to excrete *Giardia* cysts. It is important to emphasize that animals in both groups shared the same bay. Therefore, animals in the treatment group were exposed daily to environmental contamination by protozoan cysts.

4. Discussion

Transmission of the parasite in domestic cattle is particularly concerning because of the high concentration of animals in a small area of land, and the large output of feces. We suggest that a major factor is dissemination among calves. Previous studies examining *Giardia* in cattle in North America revealed a point prevalence of 6.5–11% in adult cattle, but up to 100% cumulative prevalence in calves under 6 months of age (Hoar et al., 2001; Ralston et al., 2002). Despite recognition of the prevalence *G. duodenalis*, only a handful of agents have been used in therapy, and the agents that are available may have adverse effects or may be contraindicated in certain clinical situations.

We observed no *G. duodenalis* cysts in calves treated with a single dose of secnidazole at 10 mg/kg. This result is similar to those seen in other studies in lambs (Ural et al., 2014), cats (Da Silva, et al., 2011) and mice (Franco et al., 2015). Secnidazole is a nitroimidazole class antiprotozoal derived from 5-nitroimidazole. Like other drugs of this class, enters microorganisms through passive diffusion and is activated by reduction of the nitro group (Gardner and Hill, 2001), effectively killing *Giardia*. In anaerobic microorganisms, such as *G. duodenalis*, this intracellular reduction occurs through pyruvate ferredoxin oxidoreductase and results in a concentration gradient across the cell membrane, which, in turn, increases the transport of the original drug into the cell. Since the electron affinity of 5-nitroimidazoles is greater than the reduced ferredoxin, the drug disrupts the normal flow of electrons, aerobic microorganisms have a more positive redox potential (ie, more efficient electron acceptors) than secnidazole and other 5-nitroimidazoles, which explains the selective toxicity of these drugs against anaerobic microorganisms. However, DNA is the intracellular target of 5-nitroimidazoles and when induced by the drug results in tape rupture, loss of helical structure and impaired template function. However, the lethal effect of 5-nitroimidazoles on the microorganism is attributed to a short-term reduction product, probably a nitro radical anion (Gardner and Hill, 2001). Although the mechanism of action of secnidazole in infections caused by these microorganisms has apparently not been investigated, it would have been expected to be similar to that of metronidazole.

In addition to combating infection, secnidazole treatment prevented reinfection in more than 80% of the animals. This effect is likely due to secnidazole being a pharmacological substance derived from long-acting 5-nitroimidazole. The drug is absorbed rapidly and completely, and has a half-life of 17 to 29 hours to humans (Gillis and Wiseman, 1996). The half-life of secnidazole in ruminants is unknown. It is important to remember that, in some countries, the use of drugs derived from nitroimidazoles is prohibited in food animals. However,

since the half-life of the drug is short, it would have no negative consequences for food production when used in young animals such as dairy calves, as these would only have started their productive life as cows when they are more than 2 years old.

5. Conclusion

Therefore, we conclude that secnidazole is 100% effective in the treatment of giardiasis in dairy heifers. It eliminates the excretion of *Giardia duodenalis* cysts; it is available in convenient single-dose form; and the effect persists for at least 30 days. Since giardiasis is a persistent infection, a single dose treatment option is attractive. Because of this, studies on half-life, excretion and possible side effects should be undertaken to validate that treatment with secnidazole is a safe option in combating giardiasis in young ruminants.

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Ethics committee

This study was approved by the Ethics Committee on the Use of Animals of the State University of Santa Catarina (CEUA/UDESC), protocol number 4964301116.

References

Atashi, H., Zamiri, M.J., Dadpasand, M., 2013 Association between dry period length and lactation performance, lactation curve, calf birth weight, and dystocia in Holstein dairy cows in Iran. J. Dairy Sc. 96, 6, 3632-3638.

Barbier, D., Perrine, D., Georges, P., 1990. Quantitative recovery of *Taenia saginata* eggs from sewage sludge. Med. Mal. Infect 19, 315–318.

Da Silva, A.S., Castro, V.S.P., Tonin, A.A., Brendler, S., Costa, M.M., Jaques, J.A., Bertoletti, B., Zanette, R.A., Raiser, A.G., Mazzanti, C.M., Lopes, S.T.A., Monteiro, S,G,. 2011. Secnidazole for the treatment of giardiasis in naturally infected cats. Parasitol. Int. 60, 429–432.

Faust, E.C., D'antoni, I.C., Odon, V., Miller, M.J., Perez, E.C., Sawitz, W., 1938. A critical study of clinical laboratory techniques for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. Am. J. Trop. Med. Hyg. 18, 169–183.

Franco, S.F., Silva, A.M.G., Garcia, T.I., Ramos, A.C., Colli, C.M., Pavanelli, M.F., 2015. Infecção por *Giardia intestinalis*: avaliação dos sinais clínicos e resistência medicamentosa em camundongos swiss. Rev. Saúde e Biol. 10, 23-33.

Gardner, T.B., Hill, D.R., 2001. Treatment of giardiasis. Clin. Microbiol. Rev.14, 1, 114-128.

Geurden, T., Claerebout, E., Dursin, L., Deflandre, A., Bernay, F., Kaltsatos, V., Vercruysse, J. 2006a. The efficacy of an oral treatment with paromomycin against an experimental infection with *Giardia* in calves. Vet. Parasitol. 135, 3-4, 241-247.

Geurden, T., Vercruysse, J., Claerebout, E. 2006b. Field testing of a fenbendazole treatment combined with hygienic and management measures against a natural *Giardia* infection in calves. Vet. Parasitol. 142, 3-4, 367-371.

Gillis, J.C., Wiseman, L.R., 1996. Secnidazole. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. Drugs. 51, 621–38.

Gulliksen, S.M., 2009. Calf mortality in Norwegian dairy herds. J. Dairy Sci. 92, 2782–2795.

Hoar, B.R., Atwill, E.R., Elmi, C., Farver, T.B., 2001. An examination of risk factors associated with beef cattle shedding pathogens of potential zoonotic concern. Epidemiol. Infect. 127, 147–155.

National Research Council (NRC). Nutrient Requirements of Dairy Cattle. Seventh Revised Edition; National Acedemy Press: Washington, DC, USA, 2001.

O'handley, R.M., Buret, A.G., Mcallister, T.A., Jelinski, M., Olson, M.E, 2001. Giardiasis in dairy calves: effects of fenbendazole treatment on intestinal structure and function. Int.l J. for Parasitol. 31, 1, 73-79.

Ralston, B.J., Mcallister, T.A., Olson, M.E., 2002. Prevalence and infection pattern of naturally acquired Giardiasis in beef calves and their dams from birth to weaning. In: Olson, B.E., Olson, M.E., Wallis, P.M. (Eds.), Giardia the Cosmopolitan Parasite. CABI Publishing, New York, pp. 47–52.

Sugimoto, M., Watanabe, T., Sugimoto, Y., 2012. The Molecular Effects of a Polymorphism in the 59UTR of Solute Carrier Family 44, Member 5 that Is Associated with Birth Weight in Holsteins. PLoS ONE. 7, 7, e41267.

Ural, K., Aysul, N., Voyvoda, H., Ulutas, B., Aldemir, O.S., Eren, H., 2014. Single dose of secnidazol treatment against naturally occuring *Giardia duodenalis* infection in Sakiz lambs. Rev. MVZ Córdoba, 19, 1, 4023-4032.

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

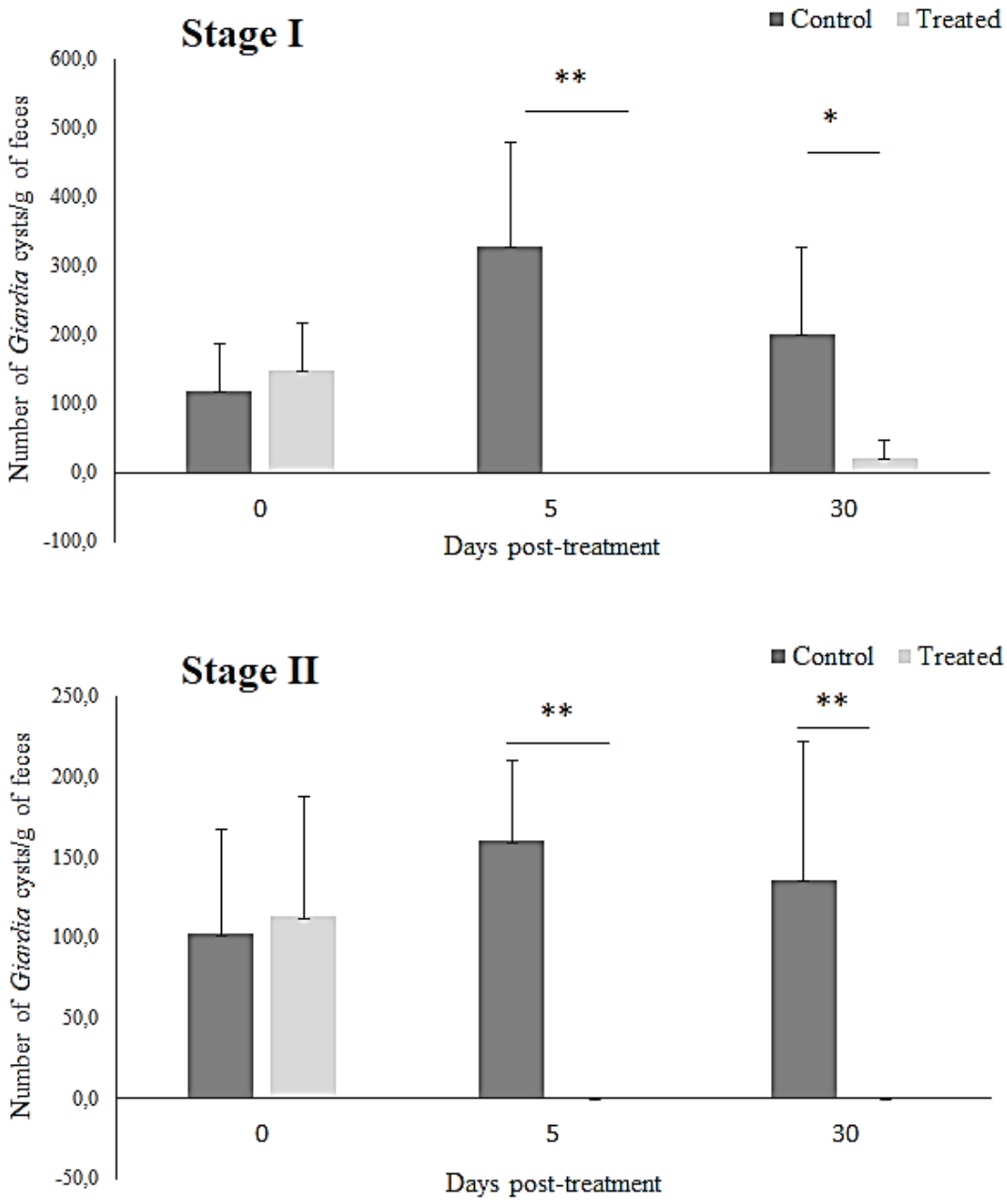

Figure 1. Number of *Giardia* cysts at day 0 and post treatment (days 5 and 30) with a single oral dose of secnidazole (10 mg/kg) at the two stages of the experiment (Step I (n = 12) and Step II (n = 12)). * P<0.05 indicates difference between groups.

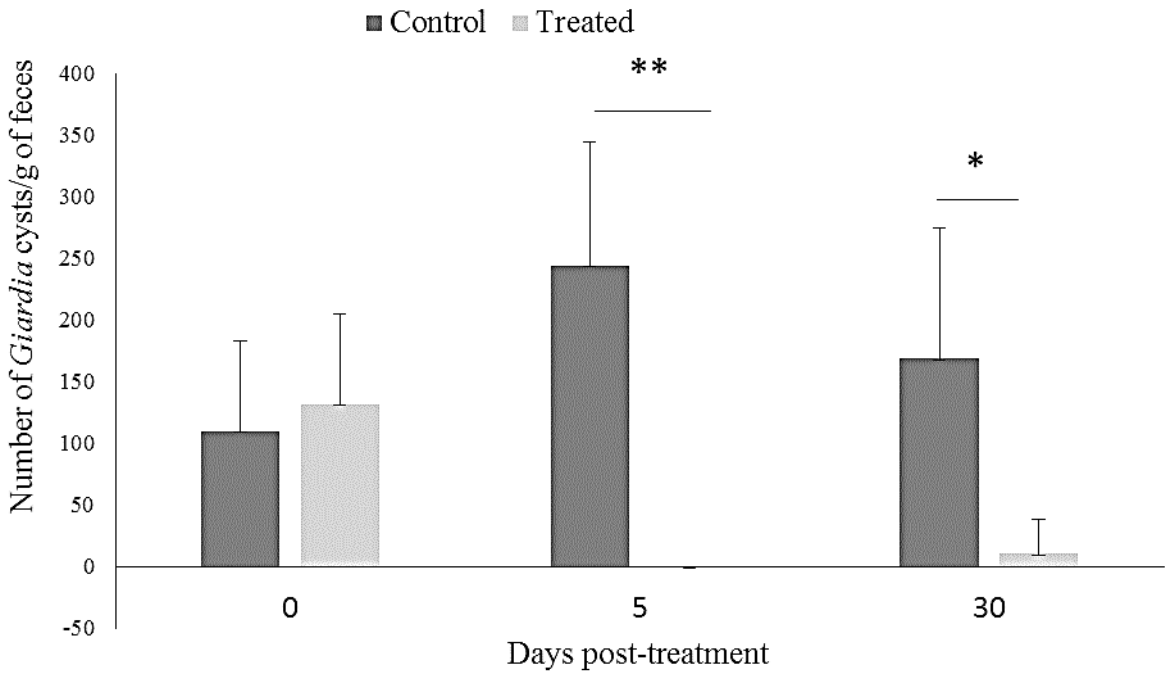

Figure 2. Response to secnidazole by *Giardia* cysts. Data plotted together (Stage I and II) (n = 24). * P<0.05 indicates difference between groups.

2.3. MANUSCRITO I

A prophylactic protocol to stimulate the immune response also control infectious disease and, consequently, minimizes diarrhea in newborn heifers

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ABSTRACT. The aim of this study was to verify whether selenium (Se) and vitamins (A and E) applied via subcutaneous associated with secnidazole via oral exert positive effects in the antioxidant and immune systems, as well as whether prevent infections caused by protozoan and bacteria, and consequently, reduce the number of cases of diarrhea in calves. Thirty-two newborn Holstein heifers were divided into two groups with sixteen animals each: the control group and the treated group that received sodium selenite (0.2 mg/kg) and vitamins A (35 mg/kg) and E (1 mg/kg) with one day of life, and a second application associated with secnidazole (400 mg/animal) on day 10 of life. Sample collection (blood and feces) were performed on days 1, 15, 30, 45 and 60 of life. Heifers from the treated group showed higher hematocrit values compared to the control group on day 60 of life, while total serum protein levels were higher on days 15 and 30. The ceruloplasmin (days 15, 30 and 60), IgG of heavy chain (days 15, 30, 45 and 60), IgG of light chain (days 45 and 60) and haptoglobin (days 15, 30, 45 and 60) were higher in the treated group compared to the control group. Serum levels of glucose decreased in treated animals on day 60 of life, while serum levels of albumin, triglycerides, urea, cholesterol, thiobarbituric acid reactive substances, reactive oxygen species and glutathione S-transferase activity did not differ between groups. Secnidazole was able to prevent infections caused by *Giardia duodenalis* in the first few days of life, but no difference was observed between groups. Moreover, there was no difference on total bacteria count and the incidence of diarrhea between groups. No difference on weight gain was observed on day 60 of life, but on day 210 of life treated animals had higher weight gain compared to the control group. Based on these evidences, we concluded that the injectable application of Se and vitamins (A and E) associated to secnidazole can improve the immunological system, and consequently, favor animal´s performance.

Keywords: metaphylactic effect, nutraceutical, selenium, vitamin, control, disease pathogenesis.

1. Introduction

Proper management of heifers is a crucial activity in milk farms, since they will be the replacements that could improve the performance of the herd in the future, and their discard represent an important production cost. The mortality and morbidity rates of young animals, including calves and heifers, may hamper dairy farm activities mainly because these animals have immature immune system and depend on colostrum supply during the first hours of life since placental transfer of immunoglobulins is minimal [1], leaving them more susceptible to infections that cause diarrhea, the main clinical sign related of death at this age, with mortality

rates of approximately 22 % [2].

Currently, the treatment of calves diarrhea involves, beyond the costs associated with the treatment, the risk of new infections and the permanent intestinal lesions in their mucosa [3]. Moreover, there is a lack of data regarding the efficiency of prophylactic treatments that could improve their immunity and, consequently, reduce intestinal infections. In this sense, the modulation of the immune system could be considered a pathway to prevent some diseases. In this regard, the idea to associate Se and the vitamins A and E comes to light, since these treatments are involved in the proper functioning of the immune system, and this supplementation can potentiate animal immunity [4,5]. As an example, Se-supplementation before calving increased the concentration of immunoglobulins in newborn heifers [6,7], and higher serum Se-concentration in heifers induced an increase on phagocytic activity and, consequently, major protection against pathogenic agents [8]. Also, a study conducted by Moeini et al. [9] demonstrated that leucocyte counts were higher in heifers supplemented with sodium selenite and DL-alpha-tocopherol, a synthetic form of vitamin E.

It is known that diarrhea can be caused by different pathogenic agents, as the protozoan *Giardia* sp., which is found with major frequency in heifers [10]. This protozoan is an intracellular parasite that infects the host during a long period of time due to a low efficacy of the immune system [11,12]. In this context, it is necessary the use of chemical substances for a complete elimination of this parasite, as the use of secnidazole, an anti-protozoan used in humans, and untested in heifers until the present moment. However, this active principle was used successfully in the treatment of giardiasis in lambs [13], cats [14] and Swiss mice [15].

Based in the beneficial properties of sodium selenite, secnidazole, and vitamins A and E, our hypothesis is that combination on them could be an efficient option prevent heifers diarrhea and reduce their mortality. Thus, the aim of this study was to test the prophylactic treatment based on the injectable application of sodium selenite and vitamins (A and E) associated to the oral treatment with secnidazole, and evaluate whether this prophylactic treatment possess positive effect on the antioxidant and immune systems, as well as whether prevents infections caused by protozoan and bacteria, and consequently, reduces the incidence of diarrhea in heifers during the lactation period.

2. Material and Methods

2.1 Chemicals

Sodium selenite was purchased from Sigma-Aldrich® and diluted in saline solution. The vitamins A and E were purchased from Biogen®. Secnidazole (active principle) was purchased

from Medley®, and the capsules containing 400 mg of secnidazole was produced in a manipulation pharmacy.

2.2 Animals

This study was conducted in a commercial dairy cattle farm located in Xanxerê (Santa Catarina State, Southern Brazil) with optimal facilities management, but with high sanitary challenges, i.e., preventive measures were unable to prevent the occurrence of diarrhea and pneumonia, causing a neonatal mortality of approximately 25 % by per month in their historic. This study was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Catarina (UDESC) under protocol number 4964301116.

Thirty-two newborn Holstein heifers born in an intervals of eight days with average weight of 49.95 (± 3.12 kg) were used as the experimental model. However, body weight was measured at three moments using a tape to determine the thoracic perimeter [16], i.e., 1 and 60 days of life (experimental period) and 210 days of life (period of growth, post-experiment). In addition, these animals were monitored up to weaning (60 days of life) through blood and fecal examination.

Newborn heifers were housed in individual stalls and received two liters of colostrum (quality evaluated using the refractometer type Brix, considering a limit of 21 %, i.e., > 50 mg of Ig/mL) in the first six hours after calving. After the consumption of colostrum, the animals received three liters of milk twice a day, with access to concentrate and water *ad libitum*. With fifteen days of life, the heifers were grouped in collective stalls with the capacity of twelve animals with automatic milking (6 liters per day), concentrate, hay and water *ad libitum*.

The concentrate was farm-made constituted of corn bran (45 %), soybean meal (33 %), soybean hull (16 %) and Bovigold Prima® (6 %), providing 22.6 % of brute protein. The hay was produced from Tifton 85 with approximately 10.7 % of crude protein.

2.3 Experimental design

The animals were randomly divided into two groups: control and treatment. Sixteen heifers of the treatment group received a prophylactic treatment of injectable sodium selenite (0.2 mg/kg) and subcutaneous application of vitamins A and E following the doses recommended by manufactures (35 mg/kg and 1 mg/kg, respectively) (volume of 1 mL/50 kg) on days 1 and 10 of life. These same animals received one capsule containing 400 mg of secnidazole orally on day 10 of life.

2.4 Sample collection

The first blood collection occurred until 24 h of life, after the consumption of colostrum (day 1), while the others collections (blood and feces) were performed on days 15, 30, 45 and 60 of life. The blood collection was performed directly from jugular vein using tubes containing EDTA as anticoagulant (10 %) and without anticoagulant. The sample collected in tubes without anticoagulant were centrifuged at 7000 rpm during 10 min to obtain serum. After, the serum samples were transferred to microtubes and stored at -20 ºC until utilization.

The fecal samples were obtained directly from rectal ampule, which were stored at 5 ºC until utilization. One portion of the feces were used to bacterial count, and the other portion to protozoan research.

2.4.1 Hematocrit

Hematocrit was performed up to 2 h after blood sampling in tubes containing EDTA. The microhematocrit was performed using capillary tubes after a centrifugation at 10,000 rpm for 3 min. After centrifugation, the percentage of erythrocytes in the total blood was evaluated using a reading card as preconized by Feldman et al. [17].

2.4.2 Serum biochemistry

Serum levels of total protein, albumin, urea, glucose, cholesterol and triglycerides were measured by a semi-automated analyzer BioPlus 2000® using commercial kits (Analisa Gold®) following the manufacturer´s recommendations. The globulin levels were obtained by the formula: (total protein – albumin).

2.4.3 Oxidant/antioxidant status

Serum lipid peroxidation was determined according to the methodology described by Jentzsch et al. [18], which measures the malondialdehyde (MDA) levels by thiobarbituric acid reactive substance (TBARS), and the results were expressed as µm MDA/mL. Serum ROS levels were quantified by fluorimetric method of diacetate dichlorofluorescein (DCFH-DCF) [19], and the results were expressed as U DCF/mL. The activity of antioxidant enzyme glutathione S-transferase (GST) was analyzed in serum using the method described by Habig et al. [20], and the results were expressed as µmolCdnb/min/mL.

2.4.4 Proteinogram

For protein fractionation, polyacrylamide gel electrophoresis containing sodium

dodecyl sulphate (SDS-PAGE) was performed, according to a technique suggested by Fagliari et al. [21] using a mini gel (10 x 10 cm). The gel was stained with Comassie Blue and photographed to identify and quantify protein fractions using the Labimage1D software (Loccus Biotechnology). A standard containing fractions with molecular weight between 10 and 250 KD (Kaleidoscope - BIORAD) was used as reference for the identification of protein fractions.

2.4.5 Parasitological examination

Parasitological examination was performed to determine the presence of oocysts of *Cryptosporidium* spp. and *Eimeria* spp., as well as cysts of *Giardia duodenalis.* For this, 2 g of feces were processed by the centrifugal-flotation technique using hypersaturated sugar solution, according to the methodology described by Monteiro [22].

2.4.6 Total bacterial count

A portion of fresh fecal sample was used to perform the Total bacterial count was performed using 1 gram of fresh feces that was serially diluted into 9 mL of buffered peptone water and plated (1 ml) in total aerobic count plates (3M Petrifilm® Plate). This plate is a system of culture medium ready for use which contains all nutrients of standard method, as well as an indicator that facilitates colony counting. Results were expressed as CFU/g of feces.

2.4.7 Fecal score

The occurrence of diahrrea was observed daily following the methodology described by Larson et al. [23], which is based on fecal score and classification linked to fluidity: (1) normal and solid; (2) pasty, but with healthy aspect; (3) aqueous consistency and (4) fluid consistency.

2.5 Statistical analysis

The data from 32 animals were evaluated firstly by descriptive statistics for contingency of information and for further assumptions, which were presented as descriptive for mean and standard deviation. All covariates were tested for normality using the Shapiro-Wilk test, and the skewness, kurtosis and homogeneity by the Levene test. A repeated measure t-test was used to examine differences of the parameters to control and treated group, and all parameters compared mean between groups tested via a Student's t-test (controlling for data dependency due to dependence in time) was used to analyze all parameters at each time period (days 1, 15, 30, 45 and 60 of the experiment). Weight gain from birth (day 1) until weaning (day 60) and

from day 1 to 210 days was evaluated by the t-test. In addition, fecal consistency was associated with total bacterial count by a logistic regression analysis. All plots visualization was performed using the ggplot2 package [24]. It was considered significantly different for $P<0.05$. The statistical process was performed using R-language, v.2.15.1 (R Development Core Team 2012).

3. Results

3.1 Mortality and pneumonia

The mortality rate was similar for both groups (6.25 %), i.e., one animal per group. Also, no significant difference was observed between groups regarding the incidence rate for pneumonia (data not shown).

3.2 Hematocrit

The treatment group showed higher hematocrit values compared to the control group on day 60 of life. Over time, the hematocrit values increased in the control group (day 15 for days 30, 45 and 60) and the treatment group (day 15 for days 45 and 60; day 30 for day 60) (Figure 1).

3.3 Serum biochemistry

Serum levels of albumin, urea, triglycerides and cholesterol did not differ between groups in all evaluated moments. However, the treatment group showed lower glucose levels compared to the control group on day 60 of life. Serum cholesterol levels decreased in both groups over time, while glucose levels changed only in the treatment group. No difference was observed over time regarding albumin, urea and triglycerides levels (Table 1).

Total protein and globulin levels were higher in the treatment group compared to the control group on days 15 and 30 of life. Regarding the time, seric levels of total proteins and globulins increased on day 1 to 15 and 30 of life, while decreased from day 15 to 45 and 60 of life, and from day 30 to 45 of life in the treatment group. Over time, serum levels of total proteins increased in the control group on day 15 to 30 of life (Figure 2).

3.4 Proteinogram

The treatment group showed higher levels of ceruloplasmin compared to the control group on days 15, 30 and 60 of life, and also for IgG heavy chain on days 1, 15, 30 and 45 of life. However, IgG light chain increased only on days 45 and 60 of life in the treatment group

compared to the control group. Moreover, the treatment group showed higher levels of haptoglobin compared to the control group on days 15, 30, 45 and 60 of life (Figure 3).

Over time, ceruloplasmin and IgG light chain levels did not differ in the control group. On the other hand, the levels of IgG heavy chain decreased on day 1 to days 15 and 30 of life and also for haptoglobin levels on day 1 to days 15, 30, 45 and 60 of life in the control group. Over the time, ceruloplasmin levels increased in the treatment group on day 1 to 15 of life, and decreased on day 15 to days 30 and 45 of life. The levels of haptoglobin increased gradually (days 15, 30 and 45) with significant differences ($P<0.05$) on day 1 to 60 of life. Over time, there was no difference in the treatment group for IgG (heavy and light chains).

3.5 Oxidant/antioxidant status

There were no significant differences between groups for ROS and TBARS levels, as well as for GST activity in all evaluated moments. Over time, the serum levels of ROS and TBARS decreased in both groups, while serum GST activity increased in the treatment group compared to the control group (Table 2).

3.6 Fecal score

We did not observe differences between groups ($P = 0.32$) regarding fecal consistency score, however, numerically the number of animals with diarrhea was lower in the treated group (46.6%) than in the control group (73.3%). For the dependency of fecal consistency regarding total bacterial count and *Giardia* spp., it was found a non-statistical association with p-value of 0.21 and 0.43, respectively.

3.7 Total bacterial count

Total bacterial count did not differ between groups in the evaluated moments. However, a significant reduction on total bacterial count was observed in both groups, i.e., on the day 15 for days 45 and 60 of life (Figure 4).

3.8 Parasitological examination

Giardia cyst were not observed in fecal samples from treated heifers, differently of the control group, where 40% had infection on day 15 of the experiment. After 30 days, *Giardia* spp. cysts and *Eimeria* spp. oocysts were found in some animals from both groups.

3.9 Body weight and weight gain

No significant difference was observed between groups regarding body weight on days 1, 60 and 210 of life. Over time, a significant increase on body weight was observed in both groups, as expected for animals in the growth phase (data no shown). When analyzing the weight gain from days 1 to 60, a numerical ($P=0.098$) and statistical difference ($P<0.05$) was observed between groups on days 1 to 210 of life (Figure 5), i.e., weight gain was higher in the treatment group. Similarly, the daily weight gains on day 210 of life was superior in the treatment group (643 grams) compared to the control group (569 grams).

4. Discussion

Secnidazole has been effective against *Giardia* spp. in some animal species such as cats, lambs and rodents [13, 14, 15], but its prophylactic effect has not been studied yet up to this moment. In this study, secnidazole was effective to prevent infections caused by *G. duodenalis* during the first thirty days of heifer´s life. Its specific mechanism of action remains unknown, but for nitroimidazoles, the pharmacological class of secnidazole, its mechanism of action is associated to the incorporation of the drug by the microorganism through passive diffusion after reduction of nitrate groups [25]. Total bacterial count was similar for both groups, which is justified since none of the drugs tested have direct antimicrobial effect. Regarding the bacterial count, a possible indirect effect was observed, i.e., the prophylactic treatment increased the immune response and, consequently, the animals were more resistant to infectious diseases. Possibly, the lower incidence of *G. duodenalis* and bacterial count seen during the experiment compared to the farm is a consequence of better hygiene and disinfection protocols, which demonstrates the importance of proper hygiene to minimize disease.

Serum levels of total proteins were higher in treated animals compared to the control group. As expected, serum levels of total proteins increased after ingestion and absorption of colostrum, being this parameter used as an indicator of passive immunity transference. This result may be due to the relation between Se and glutathione (GSH), since Se is present in the enzymatic structure (linked to the amino acid selenocistein), and GSH participates on biochemical reactions associated to protein synthesis [26]. Also, it is possible that the increase on total proteins is directly related to mineral and vitamin supplementation, since these molecules exert important effects on the immune and antioxidant systems [5]. Recently, a study conducted by Gumus and Imik [27] demonstrated that vitamin E exerts beneficial properties in broilers exposed to heat stress through a direct effect on total protein levels, in agreement to what was observed in this study. Serum globulin levels were higher in treated animals compared

to those of the control group. Globulins are proteins present in blood plasma divided in three fractions: alpha globulins, beta-globulins and gamma-globulins, being the last linked to the immunological system [28]. It is recognized that Se is intimately related to vitamin E, since these compounds exert a synergic effect on gamma-globulin fraction, where Se acts in the transference of D-alpha-tocopherol through cellular membranes, leading to its increased biological activity. Recently, Sushma et al. [29] demonstrated that sodium selenite, when used alone, does not exert any effect on serum globulins levels in lambs, suggesting that the association between sodium selenite and vitamin E potentiates the production of immune cells, increasing its seric levels in treated animals.

Serum levels of IgG (heavy and light chains) were higher in animals of the treated group compared to the control group. These results can be associated to Se-supplementation that induced an increase of lymphocyte synthesis, and thus, on the synthesis of immunoglobulins [30,31]. This occurs because Se is found in the main organs of the immune system such as bone marrow and thymus, as well as in major cells derived from stem cells like lymphocytes [32]. In this way, Se deficiency may lead to lower synthesis of immunoglobulins by lymphocytes [33]. Thus, Se-supplementation is beneficial to the immune response of heifers on the first few days of life, as already reported by Rowntree et al. [7] and Guyot et al. [6] in newborn calves from cows Se-supplemented before calving. On the contrary, other studies did not observe differences on serum IgG levels of heifers from cows Se-supplemented [9, 34]. These differences might be associated to several factors such as climate, diet and type of Se-supplementation (dose, form and administration route), and deserve further research since Se is one of the most important minerals for a fully functioning *immune system*.

Regarding ceruloplasmin and haptoglobin, seric levels of these acute-phase proteins (APPs) were higher in treated heifers. The APPs are produced in the hepatocytes after cytokine (TNF-α, IL-1β and IFN-γ) stimuli [35]. In this sense, it is possible that Se promoted an increase of IFN-γ, an important mediator of immune and inflammatory responses, increasing APPs content. In this sense, Tsuji et al. [36] demonstrated that mice Se-supplemented showed higher levels of cytokines, since IFN-γ and IL-6 were sensible to Se ingestion.

In this study, no difference was observed between groups regarding serum ROS and TBARS levels. However, individually, it was observed that animals with diarrhea showed higher levels of serum oxidant. However, it is important to emphasize that some animals of the control group did not face significant sanitary challenges, which may explain the absence of statistical difference between groups. Despite the high mortality and occurrence of diarrhea, the farm maintained hygienic conditions during the entire experiment. Thus, we believe that these

factors can explain the reduction on seric ROS and TBARS levels in both groups according to animal growth. Similarly, serum GST activity was similar in both groups, i.e., the treatment did not affect directly GST activity, an enzyme not Se-dependent [37]. However, it is important to highlight that serum GST activity increased in treated animals according to their development, which may be considered an indirect effect due to an enhanced immune response, as well as the activation of other antioxidant pathways not investigated in the present study.

The administration of sodium selenite and vitamins (A and E) did not alter seric levels of albumin, urea, cholesterol and triglycerides, with exception of glucose on day 60, as observed by Shinde et al. [38] using buffalos as the experimental model. Thus, the treatment did not interfer in the proteic and lipid metabolism. The augmentation on serum cholesterol levels in both groups was expected, as a result of the lipid metabolism development.

Overall, the hematocrit was higher in treated heifers compared to the control group. The hematocrit is the percentage of total blood volume occupied by erythrocytes, and Se deficiency causes anemia. Therefore, sodium selenite supplementation may prevent erythrocyte fragility (which was not evaluated), and thus avoid hemolysis. According to Kaushal et al. [39], Se is important in the regulation of erythrocytes homeostasis, which could explain this result.

Heifers that received sodium selenite and vitamins showed numerical weight gain in the lactation period, but significant differences between groups were observed only on day 210 of life. This result reinforces the knowledgement that heavier and healthier animals at weaning result in heavier animals in the adult life. However, newborn heifers from supplemented cows (with Se or Se associated to vitamin E) did not have higher weight gain [8,34,40]. Possibly, beneficial alterations on the immune system promoted improved animal health, and consequently, better performance and development in the growth phase.

5. Conclusion

Based on these results, we concluded that the treatment of Se and vitamins (A and E) associated to secnidazole prevented infections caused by *G. duodenalis* up to thirty days of life, although it did not influence bacterial count and the incidence of diarrhea. Moreover, this prophylactic treatment increased immunological markers (cellular and humoral) and weight gain, and thus, improved animal health.

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References

[1] Meganck, V., Hoflack, G., Opsomer, G., 2014. Advances in prevention and therapy of neonatal dairy calf diarrhea: a systematical review with emphasis on colostrum management and fluid therapy. Acta Vet. Scand. 56, 1-75. http://doi.org/10.1186/s13028-014-0075-x

[2] Trefz, F.M, Lorenz, I., Lorch, A., Constable, P.D., 2017. Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. PLoS ONE. 12, 8, e0182938. http://doi.org/10.1371/journal.pone.0182938.

[3] Pereira, R., 2017. Dairy calf treatment for diarrhea: are the drugs we use effective? WSU Extension. FS254E, 1-7.

[4] Hosnedlova, B., Kepinska, M., Skalickova, S., Fernandez, C., Ruttkay-Nedecky, B., Malevu, T.D., Sochor, J., Baron, M., Melcova, M., Zidkova, J., Kizek, R., 2017. A summary of new findings on the biological effects of selenium in selected animal species - A Critical Review. Int. J. Mol. Sci. 18, 2209, 1-47. http://doi.org/10.3390/ijms18102209

[5] Mehdi, Y., Dufrasne, I., 2016. Selenium in Cattle: A Review. Molecules. 21, 545, 1-14. http://doi.org/10.3390/molecules21040545

[6] Guyot, H., Spring, P., Andrieu, S., Rollin, F., 2007. Comparative responses to sodium selenite and organic selenium supplements in Belgian Blue cows and calves. Livest. Sci. 111, 259–263. http://doi.org/10.1016/j.livsci.2007.04.018

[7] Rowntree, J.E., Hill, G.M., Hawkins, D.R., Link, J.E., Rincker, M.J., Bednar, G.W., Kreft, R.A., Jr., 2004. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. J. Anim. Sci. 82, 2995–3005. http://doi.org/10.2527/2004.82102995x

[8] Salles, M.S.V., Zanetti, M.A., Junior, L.C.R., Salles, F.A., Azzolini, A.E.C.S., Soares, E.M., Faccioli, L.H., Valim, Y.M.L., 2014. Performance and immune response of suckling calves fed organic selenium. Anim. Feed Sci. Technol. 188, 28–35. http://doi.org/10.1016/j.anifeedsci.2013.11.008

[9] Moeini, M.M., Kiani, A., Mikaeili, E., Shabankareh, H.K., 2011. Effect of Prepartum Supplementation of selenium and vitamin E on serum Se, IgG concentrations and colostrum of heifers and on hematology, passive immunity and Se status of their offspring. Biol. Trace. Elem. Res. 144, 529–537. http://doi.org/10.1007/s12011-011-9148-0.

[10] Volpato, A., Machado, G., Tonin, A.A., Stefani, L.M., Campigotto, G., Glombowsky, P., Galli, G.M., Fávero, J.F., Da Silva, A.S., 2017 Gastrointestinal protozoa in dairy calves: identification of risk factors for infection. Rev. MVZ Cordoba. 21, 5909-5923.

[11] Guimarães, A.M., Guedes, E., Carvalho, R.A., 2001. Ocorrência de *Giardia* spp. em bezerros leiteiros no Brasil. Arq. Bras. Med. Vet. Zootec. 53, 652-653. http://dx.doi.org/10.1590/S0102-09352001000600005.

[12] Machado, P.R.L., Araújo, M.I.A.S., Carvalho, L., Carvalho, E.M., 2004. Mecanismos de resposta imune às infecções/Immune response mechanisms to infections. An. Bras. Dermatol. 79, 6, 647-664.

[13] Ural, K., Aysul, N., Voyvoda, H., Ulutas, B., Aldemir, O.S., Eren, H., 2014. Single dose of secnidazole treatment against naturally occuring *Giardia duodenalis* infection in Sakiz lambs. Rev. MVZ Córdoba. 19, 1, 4023-4032.

[14] Da Silva, A.S., Castro, V.S., Tonin, A.A., Brendler, S., Costa, M.M., Jaques, J.A., Bertoletti, B., Zanette, R.A., Raiser, A.G., Mazzanti, C.M., Lopes, S.T., Monteiro, S.G., 2011. Secnidazole for the treatment of giardiasis in naturally infected cats. Parasitol. Int. 60, 429–432. http://doi.org/10.1016/j.parint.2011.06.024.

[15] Franco, S.F., Silva, A.M.G., Garcia, T.I., Ramos, A.C., Colli, C.M., Pavanelli, M.F., 2015. Infecção por *Giardia intestinalis*: avaliação dos sinais clínicos e resistência medicamentosa em camundongos swiss. Rev. Saúde e Biol. 10, 23-33.

[16] Heinrichs, A.J., Hargrove, G.L., 1987. Standards of weight and height for Holstein heifers. J. Dairy Sci. 70, 653–660. http://doi.org/10.3168/jds.S0022-0302(87)80055-3

[17] Feldman, B.F., Zinkl, J.G., Jain, N.C., 2000. Schalm's Veterinary Hematology (5th ed.). Philadelphia: Lippincott Williams & Williams.

[18] Jentzsch, A.M., Bachmann, H., Fürst, P., Biesalski, H.K., 1996. Improved analysis of malondialdehyde in human body fluids. Free Radic. Biol. Med. 20, 2, 251-256. http://doi.org/10.1016/0891-5849(95)02043-8

[19] Ali, S.F., LeBel, C.P., Bondy, S.C., 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicol. 113, 637-648.

[20] Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 22, 7130-7139.

[21] Fagliari, J. J., Santana A.E., Lucas F.A., Campos Filho E., Curi P.R. 1998. Constituintes sanguíneos de bovinos recém-nascidos das raças Nelore (B*os indicus*) e Holandesa (*Bom taurus*) e de bubalinos (*Bubalis bubalus*) raça Murrah. Arq Bras Med Vet Zootec 50, 253-262.

[22] Monteiro, S.C., 2011. Parasitologia na Medicina Veterinária. São Paulo: Roca. 356p.

[23] Larson, L.L., Owen, F.G., Albright, J.L., Appleman, R.D., Lamb, R.C., Muller, L.D., 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60, 6, 989-991. http://doi.org/10.3168/jds.S0022-0302(77)83975-1

[24] Wickham, H., 2009. Elegant Graphics for Data Analysis. Springer, New York, USA.

[25] Gardner, T.B., Hill, D.R., 2001. Treatment of giardiasis. Clin. Microbiol. Rev. 14, 1, 114-128. http://doi.org/10.1128/CMR.14.1.114-128.2001

[26] Bock, A., Forchhammer, K., Heider, J., Leinfelder, W., Sawers, G., Veprek, B., Zinoni, F., 19991. Selenocysteine: the 21st amino acid. Mol. Microbiol. 5, 515–520. http://dx.doi.org/10.1111/j.1365-2958.1991.tb00722.x

[27] Gumus, R, Imik, H., 2016. Effects of vitamin E (α-tocopherol acetate) on serum lipid profile, Ca and P levels of broilers exposed to heat stress. Sch. J. Agric. Vet. Sci. 3, 105-110.

[28] Desai, I.D., Scott, M.L., 1965. Mode of Action of Selenium in Relation to Biological Activity of Tocopherols. Arch. Biochem. Biophys. 110, 309-315. http://doi.org/10.1016/0003-9861(65)90124-4

[29] Sushma, K., Reddy, Y.R., Kumari, N.N., Reddy, P.B., Raghunandan, T., Sridhar, K.. 2015. Effect of selenium supplementation on performance, cost economics, and biochemical profile of Nellore ram lambs. Vet. World. 8, 1150-1155.

[30] Hoffmann, P.R., Berry, M.J., 2008. The influence of selenium on immune responses. Mol. Nutr. Food Res. 52, 11, 1273–1280. http://dx.doi.org/10.1002/mnfr.200700330

[31] Fillit, H.M., Zabriskie, J.B., 1982. Cellular immunity in glomerulonephritis. Am J Pathol. 109, 2, 227–243.

[32] Spallholz, J.E., Boylan, L.M., Larsen, H.S., 1990. Advances in understanding selenium's role in the immune system. Ann N Y Acad Sci. 587, 123-39.

[33] Mckenzie, R.C., Rafferty, T.S., Beckett, G.J., 1998. Selenium: an essential element for immune function. Immunology Today. 19, 8, 342-345. https://doi.org/10.1016/S0167-5699(98)01294-8

[34] Kafilzadeh F., Kheirmanesh, H., Shabankareh, H.K., Targhibi, M.R., Maleki, E., Ebrahimi, M., Meng, G.Y., 2014. Comparing the Effect of Oral Supplementation of Vitamin E, Injective Vitamin E and Selenium or Both during Late Pregnancy on Production and Reproductive Performance and Immune Function of Dairy Cows and Calves. Scientific World Journal. 1-5. http://dx.doi.org/10.1155/2014/165841

[35] Gruys, E., Toussaint, M.J.M., Niewold, T.A., Koopmans, S.J., 2005. Acute phase reaction and acute phase proteins. J. Zhejiang Univ. Sci. B. 6, 11, 1045-1056. http://doi.org/10.1631/jzus.2005.B1045

[36] Tsuji, P.A., Carlson, B.A., Anderson, C.B., Seifried, H.E., Hatfield, D.L., Howard, M.T., 2015. Dietary Selenium Levels Affect Selenoprotein Expression and Support the Interferon-γ and IL-6 Immune Response Pathways in Mice. Nutrients. 7, 6529-6549. http://doi.org/10.3390/nu7085297.

[37] Brigelius-Flohe R., Maiorino M., 2013. Glutathione peroxidases. Biochim. Biophys. Acta. 1830, 3289–3303. http://doi.org/10.1016/j.bbagen.2012.11.020

[38] Shinde, P.L., Dass, R.S., Garg, A.K., 2009. Effect of vitamin E and selenium supplementation on haematology, blood chemistry and thyroid hormones in male buffalo (*Bubalus bubalis*) calves. J. Anim. Feed Sci. 18, 241–256. http://doi.org/10.22358/jafs/66388/2009

[39] Kaushal, N., Hegde, S., Lumadue, J., Paulson, R.F., Prabhu, K.S., 2011. The Regulation of Erythropoiesis by Selenium in Mice. Antioxid. Redox Signal. 14, 8, 1403-1412. http://doi.org/10.1089/ars.2010.3323

[40] Slavík, P., Illek, J., Rajmon, R., Zelený, T., Jílek, F., 2008. Selenium Dynamics in the Blood of Beef Cows and Calves Fed Diets Supplemented with Organic and Inorganic Selenium Sources and the Effect on Reproduction. Acta Vet. Brno. 77, 11–15. http://doi.org/10.2754/avb200877010011

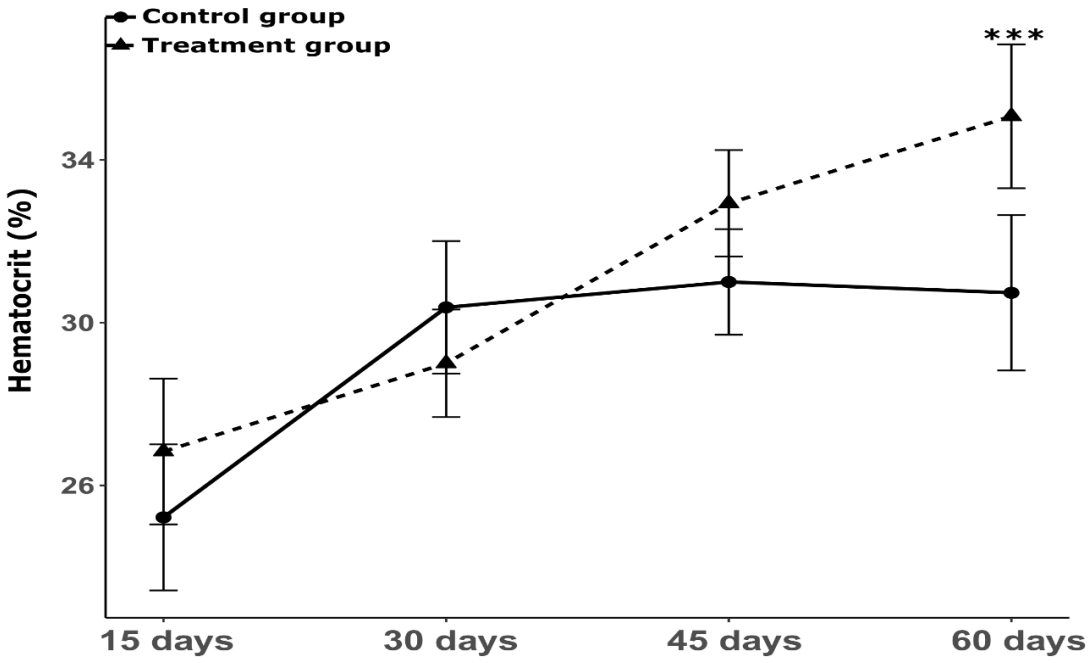

Figure 1: Hematocrit values of heifers from control and treated groups on days 15, 30, 45 and 60 of life. * P<0.05 indicates difference between groups.

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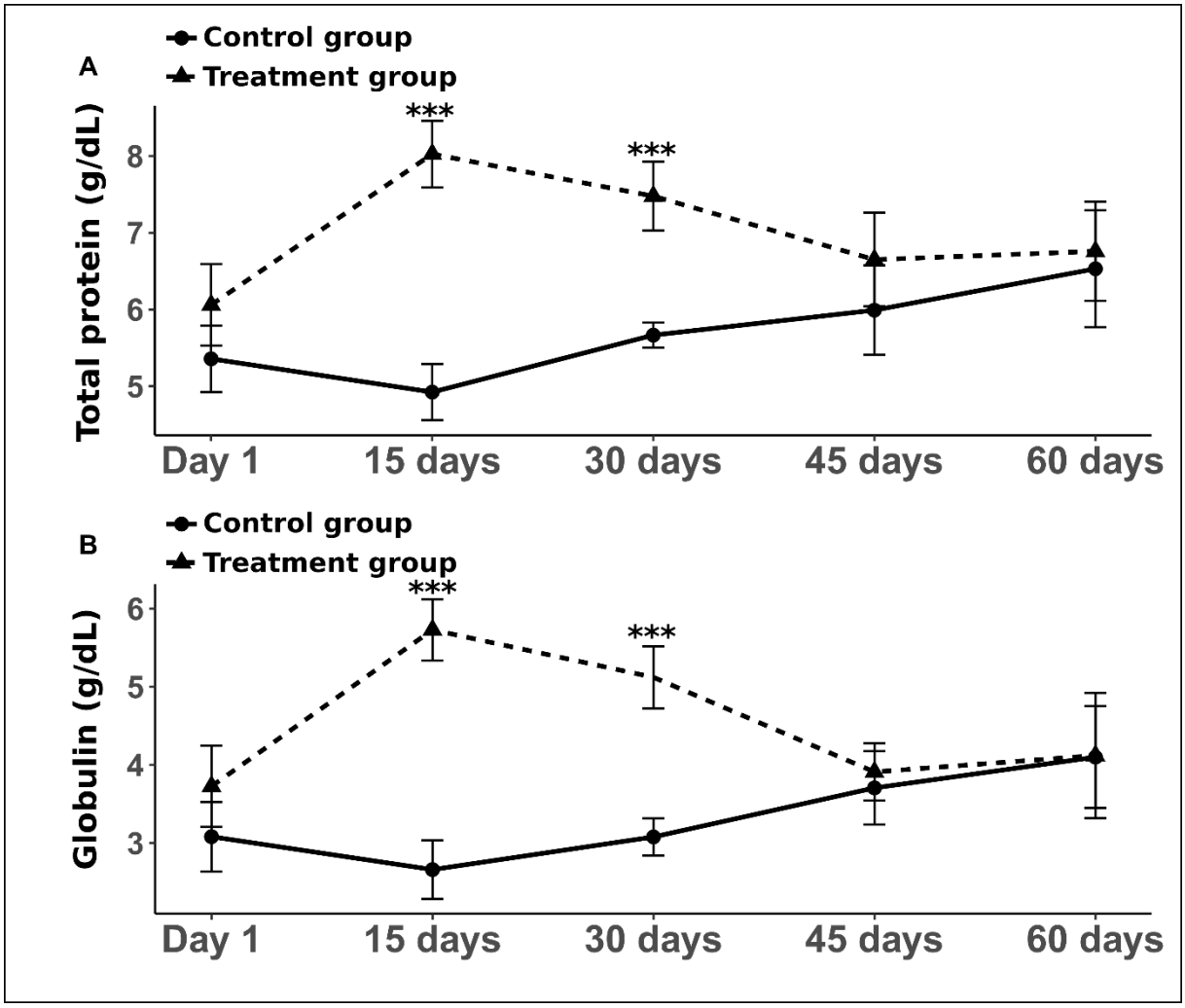

Figure 2: Serum levels of total protein [A] and globulin [B] of heifers from control and treated groups on days 1, 15, 30, 45 and 60 of life. * $P<0.05$ indicates difference between groups.

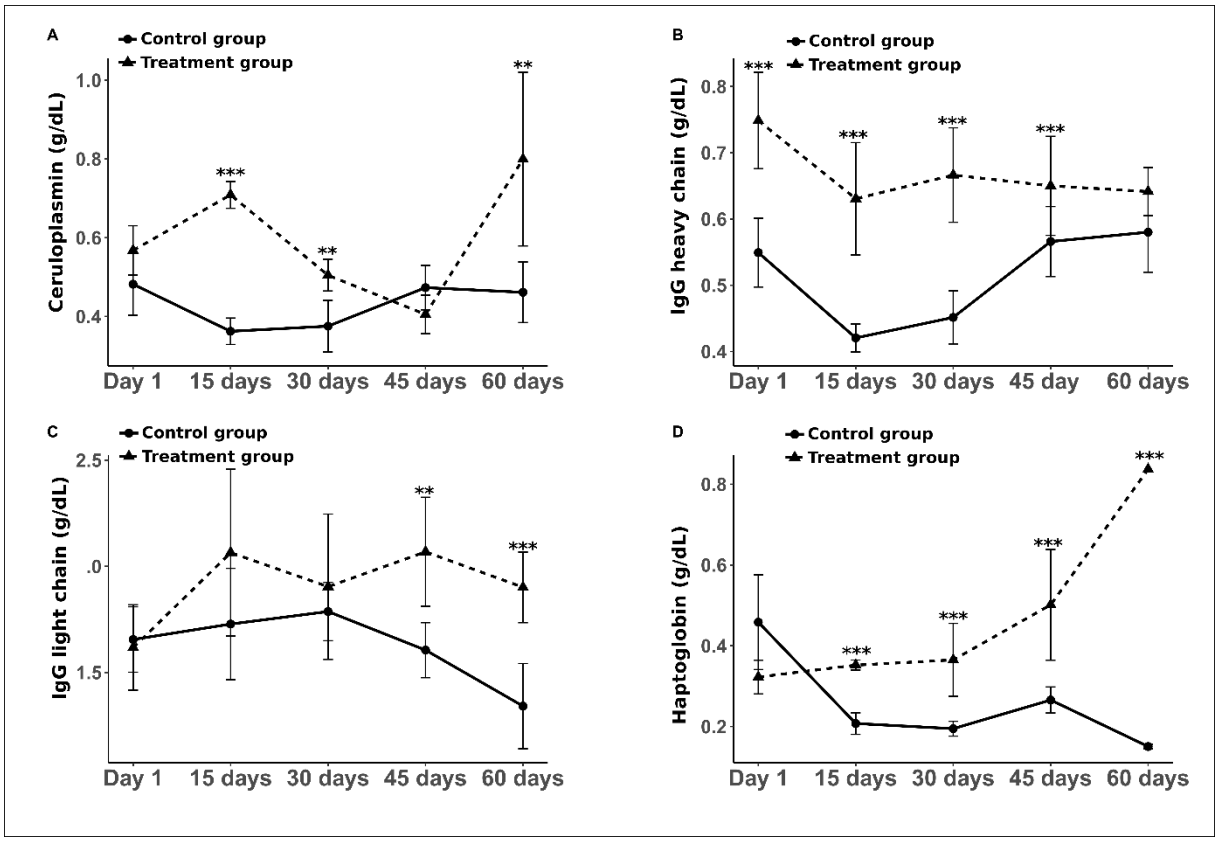

Figure 3: Ceruloplasmin [A], immunoglobulin G heavy chain (IgG) [B], IgG light chain [C] and haptoglobin [D] of heifers from control and treated groups on days 1, 15, 30, 45 and 60 of life. * P<0.05 indicates difference between groups.

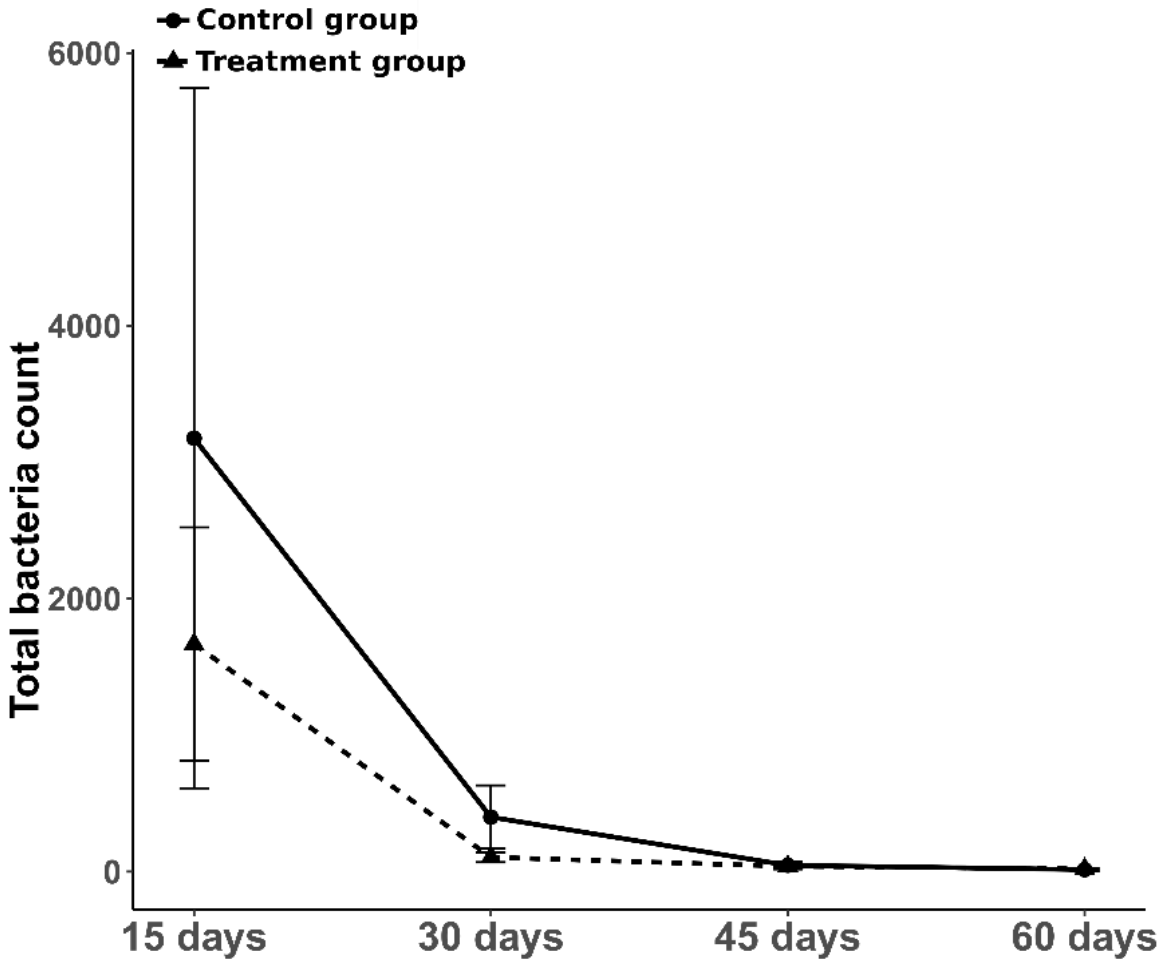

Figure 4: Total bacterial count in fecal samples of heifers from control and treated groups with 15, 30, 45 and 60 days of life.

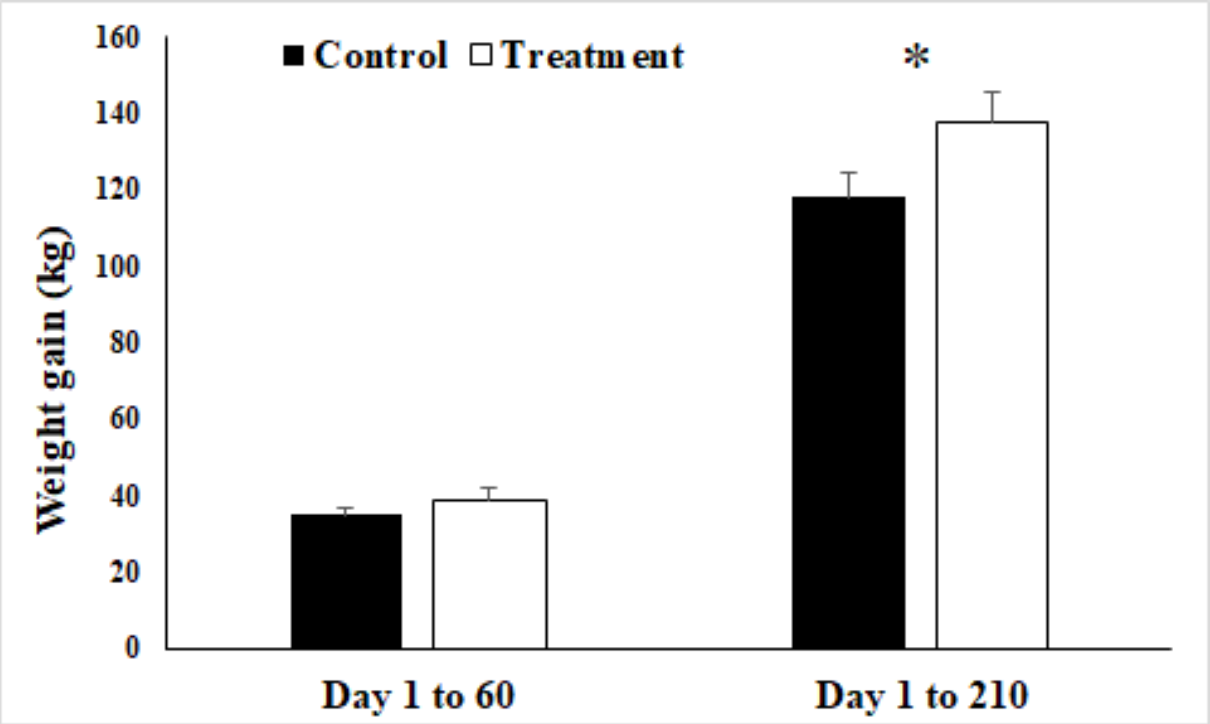

Figure 5: Weight gain between 1 and 60 days of life and between 1 and 210 days of life of heifers from control and treated groups. * $P<0.05$ indicates difference between groups.

Table 1: Serum biochemistry of heifers on days 1, 15, 30, 45 and 60 of life.

Variables	Age (days)	Control (N=15)	Treatment (N=15)	*p-value
Albumin (g/dL)	1	2.28 ±0.38	2.33 ±0.78	0.863
	15	2.26 ±0.46	2.30 ±0.41	0.882
	30	2.59 ±0.26	2.36 ±0.57	0.357
	45	2.29 ±0.42	2.74 ±0.79	0.210
	60	2.43 ±0.54	2.64 ±1.04	0.649
&p-value		0.562	0.123	
Urea (mg/dL)	1	34.6 ±18.4	28.0 ±9.31	0.248
	15	32.3 ±20.5	20.3 ±13.4	0.171
	30	31.6 ±15.1	26.0 ±10.3	0.266
	45	41.9 ±20.2	34.2 ±12.8	0.215
	60	44.3 ±32.8	31.7 ±8.11	0.168
&p-value		0.494	0.247	
Triglycerides (mg/dL)	1	35.5 ±13.0	27.4 ±10.1	0.085
	15	28.7 ±12.0	31.2 ±14.4	0.604
	30	33.3 ±15.2	32.4 ±16.7	0.892
	45	24.8 ±6.19	30.9 ±12.2	0.319
	60	29.6 ±7.84	29.4 ±12.9	0.960
&p-value		0.08	0.698	
Cholesterol (mg/dL)	1	31.4 ± 6.66^{C}	36.1 ± 10.9^{C}	0.301
	15	52.9 ± 13.7^{B}	65.7 ± 30.2^{B}	0.164
	30	75.4 ± 21.7^{A}	84.5 ± 20.9^{AB}	0.290
	45	74.3 ± 24.3^{A}	96.1 ± 26.6^{A}	0.074
	60	69.3 ± 29.7^{AB}	68.1 ± 26.1^{B}	0.915
&p-value		0.001	0.001	
Glucose (mg/dL)	1	85.1 ±19.0	68.4 ± 20.6^{C}	0.445
	15	118 ±23.4	123 ± 19.3^{A}	0.792
	30	103 ±22.9	98.5 ± 20.8^{AB}	0.583
	45	99.0 ±26.7	100 ± 21.0^{AB}	0.917
	60	111 ±23.3	85.9 ± 23.2^{BC}	0.046*
&p-value		0.257	0.001	

* P<0.05 in the same line indicates difference between groups. & P<0.05 in the same column indicates difference in the same group, as well as different letters (capital letters) indicate difference between sampling time considering the effect over time (repeated measures).

Table 2: Serum levels of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS) and glutathione S-transferase (GST) activity of heifers on days 1, 15, 30, 45 and 60 days of life.

Variables	Age (days)	Control (N=15)	Treatment (N=15)	p-value
ROS (U DCF/mL)	1	54.6 ±30.5^{A}	60.6 ±28.0^{A}	0.767
	15	31.5 ±11.4^{AB}	37.5 ±18.6^{AB}	0.307
	30	28.9 ±11.2^{AB}	34.5 ±15.8^{AB}	0.302
	45	20.3 ±6.34^{BC}	18.1 ±4.23^{C}	0.292
	60	17.6 ±6.52^{C}	19.8 ±7.91^{BC}	0.411
$^{\&}$p-value		0.001	0.001	
TBARS (µm MDA/mL)	1	0.40 ±0.46^{A}	0.32 ±0.11^{A}	0.424
	15	0.15 ±0.05^{B}	0.15 ±0.06^{B}	0.851
	30	0.14 ±0.05^{B}	0.15 ±0.07^{B}	0.753
	45	0.13 ±0.04^{B}	0.12 ±0.03^{B}	0.329
	60	0.14 ±0.08^{B}	0.13 ±0.04^{B}	0.779
$^{\&}$p-value		0.001	0.001	
GST (µmol Cdnb/min/mL)	1	16.0 ±7.19	16.2 ±6.31^{C}	0.268
	15	18.2 ±6.84	16.8 ±7.90^{BC}	0.320
	30	17.0 ±0.07	18.4 ±7.09^{BC}	0.767
	45	16.5 ±6.60	23.3 ±7.72^{AB}	0.099
	60	18.2 ±8.15	26.2 ±6.38^{A}	0.092
$^{\&}$p-value		0.797	0.001	

* P<0.05 in the same line indicates difference between groups. $^{\&}$P<0.05 in the same column indicates difference in the same group, as well as different letters (capital letters) indicate difference between sampling time considering the effect over time (repeated measures).

2.4 MANUSCRITO III

A mix of essential oils as feed additive for dairy calves reduce fecal bacterial counts and enhance growth

Running Head
Essential oils as feed additive for dairy calves

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Abstract

Dairy animals suffer with several problems linked to sanitary problems, which may lead to high mortality and severe economic losses to farmers. Therefore, the aim of this study was to evaluate whether the addition of a commercial product based on a mixture of essential oils in the diet of Holstein calves could exert beneficial effects, such as prevention of protozoans and bacterial infections, and consequently, reduction in the incidence of diarrhea and improvement of body development before mating. Thirty newborn calves were randomly divided into two groups (control and treated). The treatment consisted of 10g of the product composed of carvacrol, cinnamaldehyde, eucalyptus aroma and paprika oleoresin diluted in milk once a day for 30 days, while the control received a diet without the supplementation. Blood and fecal samples were collected, and fecal score was performed daily in order to determine the occurrence of diarrhea. Body weight was evaluated in three moments: 1, 60 and 210 days of age. Treated cows showed higher hematocrit on days 45 and 60 of life compared to the control group. Serum levels of urea, total proteins, globulins and glucose were lower in the treated group on day 60 of life, while triglycerides were lower on days 15 and 60 of life compared to the control group. Serum glutathione S-transferase activity was higher in treated animals on day 60 of life. Fecal bacterial count was lower in treated animals on days 30 and 60 of life compared to the control. The weight gain was higher in treated animals on days 60 and 210 of life compared to the control group. Based on these results, it is possible to conclude that the addition of this commercial product composed of essential oils was able to minimize fecal bacterial infections and to increase body weight gain of calves.

Keywords: carvacrol, phytotherapy, cinnamaldehyde, bacterial action, additive, supplementation.

1. Introduction

Essential oils are secondary metabolic of plants used as an interesting alternative to improve animal production (Benchaar et al., 2008; Chouhan et al., 2017) due to their antibacterial, antifungal, anticoccidian and antioxidant properties (Miguel, 2010; Lee, 2004; Kalemba and Kunicka, 2003). Some essential oils, such as *Melaleuca alternifolia*, have potent antibacterial effects against *Escherichia coli* via the denaturation of membrane proteins, which leads to the rupture of the external membrane, respiratory inhibition, and therefore, cell death (Kalemba and Kunicka, 2003). However, essential oils are composed by different molecules derived from terpenoids or phenylpropanoids (Simões, 1999). As an example, oregano essential oil is composed mainly by carvacrol (80 %), a monoterpene with antioxidant and antibacterial

properties (Jayakumar et al., 2012), as observed for cinnamaldehyde (Helander et al., 1998; Barnwal et al., 2017).

Essential or their compounds can be used as feed additives for dairy calves. Newborn animals are highly susceptible to infections, mainly by gastrointestinal infections caused by bacteria and protozoans, which cause diarrhea, and consequently, animal death (Wattiaux, 2012). In this sense, the elimination of pathogenic bacteria, the reduction of diarrhea and the improvement of the antioxidant system can contribute to enhance animal health and growth rates, promoting earlier physical and sexual maturity. Overall, the recommended age for Holstein calf´s first parturition is between 22 and 24 months with a body weight of approximately 300 kg (USDA, 2007). However, this practice is not observed in Brazil and other developed countries where the average age for the first parturition in the USA is 25.2 months (USDA, 2007). However, this reality impairs producers to have good financial return. Based on these evidences, it is interesting that the animals reach the ideal weight for reproduction as soon as possible (Radcliff et al., 2000). Therefore, the aim of this study was to evaluate whether the addition of a commercial product based on components of essential oils in the diet exerts beneficial effects on calf´s health, as well as in the prevention of protozoans or bacterial infections, and consequently, reduction in the incidence of diarrhea and higher animal body weight during the early days of life.

2. Materials and Methods

2.1 Product

The commercial mixture of components of essential oils (Activo Calf®) used in this study was produced by Grasp® (Curitiba, Brazil) and sold as a powder. This product is based on components of the following essential oils: carvacrol, cinnamaldehyde, eucalypt aroma and paprika oleoresin.

2.2 Animals and experimental design

This study was conducted in a commercial dairy farm located in Xanxerê city (Santa Catarina, Brazil) selected due to its optimal facilities and management, despite high sanitary challenge and animal mortality (neonatal mortality of approximately 25 %.), i.e., the presence of preventive measures was unable to prevent the occurrence of diarrhea and pneumonia.

Thirty newborn calves (Holstein with average weight of 42.04 ± 3.50 kg) were used as the experimental model. It is important to emphasize that animals were monitored until weaning, which occurred on day 60 of life. Newborn heifers were housed in individual stalls

and received two liters of colostrum (quality evaluated using the refractometer type Brix, considering a limit of 21 %, i.e., > 50 mg of Immunoglobulins/mL) up to the first six hours after birth. In addition, the animals received three liters of milk twice a day, with access to concentrate and water *ad libitum*. With 15 ± 3 days of life, the heifers were grouped in collective stalls with the capacity of 15 animals, with automatic milk feeding (6 liters per day), concentrate, Tifton 85 hay (10.7 % crude protein) and water *ad libitum.*

The nutritional and chemical composition of the concentrate are shown in Table 1. The concentrate was produced in the farm and it was mainly composed of corn bran and soybean meal.

The calves were randomly divided into two groups (control and oil-treated) of 15 animals each. Treated animals received 10 g of the product diluted in milk from birth up to 30 days of life twice a day via bottle.

2.3. Diarrhea incidence and body weight

The occurrence of diahrrea was observed daily following the methodology described by Larson et al. (1977), which is based on fecal score and fluidity: (1) normal and solid; (2) pasty but with health aspect; (3) aqueous consistency and (4) fluid consistency.

The body weight was measured through the correlation of the thoracic perimeter using a measure tape (Heinrichs and Hargrove, 1987) on days 1 and 60 of life (experimental period) and on day 210 of life (growth period).

2.4. Sample collection

The first blood collection occurred in the first 24 hours of life, after colostrum consumption (day 1), while the other samplings (blood and feces) were performed on days 15, 30, 45 and 60 of life. Blood collection was performed directly from the jugular vein using tubes containing EDTA (10 %) for hematocrit and into tubes without this anticoagulant. Samples collected into tubes without anticoagulant were centrifuged at 7000 rpm during 10 min to obtain sera which were transferred to microtubes and stored at -20 ºC until utilization.

Fecal samples were obtained directly from the rectal ampule, which were stored at 8 ºC until utilization. One portion of each fecal sample was used for bacterial counts, and the other portion for protozoan research.

2.5. Hematocrit

The hematocrit was performed up to 2 h after blood collection. The microhematocrit

was performed using capillaries tubes after a centrifugation at 10000 rpm during 3 min. The percentage of erythrocytes was evaluated using a reading card, as preconized by Feldman et al. (2000).

2.6. Serum biochemistry

Seric levels of total protein, albumin, urea, glucose, cholesterol and triglycerides were measured through the semi-automated analyzer BioPlus 2000® using commercial kits (Analisa Gold®) following manufacturer´s recommendations. The globulin levels were obtained by mathematic calculi (total protein – albumin).

2.7. Serum antioxidant/oxidant status

Serum lipid peroxidation was determined according to the methodology described by Jentzsch et al. (1996), which measures the levels of malondialdehyde (MDA) by thiobarbituric acid reactive substance (TBARS), and results are expressed as µm MDA/mL.

The serum ROS levels were quantified by fluorimetric method of diacetate dichlorofluorescein (DCFH-DCF) (Ali et al., 1996), and the results were expressed as U DCF/mL

The activity of the antioxidant enzyme glutathione S-transferase (GST) was analyzed in serum using the method described by Habig et al. (1974), and the results were expressed as µmolCdnb/min/mL.

2.8. Total bacterial count

A portion of fresh fecal sample was used to perform the total bacterial count. For this, 1 gram of feces was diluted in 9 mL of buffered peptone water, followed by seven more seriated dilutions. From dilution seven and eight, aliquots of 1 mL were incubated into total aerobic count plates (3M Petrifilm® Plate). This plate is a system of culture medium ready for use which contains all nutrients of the standard method, as well as an indicator that facilitates colony counting and the results were expressed as CFU/g of feces.

2.9. Parasitological examination

Parasitological examination was performed to determine the presence *Cryptosporidium* spp. and *Eimeria* spp. oocysts, as well as cysts of *Giardia duodenalis.* For this, 2 g of feces were processed using the centrifugal-flotation technique with a hypersaturated sugar solution, according to the methodology described by Monteiro (2010).

2.10. Statistical analysis

The data from 30 animals were firstly evaluated by descriptive statistics for contingency of information and for further assumptions which are presented as mean and standard deviation. All covariates were tested for normality using the Shapiro-Wilk test (Shapiro-Wilk, 1965), and the skewness, kurtosis and homogeneity by the *Levene* test. The data did not show normal distribution, and were transformed to logarithm and normalized, with the exception of the total bacterial count data. A repeated measure t-test was used to examine differences of the parameters between control and treated groups. All parameters compared mean between groups ia the Student's t-test (controlling for data dependency due to dependence in time) was used to analyze all parameters at each time period (days 1, 15 and 30, 45 and 60 of the experiment). For total bacterial counts a nonparametric test was used by Kruskal-Wallis test. The chi-square test was used to verify the occurrence of diarrhea and the presence of parasites (*Giardia* and *Eimeria*) between groups. All plots were visualized using the ggplot2 package (Wickham, 2009). It was considered significantly different when $P<0.05$. The statistical process was performed using R-language, v.2.15.1 (R Development Core Team 2012).

3. Results

3.1. Weight and weight gain

No difference was observed between groups regarding body weight on days 1, 60 and 210 of life. However, higher weight gain was observed on days 1 to 60, and 1 to 210 on treated animals compared to the control group (Figure 1). On day 210 of life, control and treated animals showed a daily weight gain of 569 and 641 g, respectively.

3.2. Hematocrit and seric biochemistry

On days 45 and 60 of life it was observed a significant difference between groups regarding hematocrit (Table 2). However, hematocrit was not affected in animals of both groups over the time ($P>0.05$).

The serum levels of urea, total proteins and globulins were lower in the treated group on day 60 of life compared to the control group, while albumin levels were not different (Table 2). Over the time, the total protein and globulin levels increased in the control group from days 1 to 60.

The serum levels of glucose, triglycerides and cholesterol are shown in the Table 2. No difference was observed between groups regarding cholesterol levels in all evaluated moments. On the other hand, serum levels of glucose were lower in treated animals on day 60 of life,

while triglycerides levels were lower on days 15 and 60 of live compared to the control group. No difference was observed over the time for all evaluated parameters.

3.3. Oxidants and glutathione S-transferase activity

No difference between groups was observed regarding ROS and TBARS levels. However, the serum GST activity was higher in treated animals on day 60 of live compared to the control group. Over time, serum levels of ROS reduced in both groups on days 1 to 45 of life ($P = 0.04$; $P = 0.001$) and 1 for 60 ($P = 0.04$; $P = 0.001$), respectively (Table 3).

3.4. Fecal bacterial count

Fecal bacterial counts were lower in treated animals on days 30 and 60 of life compared to the control group (Figure 2). Over time, it was observed a numerical reduction on bacterial counts of both groups. The reduction was significant on days 30 to 45 in the control group ($P = 0.036$) and on days 45 and 60 in treated animals.

3.5. Parasitological examination

No difference was observed between groups regarding the presence of protozoans. However, it was verified that seven control animals and three treated animals were positives for *Giardia* spp. On day 30, infections caused by *Giardia* spp. were observed in four animals from the control group, as well as two positive animals for *Eimeria* spp. In this same period, only two animals were positives for *Giardia* spp in the treated group. At 45 days of life, infection by *Giardia* spp. and *Eimeria* spp. was observed in 10 animals of each group. On day 60 of life, infections caused by both parasites remained in the control group ($n = 7$ animals), while the treated group showed only *Eimeria* spp. ($n = 3$).

3.6. Diarrhea incidence

No difference was observed between groups regarding the incidence of diarrhea. However, it is important to emphasize that this clinical sign was observed in 73.3 % of control animals and 40 % of treated animals on day 15 of life. On day 30, diarrhea was observed in 1 or 2 animals per group.

4. Discussion

Animals treated with essential oil obtained higher weight gain, which can be a direct or indirect

effect of the treatment. Indirectly, this weight gain can be linked to improved animal health due to the stimulation of the immune and antioxidant systems, as well as in the reduction of fecal bacterial counts. Directly, it can be associated with higher consumption of the concentrate stimulated by the oil product, a fact proved by a positive correlation between weight gain and concentrate levels (Bartle et al., 1994). In this sense, some studies indicated that the use of oregano and/or cinnamon essential oils as additives improve piglets performance (Franz et al., 2009).

Origanum vulgare (oregano) essential oil has the capability to induce a significant increase on GST activity (Lam and Zheng, 1991), in accordance to what was observed in this study. However, this oil has monoterpene carvacrol as its main compound (Jayakumar et al., 2012), which exerts a potent antioxidant effect (Barnwal et al., 2017). Although this treatment caused a stimulation of the antioxidant system, no effects were observed regarding levels of free radicals and lipid peroxidation.

In piglets, the addition of essential oils reduced the occurrence of diarrhea and decreased the number of *Escherichia coli* in fecal samples (Li et al., 2012). According to the literature, the carvacrol and cinnamaldehyde, which are components of the cinnamon oil, exert an inhibitory effect against *E. coli* and *Salmonella* Typhimurium (Helander et al., 1998; Friedman et al., 2004), important diarrheic agents. According to Helander et al. (1998), the action mechanism involved in carvacrol against *E. coli* is associated with disintegration of cell membrane and release of organelles in the extracellular medium. In this present study, the animals treated with the commercial product showed lower fecal bacterial counts, which can be related to an antibacterial action of the components present in this product. Even though it was not evaluated in this study, Giannenas et al. (2003) demonstrated that *O. vulgare* essential oil has potent anticoccidian effects in birds experimentally infected by *Eimeria tenella.* The hematocrit count was higher in treated animals on days 45 and 60 of life, in agreement with Al-Kassie et al. (2009) while studying broilers fed with a diet supplemented with cinnamon oil. On the other hand, this result was not observed in birds (Hashemipour et al., 2013; Saadat Shad et al., 2016) and fish (Ahmadifar et al., 2011; Yilmaz et al., 2015) fed with thymol or carvacrol.

Serum urea, total proteins and globulins levels were lower in treated animals on day 60 of life, as observed by Al-Kassie et al. (2009) in broilers that received cinnamon oil. On the other hand, levels of serum total protein were not influence by the addition of cinnamon oil in the diet of sheep (Khateri et al., 2016). The serum triglycerides levels were lower in the treated group on days 15 and 60 of life, which disagrees with Saadat Shad et al. (2016), that did not observe differences in birds treatment with carvacrol and thymol.

Serum glucose levels were lower in treated animals on day 60 of live, in disagreement with Santos et al. (2015). According to these authors, serum glucose levels were not affected in calves supplemented with a product based on essential oils. Possibly the animals from the treated group consumed preferably concentrate, while the control group consumed a liquid diet. According to Quingley et al. (1991) and Santos et al. (2015), animals that consume liquid diet showed major concentrations of glucose in comparison to those receiving solid diets, since these animals use fatty acids of short chain as a source of energy. However, we cannot assure that the essential oils influenced the consumption of concentrate, since animal consumption was not a parameter evaluated in this study.

5. Conclusion

Based on these evidences, we concluded that the addition of a commercial product composed on components of essential oils in the diet of calves was able to minimize bacterial infections and to improve their overall health during lactation, as well as it caused weight gain and enhanced animal performance. Therefore, it can be considered a potential additive to be used in the diet since affected positively the animal growth, as well as exerted antimicrobial and antioxidant effects.

Ethics Committee

This study was approved by the Ethical and Animal Welfare Committee of the Universidade do Estado de Santa Catarina (UDESC) under protocol number 4964301116.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Ahmadifar, E.; Falahatkar, B.; Akrami, R. (2011). Effects of Dietary Thymol-Carvacrol on Growth Performance, Hematological Parameters and Tissue Composition of Juvenile Rainbow Trout, *Oncorhynchus mykiss*. *Journal of Applied Ichthyology*, 27, 1057-1060.

Al-Kassie, G. A. M. (2009). Influence of two plant extracts derived from thyme and cinnamon on broiler performance. *Pakistan Veterinary*, 29, 169–173.

Bartle, S. J.; Preston, R. L.; Miller, M. F. (1994). Dietary energy sources and density: effects of roughage equivalent, tallow level, and steer type on feedlot performance and carcass characteristics. *Journal Animal Science*, 72, 8, 1943-1953.

Barnwal, P.; Vafa, A.; Afzal, S. M.; Shahid, A.; Hasan, S. K., Alpashree, S.; Sultana, S. (2017). Benzo(a)pyrene induces lung toxicity and inflammation in mice: prevention by carvacrol. *Human and Experimental Toxicology*, 1-10.

Benchaar, C.; Calsamiglia, S.; Chaves, A. V.; Fraser,G. R.; Colombatto, D.; McAllister, T. A.; Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science. and Technology*, 145, 1-4, 209-228.

Chouhan, S.; Sharma, K.; Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines*, 4, 58, 1-21.

Feldman, B. F.; Zinkl, J. G.; Jain, N. C. (2000). *Schalm's Veterinary Hematology* (5th ed.). Philadelphia: Lippincott Williams & Williams.

Friedman, M.; Buick, R.; Elliott, C.T. (2004). Antibacterial activities of naturally occurring compounds against antibiotic-resistant Bacillus cereus vegetative cells and spores, Escherichia coli, and *Staphylococcus aureus*. *Journal Food Prot*, 67, 1774–1778.

Franz, C.; Baser, K. H. C.; Windisch, W. (2010). E ssential oils and aromatic plants in animal feeding – a European perspective. A review. *Flavour Fragr. Jornal*, 25, 327–340.

Giannenas, I.; Florou-Paneri, P.; Papazahariadou, M.; Christaki, E.; Botsoglou, N. A.; Spais, A. B. (2003). Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. *Archives of Animal Nutrition*, 57, 2, 99-106.

Hashemipour, H.; Kermanshahi, H.; Golian, A.; Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry Science*, 92, 8, 2059-2069.

Heinrichs, A. J.; Hargrove, G. L. (1987). Standards of weight and height for Holstein heifers. *Journal Dairy Science*, 70, 653–660.

Helander, I. M.; Alakomi, H.; Latva-Kala, K.; Mattila-Sandholm, T.; Pol,I.; Smid, E. J.; Gorris, L. G. M.; Von Wright A. (1998). Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *Journal Agriculture Food Chemistry,* 46, 3590−3595.

Jayakumar, S.; Madankumar, A.; Asokkumar, S.; Raghunandhakumar, S; Gokula dhas, K.; Kamaraj, S.; Divya, M. G.; Devaki, T. (2012). Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. *Molecular Cellular Biochemistry*, 360, 51–60.

Kalemba, D., & Kunicka, A. (2003). Antibacterial and Antifungal Properties of Essential Oils. *Current Medicinal Chemistry*, 10, 813-829.

Khateri, N.; Azizi, O.; Jahani-Azizabadi, H. (2017). Effects of a specific blend of essential oils on apparent nutrient digestion, rumen fermentation and rumen microbial populations in sheep fed a 50:50 alfalfa hay:concentrate diet. *Asian-Australasian Journal Animal Science*, 30, 3, 370-378.

Lam, L. K. T., & Zheng, B. (1991) Effects of Essential Oils on Glutathione S-Transferase Activity in Mice. *Journal Agriculture Food Chem*istry, 39, 660-662.

Larson, L. L.; Owen, F. G.; Albright, J. L.; Appleman, R. D.; Lamb, R. C.; Muller, L. D. (1977) Guidelines toward more uniformity in measuring and reporting calf experimental data. *Journal Dairy Science*, 60, 6, 989-991.

Lee, K. W.; Everts, H.; Beynen, A. C. (2004). Essential oils in broiler nutrition. *International Journal of Poultry Science*, 3, 12, 738-752.

Li, S. Y.; Ru, Y. J.; Liu, M.; Xu, B.; Péron, A.; Shi, X. G. (2012). The effect of essential oils on performance, immunity and gut microbial population in weaner pigs. *Livestock Science*, 145, 119–123.

Miguel, M. G. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules,* 15, 9252-9287.

Monteiro, S. C. (2011). *Parasitologia na Medicina Veterinária.* São Paulo: Roca. 356p.

Quigley, J. D., & Bernard, J. K. (1992). Effects of nutrient source and time of feeding on changes in blood metabolites in young calves. *Journal Animal Science,* 70, 1543–1549.

Radcliff, R. P.; Vandehaar, M. J.; Chapin, L. T.; Pilbeam, T. E.; Beede, D. K.; Stanisiewski, E. P.; Tucker, H. A. (2000). Effects of Diet and Injection of Bovine Somatotropin on Prepubertal Growth and First-Lactation Milk Yields of Holstein Cows. *Journal of Dairy Science,* 83, 1.

Saadat Shad, H.; Mazhari, M.; Esmaeilipour, O.; Khosravinia, H. (2016). Effects of Thymol and Carvacrol on Productive Performance, Antioxidant Enzyme Activity and Certain Blood Metabolites in Heat Stressed Broilers. *Iranian Journal of Applied Animal Science*, 6, 195-202.

Simões, C. M. O. (1999). *Farmacognosia: da planta ao medicamento*, Porto Alegre: Ed. Universidade/UFRGS; Florianópolis: Ed. da UFSC.

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

Wattiaux, M. A. (2012). *Essenciais em Gado de Leite—Criação de Novilhas.* The Babcock Institute: University of Wisconsin-Madison. p. 121-124.

Wickham, H. (2009). *Elegant Graphics for Data Analysis*. Springer, New York, USA.

Yilmaz, E.; Ergün, S.; Yilmaz, S. (2015). Influence of Carvacrol on the Growth Performance, Hematological, Non-Specific Immune and Serum Biochemistry Parameters in Rainbow Trout (*Oncorhynchus mykiss*). *Food and Nutrition Sciences,* 6, 523-531.

Table 1: Nutritional and chemical composition of the concentrate.

Ingredients	Quantity (%)
Corn bran (% DM*)	45.0
Soybean meal (% DM)	33.0
Soybean hull (% DM)	16.0
Premix (% DM)[1]	6.0
Chemical composition	**Quantity**
DM (kg)	500
CP (% DM)	22.6
Ether extract (% DM)	2.9
NDF (% DM)	188.4
ADF (% DM)	119.7

* Dry matter (DM), crude protein (CP), NDF (neutral detergent fiber), ADF (acid detergent fiber). [1] Premix composition: calcium (135-165 g/kg), phosphorus (70 g/kg), sulfur (25 g/kg), magnesium (25 g/kg), potassium (30 g/kg), cobaltous (4.3 mg/kg), copper (425 mg/kg), chrome (25 mg/kg), iron (1750 mg/kg), iodine (11 mg/kg), manganese (1700 mg/kg), selenium (13 mg/kg), zinc (1700 mg/kg), biotin (1.5 mg/kg), vitamin A (350000 UI/kg), vitamin D3 (25000 UI/kg), vitamin E (2000 UI/kg), pantothenic acid (126 mg/kg), vitamin B1 (50 mg/kg), vitamin B6 (60 mg/kg), vitamin B12 (1.11 mg/kg), choline (9000 mg/kg), niacin (247.50 mg/kg), riboflavin (50 mg/kg), vitamin C (6000 mg/kg), vitamin K (20 mg/kg), D-limonene (3300 mg/kg), *Saccharomyces cerevisiae* (0.75×10^9 UFC/kg), fluorine (700 mg/kg) and bicarbonate (135 g/kg).

Table 2: Hematocrit (total blood), urea, total proteins, albumin, globulins, glucose, triglycerides and cholesterol in serum of calves not treated (control) and treated with essential oils.

Variable	Day	Control	Treated	P-value
Hematocrit (%)	1	28.6 ± 1.15	29.6 ± 7.6	0.870
	15	25.2 ± 6.70	26.7 ± 8.0	0.972
	30	29.6 ± 5.90	29.5 ± 4.0	0.822
	45	30.4 ± 4.80	35.1 ± 2.9	0.042*
	60	30.6 ± 5.19	35.5 ± 2.5	0.027*
Urea (mg/dL)	1	30.5 ± 8.40	28.2 ± 7.4	0.742
	15	23.1 ± 4.00	25.8 ± 10.1	0.650
	30	28.7 ± 10.6	21.2 ± 6.6	0.146
	45	37.8 ± 12.7	27.6 ± 6.7	0.087
	60	36.10 ± 6.1	24.8 ± 7.7	0.010*
Total proteins (g/dL)	1	5.60 ± 1.31	6,4 ± 3.1	0.546
	15	7.00 ± 1.50	5.7 ± 1.9	0.649
	30	7.22 ± 2.20	6.11 ± 1.57	0.334
	45	7.53 ± 1.58	6.2 ± 1.4	0.092
	60	8.33 ± 1.68	5.97 ± 1.18	0.001*
Albumin (g/dL)	1	2.07 ± 0.56	2.12 ± 0.88	0.733
	15	2.74 ± 0.53	2.57 ± 1.22	0.640
	30	2.78 ± 0.52	2.71 ± 0.90	0.970
	45	3.11 ± 0.48	2.65 ± 1.0	0.270
	60	2.85 ± 0.60	2.46 ± 0.86	0.481
Globulins (g/dL)	1	3.60 ± 1.44	4.7 ± 2.68	0.240
	15	4.26 ± 1.56	3.4 ± 2.26	0.470
	30	4.63 ± 1.90	3.98 ± 1.57	0.554
	45	4.61 ± 1.94	3.95 ± 2.2	0.424
	60	5.66 ± 1.87	3.72 ± 1.1	0.01*
Glucose (mg/dL)	1	90.9 ± 36.70	105.80 ± 37.70	0.249
	15	125.3 ± 60.8	109.60 ± 31.10	0.462
	30	103.2 ± 23.60	97.30 ± 37.00	0.639
	45	99.00 ± 33.30	95.23 ± 36.0	0.528
	60	110.7 ± 34.5	78.07 ± 15.1	0.038*

	1	35.20 ± 13.00	34.70 ± 19.9	0.704
	15	28.60 ± 12.20	16.40 ± 5.22	0.044*
Triglycerides	30	33.2 ± 15.70	21.70 ± 8.24	0.071
(mg/dL)	45	35.9 ± 10.70	27.60 ± 7.60	0.130
	60	36.0 ± 6.15	24.80 ± 7.70	0.029*
	1	21.42 ± 6.60	32.50 ± 14.60	0.187
	15	51.7 ± 12.70	56.70 ± 29.30	0.732
Cholesterol	30	72.8 ± 20.6	62.10 ± 18.20	0.334
(mg/dL)	45	79.50 ± 22.00	58.60 ± 17.60	0.199
	60	72.00 ± 28.8	66.60 ± 21.40	0.527

* P<0.05 in the same line indicates significant difference between groups.

Table 3: Levels of reactive oxygen species (ROS), and thiobarbituric reactive acid substances (TBARS), and gluthatione S-transferase (GST) activity in serum of calves from control and treated groups.

Variable	Days	Control	Treated	P-value
ROS (U DCF/mL)	1	44.2 ± 22.9	57.7 ± 20.5	0.393
	15	31.5 ± 11.3	29.9 ± 13.1	0.870
	30	28.8 ± 11.5	25.9 ± 12.2	0.632
	45	20.2 ± 6.5	16.9 ± 4.1	0.155
	60	18.7 ± 4.9	18.1 ± 2.7	0.789
TBARS (µm MDA/mL)	1	4.0 ± 4.5	4.6 ± 6.2	0.840
	15	1.4 ± 0.4	1.3 ± 0.3	0.804
	30	1.4 ± 0.4	1.4 ± 0.4	0.974
	45	1.29 ± 0.4	1.3 ± 0.4	0.903
	60	1.4 ± 0.7	1.11 ± 0.3	0.794
GST (µmol Cdnb/min/mL)	1	16.0 ± 7.1	19.5 ± 7.3	0.740
	15	27.3 ± 14.9	21.8 ± 9.8	0.279
	30	17.2 ± 7.4	21.3 ± 8.6	0.538
	45	17.6 ± 7.2	21.5 ± 4.1	0.431
	60	16.4 ± 6.7	23.4 ± 6.08	0.040*

* $P<0.05$ in the same line indicates significant difference between groups.

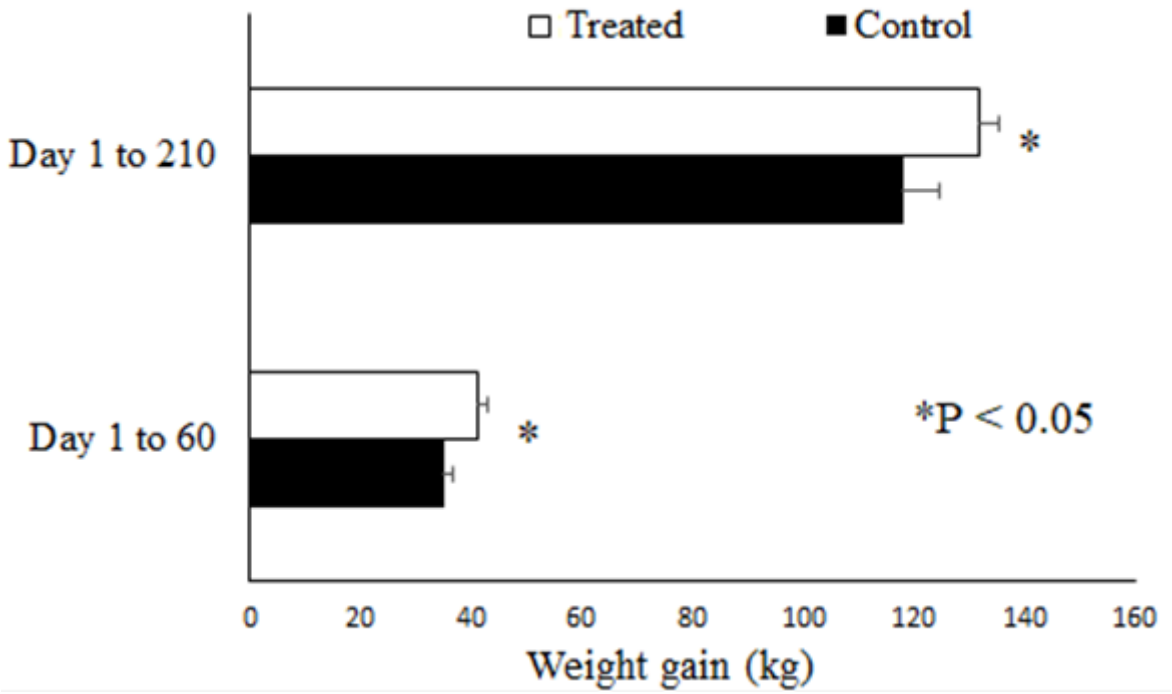

Figure 1: Weight gain of calves treated with a mixture containing components of essential oils. * P<0.05 indicates significant difference between groups.

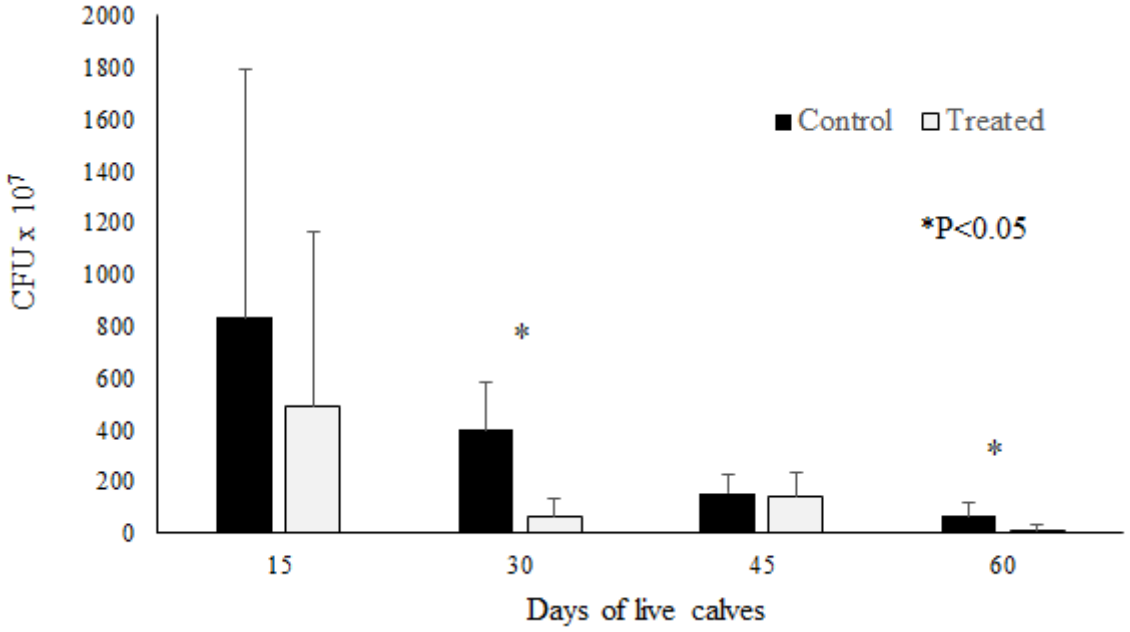

Figure 2: Total bacterial count in fecal samples of calves treated with a mixture containing components of essential oils. * P<0.05 in the same line indicates significant difference between groups.

3. CONSIDERAÇÕES FINAIS

Atualmente o leite é um dos produtos pecuários mais importantes para a economia brasileira. Setor caracterizado por pequenas propriedades rurais e muitas delas de âmbito familiar, emprega milhares de trabalhadores. Apesar de o Brasil ser um dos principais produtores mundiais de leite, a produção não atende as demandas nacionais. Com perspectivas de crescimento no mercado interno e início das exportações, a bovinocultura de leite pode avançar muito com a ajuda das pesquisas, como as desenvolvidas nessa dissertação que focaram na fase mais crítica de criação, isto é, bezerras lactantes. Cabe ressaltar que esse tipo de pesquisa não apenas contribui no aumento da produtividade, mas na eficiência da criação de bezerras.

Por se tratarem de animais jovens, cujo sistema imunológico é imaturo, são susceptíveis a contraírem infecções. As bezerras acometidas perdem peso, reduzem o consumo de concentrado, atrasam o seu crescimento e, consequentemente, o início da sua vida produtiva. Com isso, os produtores sofrem perdas econômicas com antibióticos, atraso na reposição do rebanho e risco de perder os animas. No entanto, essa pesquisa mostrou que a aplicação de selênio e vitaminas A e E contribui para melhoria da resposta imune, e consequentemente favorece a saúde dos animais que podem tornar-se mais resistentes a doenças. Outra alternativa profilática é a adição de fitoterápico como cravacrol e o cinamaldeídon na dieta das bezerras que foi capaz de minimizar infecções bacterianas e, consequentemente, promover maior ganho de peso corporal nos bezerros.

Primeiramente, identificamos que o protozoário do gênero *Giardia* é comum em bezerras leiteiras na região Oeste catarinense, assim como *Cryptosporidium* e *Eimeria*. Assim como, observamos os fatores de risco envolvidos nas infeções causadas pelos três principais protozoários encontrados nas bezerras. Ficou muito claro em nosso estudo a importância das medidas higiênicas e profiláticas adequadas para minimizar a incidência desses parasitos na criação de bezerras. Além disso, a prevenção é sempre preferível em relação ao controle, pois é mais barato, reduz o número de animais doentes e minimiza os efeitos da doença clínica.

O secnidazol pode ser uma droga eficaz na prevenção e tratamento de infecções causadas por *Giardia*. Além disso, é um tratamento de dose única e, em razão disso, facilita o manejo da propriedade e não estressa os animais. De modo geral, essa dissertação reúne diferentes protocolos alternativos para tratamento ou prevenção de doenças parasitarias em bezerras.

REFERÊNCIAS

BARNWAL, P., et al. Benzo(a)pyrene induces lung toxicity and inflammation in mice: prevention by carvacrol. **Human and Experimental Toxicology**, p. 1-10, 2017.

BARTLE, S. J.; PRESTON, R. L.; MILLER, M. F. Dietary energy sources and density: effects of roughage equivalent, tallow level, and steer type on feedlot performance and carcass characteristics. **Journal Animal Science**, v. 72, n. 8, p. 1943-1953, 1994.

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

CEPEA - CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA. **Boletim do leite**. 2016. Disponível em: <https://www.cepea.esalq.usp.br/upload/revista/pdf/0271759001483724190.pdf>. Acesso em: 06 mar. 2018.

CEPEA - CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA. **PIB do Agronegócio Brasileiro.** 2017. Disponível em: <https://www.cepea.esalq.usp.br/br /pib-do-agronegocio-brasileiro.aspx>. Acesso em: 06 mar. 2018.

CHAPPUIS, G. Neonatal immunity and immunisation in early age: lessons from veterinary medicine. **Vaccine**, v. 16, n. 1415, p. 1468-1472, 1998.

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 Technology**, v. 145, p. 396-408, 2008.

CHOUHAN, S.; SHARMA, K.; GULERIA, S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. **Medicines**, v. 4, n. 58, p. 1-21, 2017.

COUTO, M. C. M.; BOMFIM, T. C. B. Espécies de *Cryptosporidium* que infectam bovinos: características etiológicas e epidemiológicas. **Veterinária Notícias**, v. 18, p. 94-109, 2012.

EMBRAPA - EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Importância Econômica**. Disponível em: <http://www.cnpgl.embrapa.br/sistemaproducao/12-import%c3%a2ncia-econ%c3%b4mica>. Acesso em: 02 mar. 2018.

ENJALBERT, F.; LEBRETON, P.; SALAT, O. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. **Journal of Animal Physiology and Animal Nutrition,** v. 90, p. 459-466, 2006.

EUROPEAN COMMISSION. **Residues of veterinary medicinal products - Report on the implementation of national residue monitoring plans in the member states in 2015 (council directive 96/23/ec).** 2015. Disponível em: <https://ec.europa.eu/food/sites/food/files/safety/docs/cs_vet-med-residues_workdoc_2015_en.pdf>. Acesso em: 15 mar. 2018.

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **Milk and dairy products in human nutrition.** Disponível em: <www.fao.org/docrep/018/i3396e/i3396e.pdf>. Acesso em: 06 mar. 2018.

FISCHER, A., et al. Produção e produtividade de leite do oeste catarinense. **RACO, Unoesc**, v. 10, n. 2, p. 337-362, jul./dez. 2011. Disponível em: <https://editora.unoesc.edu.br/index.php/race/article/download/1681/pdf>. Acesso em: 08 mar. 2018.

FRANCO, S. F., et al. Infecção por *Giardia intestinalis*: avaliação dos sinais clínicos e resistência medicamentosa em camundongos swiss. **Revista de Saúde e Biologia**, v. 10, p. 23-33, 2015.

FRANZ, C.; BASER, K. H. C.; WINDISCH, W. Essential oils and aromatic plants in animal feeding – a European perspective. A review. **Flavour and Fragrance Journal**, v. 25,p. 327–340, 2010.

GARDNER, T. B.; HILL, D. R. Treatment of giardiasis. **Clinical Microbiology Reviews**, v. 14, n. 1, p. 114-128, 2001.

GIARETTA, P. R., et al. **Eimeriose em bezerros no Rio Grande do Sul.** In: VII Encontro Nacional de Diagnóstico Veterinário. Cuiabá: VII Endivet, 2014.

GUIMARÃES, A. M.; GUEDES, E.; CARVALHO, R. A. Ocorrência de *Giardia* spp. em bezerros leiteiros no Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 53, p. 652-653, 2001.

GILLIS, J. C.; WISEMAN, L. R. Secnidazole. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. **Drugs**, v. 51, p. 621–38, 1996.

GUILLIKSEN, S. M., et al. Calf mortality in Norwegian dairy herds. **Journal of Dairy Science**, v. 92, n. 6, p. 2782–2795, 2009.

GUYOT, H., et al. Comparative responses to sodium selenite and organic selenium supplements in Belgian Blue cows and calves. **Livestock Science**, v. 111, p. 259–263, 2007.

HELANDER, I. M., et al. Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. **Journal Agriculture Food Chemistry**, v. 46, p. 3590−3595, 1998.

HOFFMANN, P. R.; Berry, M. J. The influence of selenium on immune responses. **Molecular Nutrition Food Research**, v. 52, n. 11, p. 1273–1280, 2008.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Pesquisa Pecuária municipal.** 2016**.** Disponível em: < http://www.ibge.gov.br/estatisticas-novoportal/economicas/agricultura-e-pecuaria/9107-producao-da-pecuaria-municipal.html?=&t=resultados >. Acesso em: 05 mar. 2018.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Projeções Da População**. 2013. Disponível em: < https://www.ibge.gov.br/estatisticas-novoportal/sociais/populacao/9109-projecao-da-populacao.html?=&t=resultados>. Acesso em: 02 mar. 2018.

IFCN - INTERNATIONAL FARM COMPARISON NETWORK. IFCN Dairy reports 2016. Disponível em: <https://ifcndairy.org/ifcn-products-services/dairy-report/>. Acesso em: 02 mar. 2018.

JAYAKUMAR, S., et al. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. **Molecular and Cellular Biochemistry**, v. 360, p. 51–60, 2012.

JESSE, F. F. A., et al. Clinico-Pathological Findings of Septicaemic Colibacillosis in a Calf. **Journal of Dairy, Veterinary & Animal Research**, v.4, n. 3, p. 00124, 2016.

JOCHIMS, F.; DORIGON, C.; PORTES, V. M. O leite para o oeste catarinense. **Agropecuária Catarinense**, Florianópolis, v. 29, n. 3, p. 18-21, set./dez. 2016. Disponível em: <http://publicacoes.epagri.sc.gov.br/index.php/rac/article/viewfile /67/44>. Acesso em: 05 mar. 2018.

KALEMBA, D.; KUNICKA, A. Antibacterial and Antifungal Properties of Essential Oils. **Current Medicinal Chemistry**, v. 10, p. 813-829, 2003.

KOYUNCU, M.; CANBOLAT, O. Effect of Carvacrol on Intake, Rumen Fermentation, Growth Performance and Carcass Characteristics of Growing Lambs. **Journal of Applied Animal Research**, v. 38, n. 2, p. 245-248, 2010.

KRUSE, P. E. The importance of colostral immunoglobulins and their absorption from the intestine of the newborn animals. **Annales Recherches Veterinaires**, v. 14, n. 4, p. 349-353, 1983.

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**, v. 91, p. 453-462, 2001.

LEE, K. W.; EVERTS, H.; BEYNEN, A. C. Essential oils in broiler nutrition**. International Journal of Poultry Science**, v. 3, n. 12, p. 738-752, 2004.

LI, S. Y., et al. The effect of essential oils on performance, immunity and gut microbial population in weaner pigs. **Livestock Science**, v. 145, p. 119–123, 2012.

LIMA, L. G.; DOMINGUES, J. L. Uso de selênio na produção de bovinos. **Revista Eletrônica Nutritime**, v. 4, p. 462-474, 2007.

MARQUI, F. N., et al. **Identificação de cistos de *Giardia* spp. em bezerros leiteiros sob diferentes sistemas de criação.** In: 38º Congresso Brasileiro de Medicina Veterinária. Florianópolis: 38º Conbavet, 2011.

MARTINS-VIEIRA, M. B. C.; BRITO, L. A. L.; HELLER, L. Oocistos de *Cryptosporidium parvumem* fezes de bezerros infectados experimentalmente. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 61, p. 1454-1458, 2009.

MEGANCK, V.; HOFLACK, G.; OPSOMER, G. Advances in prevention and therapy of neonatal dairy calf diarrhea: a systematical review with emphasis on colostrum management and fluid therapy. **Acta Veterinaria Scandinavica**, v. 56, p. 1-75. 2014.

MIGUEL, M. G. Antioxidant and anti-inflammatory activities of essential oils: a short review. **Molecules**, v. 15, p. 9252-9287, 2010.

MEHDI, Y.; DUFRASNE, I. Selenium in Cattle: A Review. **Molecules**, v. 21, n. 545, p. 1-14, 2016.

MILLEMANN, Y. Diagnosis of neonatal calf diarrhea. **Revue de Médecine Vétérinaire**, v. 160, n. 8-9, p. 404-409, 2009.

MILKPOINT. **Atividade leiteira cresce no Oeste de Santa Catarina**. Disponível em: <http://www.milkpoint.com.br/noticias-e-mercado/giro-noticias/atividade-leiteira-cresce-no-oeste-de-santa-catarina-15150n.aspx>. Acesso em: 06 mar. 2018.

MEIRELES, G.S., et al. Surto de coccidiose em bezerros búfalos (Bubalusbubalis) por Eimeria bareillyi GIL et al., 1963 (APICOMPLEXA: EIMERIIDAE) - Relato de casos. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 34, p. 116-120, 2012.

MOEINI, M. M., et al. Effect of Prepartum Supplementation of Selenium and Vitamin E on Serum Se, IgG Concentrations and Colostrum of Heifers and on Hematology, Passive Immunity and Se Status of Their Offspring. **Biological Trace Element Research**, v. 144, p. 529–537, 2011.

MONTEIRO, S.C. 2011. **Parasitologia na medicina veterinária**. São Paulo: Roca. 356p.

NOWOTARSKA, S. W. Mechanisms of Antimicrobial Action of Cinnamon and Oregano Oils, Cinnamaldehyde, Carvacrol, 2,5-Dihydroxybenzaldehyde, and 2-Hydroxy-5-Methoxybenzaldehyde against *Mycobacterium avium* subsp. *paratuberculosis* (Map). **Foods**, v. 6, n. 72, p. 1-16, 2017.

NRC - NATIONAL RESEARCH COUNCIL. **Nutrient Requirements of Dairy Cattle**. Seventh Revised Edition; National Acedemy Press: Washington, DC, USA, 2001.

PEHRSON, B., et al. The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of suckler cows and on the selenium status of their calves. **Journal Animal Science**, v. 77, p. 3371–3376, 1999.

POLITIS, I., et al. Effects of vitamin E on immune function of dairy cows. **American Journal of Veterinary Research**, v. 56, n. 2, p. 179-184, 1995.

ROWNTREE, J. E., et al. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. **Journal Animal Science**, v. 82, p. 2995–3005, 2004.

SANTIAGO-NETO, W., et al. Relação da idade na presença de bactérias resistentes a antimicrobianos em rebanhos leiteiros no Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 34, n. 7, p. 613-620, 2014.

SANTOS, G.T. et al. **Bovinocultura Leiteira: Bases Zootécnicas, fisiológicas e de produção.** Eduem, Maringá, 2010.

SANTOS, F. H. R, et al. Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. **Animal**, v. 9, n. 6, p. 958–965, 2015.

SILVA JÚNIOR, F. A., et al. Fatores de risco associados à infecção por *Cryptosporidium* spp. e *Giardia duodenalis* em bovinos leiteiros na fase de cria e recria na mesorregião do Campo das Vertentes de Minas Gerais. **Pesquisa Veterinária Brasileira**, v. 31, p. 690-696, 2011.

SIMÕES, C. M. O. **Farmacognosia: da planta ao medicamento**. Porto Alegre: Ed. Universidade/UFRGS; Florianópolis: Ed. da UFSC,1999.

SPALHOLZ, J. E., et al. Advances in Understanding Selenium's Role in the Immune System. **Annals New York Academy Of Science**, v. 587, p. 123-139, 1990.

TANAKA, J.; FUJIWARA, H.; TORISU, M. Vitamin E and immune response I. Enhancement of helper T cell activity by dietary supplementation of vitamin e in mice. **Immunology**, v. 38, p. 727-734, 1979.

TREFZ, M. F., et al. Clinical signs, profound acidemia, hypoglycemia, and hypernatremia are predictive of mortality in 1,400 critically ill neonatal calves with diarrhea. **PLoS ONE**, v.12, n.8,e0182938, 2017.

ÜNAL, A., et al. Effect of different dosages of oregano oil on performance and some blood parameters in lambs. **Ankara Üniv Vet Fak Derg**, v. 61, p. 199-204, 2014.

URAL, K., et al. Single dose of secnidazole treatment against naturally occuring *Giardia duodenalis* infection in Sakiz lambs. **Revista MVZ Córdoba**, v. 19, n. 1, p. 4023-4032, 2014.

XIAO, L.; SAEED, K.; HERD, R. P. Efficacy of albendazole and fenbendazole against *Giardia* infection in cattle. **Veterinary Parasitology**, v. 61, n. 1-2, p. 165-170, 1996.

ZANETTI, M. A., et al. Efeitos da suplementação de selênio e vitamina E em bovinos leiteiros. **Revista Brasileira de Zootecnia**, v. 27, n. 2, p. 405-408, 1998.

WALKER, P. G., et al. A Reliable, Practical, and Economical Protocol for Inducing Diarrhea and Severe Dehydration in the Neonatal Calf. **Canadian Journal of Veterinary Research**, v. 62, p. 205-213, 1998.

WATTIAUX, M. A. **Essenciais em Gado de Leite—Criação de Novilhas**. The Babcock Institute: University of Wisconsin-Madison. p. 121-124, 2012.

WONG, S. Y. Y. Antibacterial activities of naturally occurring compounds against *Mycobacterium avium* subsp. *paratuberculosis*. **Applied and Environmental Microbiology**, v. 74,p. 5986–5990, 2008.

YANG, W. Z., et al. Cinnamaldehyde in feedlot cattle diets: intake, growth performance, carcass characteristics, and blood metabolites. **Journal Animal Science**, v. 88, n. 3, p. 1082-1092, 2010.

ANEXO I

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Protocolos profiláticos na prevenção de diarreia e melhoria da saúde em bezerras leiteiras", protocolada sob o CEUA nº 4964301116, sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 19/06/2017.

We certify that the proposal "Prophylactic protocols to prevent diarrhea and improve health in dairy heifers", utilizing 45 Bovines (45 females), protocol number CEUA 4964301116, under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 06/19/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 01/2017 a 12/2017 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	1 a 60 dias	N:	45
Linhagem:	holandês			Peso:	30 a 80 kg		

Resumo: A bovinocultura de leite tem crescido expressivamente nos últimos anos aumentando a importância da criação de bezerras para renovação do rebanho leiteiro. No entanto, alguns desafios são enfrentados pelos produtores e entre eles estão às elevadas taxas de mortalidade neonatal. Em algumas grandes propriedades produtoras de leite no oeste de Santa Catarina a mortalidade varia de 30 a 50%, muito superior a outras regiões brasileiras. A principal causa de morte precoce de bezerras é a diarreia infecciosa, principalmente causada por protozoários e bactérias. As infecções causadas por esses agente etiológicos em animais domésticos provocam sérios problemas a saúde, além de ocasionarem prejuízos econômicos aos produtores por gerarem perdas produtivas e custos com tratamento. Portanto, o objetivo deste estudo é testar dois protocolos profiláticos para evitar ou minimizara diarreia em berras nos primeiros dias de vida, e assim contribuir para melhorar a saúde e desempenho animal, reduzindo a mortalidade. Se esses objetivos forem alcançados, os produtores rurais serão beneficiados, assim como a região pode evoluir no potencial de seu rebanho, com crescimento e destaque cada vez maior da baia leiteira catarinense. Os protocolos testados serão: (A) selenito de sódio + vitamina A e E + secnidazole; e (B) Mix de óleos essências + secnidazole. Um grupo de bezerras não receberá nenhum dos tratamentos, ou seja, serão utilizadas como grupo controle. Para este estudo, serão utilizadas 45 bezerras recém-nascidas, da raça Holandês, sendo que todas terão o mesmo manejo e alimentação, diferindo apenas os tratamentos. Durante o experimento os animais serão monitorados diariamente, pesados em intervalos de 15 dias, assim como, submetidos a coletas de amostras de sangue e fezes também em intervalos de 15 dias. As amostras de sangue serão utilizadas para avaliação da saúde animal, por meio de exame hematológico, bioquímico sérico, imunológico e níveis de antioxidante. Além disso, as amostras de fezes coletadas durante o experimento terão três finalidades: 1) exame parasitológico de fezes; 2) contagem bacteriana total e de bactérias patogênicas; 3) teste de ecotoxicologia terrestre.

Local do experimento: Propriedade comercial de produção de leites localizada no municipio de Xanxerê.

Lages, 22 de junho de 2017

UDESC
UNIVERSIDADE DO ESTADO DE SANTA CATARINA
LAGES
CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

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Coordenadora da Comissão de Ética no Uso de Animais
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CEUA N 4964301116

ANEXO II

Cetea UDESC
Comitê de Ética em Experimentação
Centro de Ciências Agroveterinárias

CARTA DE APROVAÇÃO

O Comitê de Ética em Experimentação Animal da UDESC analisou o(s) projeto(s):

Protocolo: 1.24.15
Título: Leptospirose em bovinos: Efeitos da vacinação sobre a resposta imune e sua relação com a excreção de leptospiras.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.25.15
Título: Toxoplasmose e neosporose em bovinos: identificação de fatores de risco para infecção de rebanho leiteiro do Oeste de Santa Catarina, Brasil.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.26.15
Título: Ocorrência de protozoários gastrointestinais em bezerros na microrregião de Chapecó, Santa Catarina, Brasil.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.28.15
Título: Variáveis bioquímicas e hematológicas de ovelhas Lacaune no período de pré e pós-parto e possível influencia da carga parasitária gastrointestinal sobre essas analises.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.30.15
Título: Avaliação do efeito de sais aniônicos em vacas leiteiras no período de transição (pré-parto) sobre níveis séricos de cálcio, ferro, potássio e oxido nítrico, assim como variáveis bioquímicas relacionados ao metabolismo lipídico e proteico.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.32.15
Título: Óleo essencial de melaleuca na forma pura e nano estruturada: eficácia no controle de carrapato em vacas leiteira.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Protocolo: 1.33.15
Título: Influência da fonte de leite (materno e fórmula) na alimentação de cordeiros sobre a resposta imune, metabolismo proteico e lipídico e níveis antioxidantes.
Coordenador/Pesquisador: Aleksandro Schafer da Silva

Cetea UDESC
Comitê de Ética em Experimentação
Centro de Ciências Agroveterinárias

O Comitê de Ética em Experimentação Animal (CETEA) APROVOU o(s) projeto(s) acima relacionado(s) em seus aspectos éticos e metodológicos, para utilização de animais em pesquisa, de acordo com as diretrizes e normas nacionais e internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008 que disciplina a criação e utilização de animais em atividades de ensino e pesquisa no Brasil.

Lages, 02 de outubro de 2015.

Prof. Ubirajara Maciel da Costa
Coordenador do CETEA/UDESC

ANEXO III

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ORIGINAL

Gastrointestinal protozoa in dairy calves: identification of risk factors for infection

Protozoos gastrointestinales en terneros lecheros: identificación de factores de riesgo para la infección

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ANEXO IV

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Secnidazole for control of giardiasis in dairy calves

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