UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE EDUCAÇÃO SUPERIOR DO OESTE – CEO
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

DISSERTAÇÃO DE MESTRADO

Estratégias nutricionais na criação de bezerros leiteiros para potencializar saúde e crescimento: Uso de minerais injetáveis além de curcumina e cromo via dieta

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CHAPECÓ, 2020.

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ESTRATÉGIAS NUTRICIONAIS NA CRIAÇÃO DE BEZERROS LEITEIROS PARA POTENCIALIZAR SAÚDE E CRESCIMENTO: USO DE MINERAIS INJETÁVEIS ALÉM DE CURCUMINA E CROMO VIA DIETA

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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Chapecó, SC, Brasil

2020

Ficha catalográfica elaborada pelo programa de geração automática da

Biblioteca Setorial do CEO/UDESC,

com os dados fornecidos pelo(a) autor(a)

Glombowsky, Patrícia
Estratégias nutricionais na criação de bezerros leiteiros para potencializar saúde e crescimento: Uso de minerais injetáveis além de curcumina e cromo via dieta / Patrícia Glombowsky. -- 2020.
120 p.

Orientador: Aleksandro Schafer Da Silva
Coorientadora: Ana Luíza Bachmann Schogor
Dissertação (mestrado) -- Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia, Chapecó, 2020.

1. Minerais injetáveis. 2. Curcumina. 3. Cromo. 4. Digestibilidade. 5. Aleitamento. I. Schafer Da Silva, Aleksandro. II. Bachmann Schogor, Ana Luíza. III. Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia. IV. Titulo.

Universidade do Estado de Santa Catarina
UDESC Oeste
Programa de Pós-Graduação em Zootecnia

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

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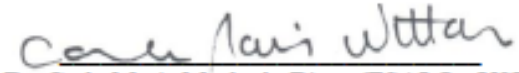
Elaborada por
Patrícia Glombowsky

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

Comissão Examinadora:

Dr. Aleksandro Schafer Da Silva (UDESC)



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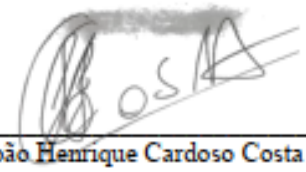

Dr. João Henrique Cardoso Costa (UK)

Chapecó, 30 de setembro de 2020.

AGRADECIMENTOS

Em especial agradeço a Deus que permitiu que tudo isso acontecesse, pela força de nunca desistir e superar todos os desafios.

Aos meus pais por todo amor incondicional, incentivo em todas minhas escolhas, por nunca ter deixado eu desistir de todos meus sonhos, também, peço desculpa pelos momentos que não me fiz presente. Amo vocês!

Ao meu eterno namorado Natan, por fazer de meus sonhos o seus e juntos lutarmos para conquistá-los, obrigada por tudo, te amo!

Agradeço a Universiade do Estado de Santa Catarina pela oportunidade de formar minha profissão como Zootecnista e agora conquistar o Mestrado em Zootecnia, claro que tudo isso não seria possível sem o incentivo e exemplo do orientador Aleksandro junto a todos os demais professores. Também agredeço o program UNIEDU pela bolsa concedida, obrigada a todos.

Nada do que realizei e conquistei até aqui seria possível sozinha, assim agradeço a todos envolvidos principalmente na realização dos experimentos, ao grupo GANA obrigada pela parceria de 6 anos.

Nesse período, comecei a trabalhar na empresa Orgânica, ganhei uma grande família, agradeço por todo apoio, em especial as irmãs que ganhei Angélica, Karina e Sônia, obrigada por tudo, amo vocês!

Muito obrigada!

“Saiba que onde quer que você esteja na vida, nesse instante, é, ao mesmo tempo, temporário e exatamento onde deveria estar. Você chegou a este momento para aprender o que precisa aprender, para que possa se tornar a pessoa que precisa ser para criar tudo que jamais desejou para sua vida”.

Seth Godin

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

ESTRATÉGIAS NUTRICIONAIS NA CRIAÇÃO DE BEZERROS LEITEIROS PARA POTENCIALIZAR SAÚDE E CRESCIMENTO: USO DE MINERAIS INJETÁVEIS ALÉM DE CURCUMINA E CROMO VIA DIETA

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Chapecó, 30 de setembro de 2020

Estratégias para criação de bezerros tem sido foco de diversas pesquisas no Brasil e no exterior. Apesar disso, existe muitas outras alterativas que precisam ser avaliadas cientificamente. Portanto, o objetivo aqui foi avaliar se a inclusão de cromo orgânico e curcumina via dieta, assim como uso de minerais injetáveis em bezerras leiteiras teria a capacidade de melhorar o sistema imunológico e respostas antioxidantes, minimizar efeitos negativos oriundos de distubios metabólicos e infeciocosos, e assim potencializar o desempenho de crescimento durante o aleitamento e desaleitamento. Para isso, nosso estudo foi dividido em três etapas, que caracterizam três estratégias diferentes. Em um primeiro momento avaliou-se o efeito nutracêutico da aplicação de mineral por via injetável (selênio, cobre, potássio, magnésio e fósforo). Usamos dose de 3 mL/animal nos dias 2 e 14 de vida em cinco animais e usamos outros cinco como controle. O estudo teve duração de 30 dias; nesse período foi coletado amostra de sangue e pesamos os bezerros. A aplicação de minerais aumentou a atividade de enzimas do sistema antioxidante, modulou a resposta imune e diminui casos de diarreia em bezerras nos primeiros 30 dias de vida. Em um segundo estudo, avaliamos a inclusão de curcumina na dieta de bezerras em diferentes fases (aleitamento e desaleitamento), para isso, foram utilizadas 33 bezerras dividos 3 grupos conforme a idade, caracterizando três estudos, denominados como: experimento 1 (aleitamento) idade média de 18±7 dias, experimento 2 (aleitamento) idade média de 64±4 dias e o experimento 3 (desaleitamento) 95±8 dias. O período experimental foi de 15 dias, sendo que nosso delineamento nos três estudos foi composto por 2 grupos (tratado - animal recebeu 200 mg de curcumina/animal/dia e controle. Foram feitas coletas de sangue e fezes nos dias 0, 10 e 15 do experimento para avaliar a contagem de oocistos de *Eimeria* por grama de fezes e escore fecal; no sangue realizou-se hemograma, avaliou perfil antioxidante e oxidante e bioquímica sérica e pesagem dos animais. Por fim, em laboratório foi feita análise de digestibilidade in vitro dos alimentos ofertados durante o experimento. A curcumina como aditivo apresentou efeito coccidiostático e aumentou o ganho de peso dos bezerros. Outros resultados que merecem destaque nesse estudo foi o efeito antioxidante associado a uma redução de células imunológicas; o que pode ter efeito indireto sobre o desempenho desses bezerros; pois é sabido que existe uma correlação negativa entre estresse oxidativo/resposta inflamatória e desempenho zootécnico. Também, verificamos que adição de curcumina, in vitro, aumenta a digestibilidade de alimentos. No terceiro estudo, avaliou-se a inclusão de cromo na dieta de

bezerros por duas vias de fornecimento (sucedâneo e concentrado). Para isso, utilizou-se 24 bezerros machos (holandês) com idade média de 8±4 dias e 39,81±6,9 kg de peso corporal, divididos em 3 grupos: SC (n=8) - recebeu 4 mg de Cr/animal/dia via sucedâneo durante os 60 dias experimentais de aleitamento; CC (n=8) - recebeu 4 mg de Cr/animal/dia via concentrado; C (n=8) - animais não receberam suplementação de cromo. O experimento teve duração de 75 dias, divididos em dois estágios, 1º período de aleitamento (1-60 dias) e 2º periodo de desaleitamento (61-75 dias). Foi realizado análises de desempenho, hemograma, bioquímica sérica e perfil oxidante e antioxidante. Observamos melhor desempenho de crescimento de bezerros que receberam cromo na dieta, independente da via, o que justifica esses animais terem maior eficiência alimentar. Cromo via sucedâneo aumentou a digestibilidade da proteína nos bezerros. Gostaríamos de destacar também aqui o aumento nos níveis da glicose e cromo no sangue desses bezerros, mostrando que a ingestão de como orgânico resultou em absorção eficiente. Com base nos estudos, podemos concluir que as estratégias para potencializar saúde e desempenho dos bezerros teve efeito positivo nos três experimentos realizados; e mostram-se como uma alternativa viável e rentável no sistema de produção; principalmente se reduzir mortalidade de bezerras, categoria animal com preço de venda e compra elevado.

Palavras-chave: Minerais injetáveis, Curcumina, Cromo, Digestibilidade, Aleitamento, Desaleitamento.

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

NUTRITIONAL STRATEGIES IN THE RAISING OF DAIRY CALVES TO POTENTIALIZE HEALTH AND GROWTH: USE OF INJECTABLE MINERALS IN ADDITION TO CURCUMIN AND CHROMIUM BY FEED

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Chapecó, September, 30th, 2020

Strategies for raising calves have been the focus of several studies in Brazil and elsewhere. Nevertheless, many other alternatives need to be evaluated scientifically. Therefore, our objective was to determine whether the inclusion of organic chromium and curcumin in feed, as well as the use of injectable minerals in dairy calves, would improve immune and antioxidant responses, and minimize negative effects from metabolic and infectious disorders, thereby enhancing growth performance during suckling and weaning. We divided our study into three stages, characterizing three different strategies. First, the nutraceutical effect of the mineral application by injectable route was evaluated (selenium, copper, potassium, magnesium and phosphorus). We used 3 mL/animal on days 2 and 14 of life in five animals and another five as a control. The study lasted 30 days; blood samples were collected and calves were weighed. The application of minerals increased the activity of enzymes in the antioxidant system, modulated the immune response and decreased cases of diarrhea in calves in the first 30 days of life. In a second study, we evaluated the inclusion of curcumin in the feed of heifers in two stages (suckling and weaning). We divided 33 heifers into three groups according to age, featuring three studies: experiment 1 (suckling) age mean 18 ± 7 days, experiment 2 (suckling) mean age 64 ± 4 days, and experiment 3 (weaning) 95 ± 8 days. The experimental period was 15 days, and our design in the three studies was composed of two groups (treated: animal receiving 200 mg of curcumin/animal/day; control: with curcumin in the feed. Blood and feces were collected on days 0, 10 and 15 of the experiment to assess the count of *Eimeria* oocysts per gram of feces and fecal score. Hemogram were also carried out. Antioxidant and oxidant profiles and serum biochemistries were measured and the animals were weighed. We analyzed the in vitro digestibility of the food offered during the experiment. Curcumin as an additive showed a coccidiostatic effect and increased calf weight gain. Other results worth mentioning in this study were the antioxidant effect associated with a reduction and immune cells; there was an indirect effect on the performance of these calves, as it is known that there is a negative correlation between oxidative stress/inflammatory response and zootechnical performance. We also found that adding curcumin in vitro increased the digestibility of food. In the third study, the inclusion of chromium in the calf diet was evaluated by two routes of supply (milk replacer and concentrate). For this, 24 dairy calves (holstein) were used, with an average age of 8 ± 4 days and 39.81 ± 6.9 kg of body weight, divided into three groups: SC (n = 8): received 4 mg of Cr/animal/day via milk replacer during the 60 experimental days of suckling; CC (n = 8): received 4 mg Cr/animal/day via

concentrate; C (n = 8): animals did not receive chromium supplementation. The experiment lasted 75 days, divided into two stages, 1st suckling period (1–60 days) and 2nd weaning period (61–75 days). Performance analysis, hemogram, serum biochemistries and oxidative and antioxidant profiles were measured. We observed better growth performance of calves that received chromium in the feed, regardless of the route, which explains why these animals had greater feeding efficiency. Chromium via substitute increased protein digestibility in calves. We would also like to highlight here the increase in the levels of glucose and chromium in the blood of these calves, suggesting that the intake of as organic resulted in efficient absorption. Based on the studies, we can conclude that the strategies to enhance calf health and performance had a positive effect on the three experiments performed; our findings suggest these additives are viable and profitable alternatives in production systems; they reduces mortality in calves, an animal category with a high sale and purchase price.

Keywords: Injectable minerals, Curcumin, Chromium, Digestibility, Suckling, Weaning.

SUMÁRIO

1. CAPÍTULO I
REVISÃO DE LITERATURA

1.1 BOVINOCULTURACULTURA DE LEITE

A produção, industrialização e comercialização do leite é considerada uma atividade importante ao ambiente produtivo e aos impactos a economia mundial, principalmente em países em desenvolvimento e onde a agricultura familiar predomina. Em 2018, o Brasil foi considerado o 4º maior produtor de leite mundial, produziu 34,23 bilhões de leite, desse total foram captados pelos laticínios 24,45 bilhões (EMBRAPA, 2018; IBGE, 2018). Mesmo com essa produção de leite, o Brasil necessita importar para garantir o alimento a todos os consumidores, no ano de 2018 foi importado em média 99,86 milhões de litros ao mês (MDIC, 2018).

Diante desse cenário, dados do IFCN (Internacional Farm Comparison Network) destacam que o brasileiro consome em média 173 kg de produtos lácteos durante o ano, essa média está abaixo do recomendado pela FAO (Food and Agriculture Organization of the United Nations) que é 200 kg/pessoa/ano. Portanto, o Brasil tem oportunidade de crescimento visando que a média consumida precisar ser estimulada para atingir a recomendação da FAO e a produção interna precisar aumentar para garantir o suprimento da demanda e poder exportar, de tal modo valorizar o produto lácteo brasileiro.

O leite está entre os produtos de maior importância da agropecuária brasileira, está à frente de produtos tradicionais como café, arroz, entre outros (CEPEA, 2017). A cadeia produtiva do leite ainda atinge a geração de empregos, ou seja, o país apresenta mais de 1 milhão de propriedades com atividade leiteira, e emprega diretamente e indiretamente mais de 3,6 milhões de pessoas (EMBRAPA, 2018). Em 2017, o agronegócio apresentou participação de 20% no PIB (Produto Interno Bruto), deste 6,05% são do ramo pecuário (CEPEA, 2017). O leite contribuiu com 22,4% do Valor Bruto da Produção Pecuária, apenas superado pela contribuição da carne bovina (EMBRAPA, 2018). Perante o exposto, é notável essa importância ao leite na pecuária, ao rendimento do PIB e ao Brasil.

Além de toda importância a economia, o leite é um alimento de elevado valor biológico, devido ser natural e nutritivo, apresenta fontes importantes de proteínas,

carboidratos, lipídios, minerais e vitaminas. Destaca-se a importância do cálcio para a nutrição humana, sendo que a cada litro de leite tem aproximadamente 1.200 mg de cálcio, desse total quando ingerido pelo humano cerca de 30% é absorvido, quando comparado ao cálcio do espinafre apenas 5% é aproveitado (SBAN, 2015).

Perante da importância visualizada da atividade para economia e aos produtores e diante da oportunidade de crescimento das propriedades, é necessário intensificar e melhorar para atingir os objetivos, para isso ser possível é importante condicionar o animal ao manejo adequada, alimentação balanceada, sanidade, melhorias genéticas, entre outros. O melhoramento genético ocorre dentro das propriedades através da seleção de animais com as características desejadas pelo produtor, como aumento de produção, maior concentração de sólidos no leite, melhorias fenotípicas e conformação. Já se sabe que o melhor caminho para atingir os objetivos é através da criação dos animais na propriedade, dentre o período de criação até a fase produtiva a categoria de bezerra é o momento que apresenta maiores desafios devido a vulnerabilidade do animal.

1.2 CRIAÇÃO DE BEZERRAS

O sucesso da criação de bezerras e novilhas está diretamente relacionado a reposição do rebanho considerada ideal de 25% ao ano, com isso possibilita a redução da idade ao primeiro parto com o menor custo, melhorias de manejo sanitário da propriedade, ou seja, sem a necessidade de entrada de animais de outros rebanhos (SANTOS et al., 2010). Além disso, muitos estudos apontam que o êxito no período de criação da bezerra refletirá no período produtivo da fêmea (LAPORTA et al., 2020).

Durante esse período são observados vários pontos críticos que devem ser muito bem feitos para obter bezerras saudáveis, dentre os aspectos mais importantes a instalação, colostragem, cura de umbigo, fornecimento de dieta líquida e sólida de qualidade apresentam grande influência nos resultados de desempenho, morbidade e mortalidade (MEGANCK, et al., 2014). Outro tópico que está sendo pesquisado e já se sabe sobre a influência no desenvolvimento da bezerra é o período de gestação principalmente o terço final, referente a alimentação, manejo e instalações (SANTOS et al., 2010).

Sabe-se que durante os últimos dois meses de gestação ocorre o maior desenvolvimento fetal, cerca de 60% do peso corporal do nascimento é gerado nesse período (SOUZA, 2011). Outra grande influência da gestação sobre o desenvolvimento da bezerra é a colostrogênese, que também acontece principalmente nessa fase final e é considerada a mais importante devido a passagem da imunidade passiva ocorrer através da colostragem (MEGANCK et al., 2014). A placenta da vaca é tipo sidesmocorial que apresenta como função proteção do feto contra ação de microrganismo como bactérias e vírus, entretanto, impede a passagem de anticorpos, assim o bezerro recebe os anticorpos através do colostro fornecido na primeira mamada imediatamente após o nascimento (MACHADO et al., 2004).

Antes do nascimento o manejo nutricional da mãe através do fornecimento de dieta balanceada será fundamental para o desenvolvimento da prole, como também prevenção de problemas metabólicos para fêmea na transição (SANTOS et al., 2010). Dentre os nutrientes que desempenham funções essenciais para a mãe e ao feto, a limitação de proteína pode comprometer o crescimento fetal (VAN EMON et al., 2014). Além disso, os microminerais também se destacam no fornecimento de dietas adequadas, exercem funções direta sobre o sistema imune e como antioxidantes ou como cofatores (KINCAID et al., 2004).

Durante fase de aleitamento, as bezerras são constantemente desafiadas por fatores extrínsecos e nesse momento o organismo depende das defesas adquiridas através da ingestão do colostro, esse manejo é considerado crítico e refletirá durante a vida da fêmea (SOUZA, 2011). Além desse fator, tem se observado outros que afetam o bem-estar e desempenho, o que inclui as instalações, ambiente, manejo nutricional, tratador, sanidade e manejos necessários durante esse período como transporte, mochamento, remoção de tetos a mais e desmama (SILVA, 2015).

Dentre os fatores extrínsecos citados, as instalações são consideradas a de maior impacto devido ser precárias, improvisadas e que não atendem as necessidades básicas da cria em algumas propriedades, dentre as necessidades básicas e essenciais ao conforto da bezerra deve ser cuidado a ventilação, isolamento e temperatura. Para esse ambiente ser julgado como adequado necessita proporcionar conforto físico, térmico, comportamental e psicológico (FURTADO et al., 2012).

Quando tratado sobre conforto térmico animais adultos geralmente sofrem de estresse por calor e busca-se estratégias que minimizem os efeitos negativos. Entretanto, animais

jovens podem ser afetados pelo estresse por calor e frio principalmente, por esse motivo é necessário buscar alternativas que mantenham o animal sobre as condições de conforto, a literatura preconiza que a faixa de temperatura de conforto para animais jovens é entre 15 a 25 ºC (PICCIONE et al., 2003). Quando a temperatura ambiente não se encontra dentro da faixa de conforto o organismo do animal responde de maneiras diferentes para tentar compensar esse estresse, por exemplo, abaixo de 15 ºC o organismo do bezerro realiza gasto de energia para manter a temperatura corporal, consequentemente diminuirá o ganho peso (NONNECKE et al., 2009). No outro extremo, quando as temperaturas são acima de 25 ºC e o organismo animal diminui o consumo para não ocorrer incremento calórico e conseguir dissipar calor metabólico na tentativa de manter a homeotermia, consequentemente também não ganhará peso devido ao baixo consumo (PICCIONE et al., 2003).

Durante o período de aleitamento os principais objetivos é que a cria desaleite com dobro do peso do nascimento e consuma concentrado precocemente (SANTOS et al., 2010). Para garantir o sucesso nessa fase existem estratégias de aleitamento, sendo a “convencional” que consiste no fornecimento de 10% do peso corporal em leite e o “intensivo” que é caracterizado por fornecer de 15 a 20% (RUFINO et al., 2019). Além disso, outra estratégia utilizada na dieta líquida é o aumento da concentração de sólidos totais, ou seja, 1 litro de leite possui aproximadamente 12% de sólidos totais através dessa estratégia estudos apontam que é possível chegar até 20% de sólidos com benéficos a bezerra, através da melhora no desenvolvimento corporal (AZEVEDO et al., 2016). Esse aumento pode ser feito através da adição de sucedâneos lácteos de boa qualidade e com a garantia do aumento através do acompanhamento do equipamento refratômetro.

Essa estratégia alimentar apresenta um grande impacto sob a eficiência alimentar durante a fase de cria e até mesmo na vida adulta (FURINI et al., 2018). Por isso adicionar sucedâneo ao leite vai modificar a osmolaridade intestinal, altera a permeabilidade intestinal, digestibilidade de nutrientes e em alguns casos é observado como desvantagem o aumento de diarreias, por isso deve ser aplicado com cautela (GLOSSON et al., 2015). Outra estratégia nutricional aplicada aos bezerros é a disponibilização de alimento concentrado a partir do 2º dia de vida, que apresente um valor nutricional adequado e que os nutrientes sejam mais disponível ao animal (AZEVEDO et al., 2016).

As estratégias alimentares citadas acima podem também ser empregadas ao uso de sucedâneo em substituição ao leite, essa troca apresenta como vantagens economia devido ao baixo custo quando comparado ao leite, disponibilidade de maior volume de leite comercializado e conhecimento sobre a composição da dieta fornecido (TERRE et al., 2007). Entretanto, é necessário conhecer os constituintes do sucedâneo, pois o baixo custo pode estar aliado a alimentos de má qualidade, assim, os animais apresentará atraso no crescimento, maior incidência de doenças, até visualizado aumento da taxa de mortalidade (HILL et al., 2010).

O estímulo de consumo de concentrado durante a fase de aleitamento é fundamental para colonização da microbiota do rúmen e desenvolvimento dos órgãos do trato gastrointestinal em tamanho (SOUZA, 2011). Além disso, no rúmen o concentrado estimula o crescimento no número de papilas e no seu tamanho, como esse desenvolvimento inicia-se a degradação de alimento no rúmen e produção de AGV’s importantes que serão a principal fonte de energia ao ruminante (BERCHIELLI et al., 2006). Dentre os alimentos utilizados para constituir o concentrado, recomenda-se o uso de farelo de milho e trigo como fonte de carboidratos, farelo de soja como fonte proteica, é aconselhado evitar uso de ureia nesse período de introdução alimentar, também, e é indispensável a formulação de núcleo vitaminico/mineral com aditivos para suprir as exigências e potencializar o crescimento dos bezerros (AZEVEDO et al., 2016).

Dentre dos ativos alimentares comumente utilizados para bezerros, os promotores de crescimento são os que apresentam maior impacto sobre o desempenho (SALLES e LUCCI, 2000). Entretanto, atualmente o mercado consumidor busca por alimentos mais seguros, sem resíduos químicos e que apresentem menor impacto sobre o custo de produção (Sandi e MUHLBACH, 2001). Desta maneira, cresce as pesquisas que avaliam os benefícios da inclusão de aditivos, como prébioticos, probioticos, fitoterápicos, extratos, entre outros, na tentativa de não prejudicar o desempenho com a substituição e verificar possíveis efeitos tóxicos (OLIVEIRA et al., 2005).

1.3 CURCUMINA

A curcumina é um componente biologicamente ativo presente na *Curcuma longa* L., planta pertence à família Zingiberaceae; conhecida popularmente como açafrão, batatinha amarela, açafrão da terra, entre outros (ABRANCHES, 2015). É originária da Índia e da Ásia e rapidamente de difundiu pelo mundo devido as propriedades medicinais, no Brasil a introdução foi realizada na década de 80 (ALMEIDA, 2008).

Antigamente não se conhecia os efeitos farmacológicos, assim era utilizada como corante devido a coloração amarelada de fácil pigmentação (GRANDI, 2014). Com o avanço de estudos sobre os benefícios do consumo de fitoterápicos, descobriu-se os constituintes químicos da *Curcuma longa* que apresentam benefícios a saúde (ALMEIDA, 2008). É composta por curcuminoides sendo a curcumina como principal ativo (60 a 76% de concentração), desmetoxicurcumina e bisdesmetoxicurcumina, esses são responsáveis pela pigmentação do rizoma (EL-BAHR, 2015). Além de outros componentes, como carbinol, resina, amido, polissacarídeos, entre outros em menores quantidades (GRANDI, 2014).

A curcumina é insolúvel em água, entretanto, apresentam alta solubilidade em álcoois metílicos e etílicos (ORSOLIN e NEPOMUCENO, 2009). Além disso, é considerada estável em diferentes variações de pH, por exemplo, pH ácido do estômago, assim possibilita o consumo pelo humano e animais sem perder as propriedades (SANDUR et al., 2007). Já a porção de óleo essencial da *C. longa* é composta de sesquiterpenos ar-turmerone, ar-cucurmeno, ar-turmerol, curlone, zingiberene e curcumene (SINGH et al., 2010; DOHARE et al., 2008).

Esses compostos da planta citados anteriormente apresentam ação terapêutica sobre o organismo, e são conhecidos pelo efeito anti-inflamatório, antioxidante, antimicrobiano, antiviral, antifúngico, antitumoral, antiparasitário, inibidor da carcinogênese, hepatoprotetor, imunomodulador, entre outros (KIM et al., 2014; YU et al., 2002; BASTOS et al., 2009). A ação anti-inflamatória da curcumina ocorre devido a presença dos compostos fenólicos na molécula que possui a capacidade de inibir as moléculas envolvidas na cascata do processo inflamatório, como fosfolipase A, LOX – lipoxigenases, tromboxanos, prostaglandinas, óxido nítrico, leucotrienos, entre outros (CHAINANI-WU, 2003; ROSA, 2009; KHALAF et al., 2010).

A curcumina tem a capacidade de inibir a atividade de NF-κB (Fator nuclear kappa – potenciador de células b ativadas) apresenta função importante sobre o fator de transcrição e é encontrada em todos os tecidos do organismo (JOE et al., 2004). Atua também sobre a inibição da produção de fator de necrose tumoral alfa (TNF-α), interleucina-1 (IL-1), e interleucina-6 (IL-6) (LIU et al., 2016), que são importantes marcadores de resposta inflamatória. Além disso, a curcumina intercepta e neutraliza potentes pró-oxidantes e substâncias cancerígenas (AGGARWAL e HARIKUMAR, 2009).

A ação antioxidante da molécula de curcumina é devido a capacidade sequestrar radicais livres no organismo, como espécies reativas ao oxigênio e doar elétrons de hidrogênio que permite a estabilidade de células, inibindo o efeito cascata do estresse oxidativo, isso é consequência do desiquilíbrio entre a concentração de radicais livres presentes e o sistema antioxidante (BARBOSA et al., 2010). O sistema antioxidante é dividido em dois grupos, o enzimático e não enzimático. O primeiro é composto por importantes enzimas que auxiliam na manutenção do estresse oxidativo, como glutationa peroxidase (GPx), glutationa (GSH), superóxido dismutase (SOD), catalase (CAT), peroxirredoxina, entre outros; e não enzimático, constituído por minerais, vitaminas e compostos fenólicos que apresentam propriedades antioxidantes (SCOTTI et al., 2007). Estudos recentes que avaliaram a suplementação da curcumina a humanos e animais observam a diminuição dos impactos causados pelo estresse oxidativo ao organismo, essa influência é visualizada através do aumento das enzimas antioxidantes citadas anteriormente (HONG et al., 2020; MANJU et al., 2017; BHATT et al., 2014).

Estudos que avaliaram o fornecimento de curcumina a animais de produção como frangos de corte, cordeiros, ovelhas e para cães que são animais de companhia, observaram aumento nas enzimas antioxidantes, como CAT, GPx, SOD e ACAP (capacidade antioxidante total), assim estabilizando ou eliminando radicais livres do organismo (GALLI et al., 2019; MOLOSSE et al., 2018; JAGUEZESKI et al., 2018; CAMPIGOTTO et al., 2020). Esses efeitos relatados são de grande impacto na produção, principalmente quando apresenta ação sobre os radicais livres, pois, são maléficos devido causar peroxidação lipídica nas células, ruptura de DNA, proteínas teciduais e inativação de enzimas (MOHANTY et al., 2012).

A curcumina também apresenta ação antimicrobiana, devido apresentar interação com a proteína FtsZ do citoesqueleto bacteriano, essa proteína participa da divisão celular de

algumas bactérias (KAUR et al., 2010). Além disso, alguns autores afirmam que essa característica antimicrobiana aconteça pela presença dos grupos metoxila e hidroxila (HAN e YANG, 2005). A inibição do desenvolvimento bacteriano já foi visualizada em diferentes classes bacterianas importantes para a produção animal (DUBEY et al., 2008; HAN e YANG, 2005; MUN et al., 2013).

Além disso, atualmente foi descoberto o efeito antiparasitário, em ovinos observou o efeito da curcumina sobre a *Eimeria* spp., autores observaram 100% de eficácia no uso (MALLO et al., 2017; CERVANTES-VALENCIA et al., 2016). Em cordeiros quando suplementados com curcumina foi observado melhora no ganho de peso, estimulação do sistema imunológico e diminuição do estresse oxidativo (MOLOSSE et al., 2019; CERVANTES-VALENCIA et al., 2016). Diante de todos os benefícios no uso da curcumina em ovinos, em específico a cordeiros e haver poucos estudos na literatura com ruminantes, os bezerros apresentam características fisiológicas e manejo semelhantes que podem determinar a contribuição da curcumina para essa categoria também.

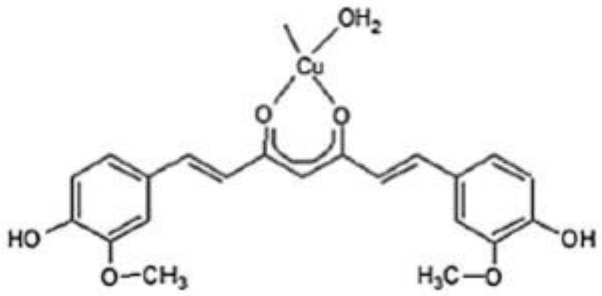

Figura 1. Molécula estrutural da curcumina, componente ativo encontrado na Curcuma longa após purificação. **FONTE:** García-Niño et al., 2014.

1.4 SUPLEMENTAÇÃO MINERAL INJETÁVEL

Os minerais são considerados essenciais ao organismo animal, atuam sobre diversos processos fisiológicos, como sistema imunológico, reprodução, antioxidante e crescimento (SPEARS, 2000). Quando fornecido complexos de minerais é importante cuidar o as

quantidades fornecidas, pois à falta de algum mineral pode ocasionar deficiência de outro, e em casos mais graves levar a doenças e retardo de crescimento. Além disso, atingir os níveis de exigências também é essencial para garantir a absorção e utilização de todos, pois, os minerais apresentam característica de antagonismo e sinergismo, ou seja, a presença de um nutriente (mineral) pode diminuir ou aumentar a absorção de outro (BERCHIELLI, 2011).

Diante dessa importância, para animais em aleitamento essa suplementação e suprimento das exigências torna-se um grande desafio visando que o principal alimento é o leite integral sem alterações (DRACKLEY, 2008). O leite pode ter sua qualidade e composição alterada devido a vários fatores, entre eles, o melhoramento genético, intensificação do sistema de produção e alimentação da vaca. Já se sabe que o leite é deficiente de alguns minerais importante, como exemplo o manganês, selênio e cromo que são essenciais ao crescimento e esse déficit pode apresentar prejuízos ao animal (DRACKLEY,2008).

A suplementação de minerais pode acontecer por diversas vias, através de misturas de núcleo adicionados a concentrados e de pronto uso disponível para o animal, bolus intraruminal e soluções injetáveis (ARTHINGTON et al., 2014). A aplicação injetável de minerais pode ser uma alternativa viável a criação de bezerras, devido suprir as exigências e minimizar as perdas durante o fornecimento (ARTHINGTON et al., 2014). Atualmente cresce o desenvolvimento de produtos, assim como de estudos que avaliam os benefícios do fornecimento de minerais injetáveis em animais de produção (PETERS et al., 2008; SOLDÁ et al., 2016; CAZAROTTO et al., 2018; TOMASI et al., 2018; BORDIGNON et al., 2019;).

Em cordeiros durante o período de aleitamento até 30 dias de vida, sob condições de desafios e estresse o fornecimento metafilático de minerais, como zinco, cobre, manganês e selênio melhorou a atividade do sistema antioxidante, estimulação da produção de IgG e IgM, isso refletiu em maior ganho de peso (CAZAROTTO et al., 2018). Tomasi et al. (2018) avaliou o fornecimento da associação dos minerais cobre e zinco injetável a bezerros no primeiro dia de vida em condições de estresse por calor, obtiveram resultados semelhantes de fortalecimento do sistema imunológico e antioxidante, consequentemente melhor desempenho.

Bezerros em aleitamento apresentam elevado desafio sanitário principalmente devido a janela imunológica que acontece nesse período, comumente próximo aos 21 dias de vida ocorre um declínio da resposta imune passiva para o desenvolvimento da imunidade ativa

(BITTAR et al., 2016). Diante disso, o contato com patógenos presente no ambiente pode dificultar o desenvolvimento e expressão do potencial genético do animal, quando suplementado com um complexo contendo zinco, cobre, manganês e selênio em duas doses subcutâneas aos 3 e 30 dias de vida, observou-se redução na incidência de diarreia (suplementado: 41,7% e controle: 49,7%) e pneumonia (suplementado: 41,7% e controle: 49,1%), os autores consideram que essa diminuição de problemas comuns em criação de bezerras ocorreu pela maior produção de neutrófilos e aumento nos níveis da enzima Glutationa Peroxidase (GPx) (TEIXEIRA et al., 2014).

Como é sabido, durante o período de aleitamento a bezerra passa por diversos desafios, outro importante é o desaleitamento que consiste na troca de dieta líquida para sólida é considerado estressante e marcado pela perda de peso. Algumas estratégias utilizadas é a suplementação injetável de complexo de minerais e vitaminas, em estudo que avaliou a suplementação de cobre, zinco, manganês e vitaminas A e E receberam o complexo 7 dias antes do desmame e no dia, concluíram que a suplementação estimulou o sistema antioxidante, melhorou a resposta imunológica e proporcionou melhor ganho de peso durante o período de estresse (MATTIOLI et al., 2020). Bordignon et al. (2019) obteve resultados semelhantes quando avaliou o fornecimento de complexo de minerais e vitaminas injetáveis a bezerras em fase de desaleitamento em condições de estresse por calor, diante desse desafio as bezerras suplementadas apresentaram maior ganho de peso e notou-se aumento de leucócitos, neutrófilos e monócitos, além disso, houve redução dos radicais livres e também como já esperado aumento das enzimas antioxidantes.

1.5 CROMO ORGÂNICO

Cromo é um mineral considerado elemento químico de símbolo Cr, descoberto 1797 pelo francês Louis Nicolas Vauquelin (ICDA, 2006). Encontrado no estado oxidado na forma trivalente (Cr3+) devido a estabilidade, a forma biologicamente ativa é uma molécula organometálica conhecida como fator de tolerância a glicose (GTF) que aumenta a interação da insulina com as células alvo, com função de potencializar sua atividade (Anderson et al., 2001). A estrutura ainda não está bem esclarecida, mas sabe-se que apresenta um átomo de

Cr3+, ácido nicotínico e alguns aminoácidos como a glicina, cisteína e ácido glutâmico. O organismo sem Cr3+ o GTF é inativo (VICENT e DAVIS, 2001).

O mineral ainda não é definido como essencial aos animais, entretanto existe diversas pesquisas que o classificam como essencial devido as propriedades de ativar enzimas, estabilizar proteínas e ácidos nucleicos (VINCENT, 2003; YARI et al., 2010; GHORBANI et al., 2012). Em acréscimo possui influência ao metabolismo de carboidratos, proteínas e lipídios, e assim, estimula o fortalecimento do sistema imune e desempenho de animais de produção (LAY e LEVINA, 2008). Dentre essas funções destaca-se a influência ao metabolismo da glicose, sendo que estudos antigos já observavam o aumento do GTF, que consiste no aumento da capacidade de mobilizar glicose para dentro das células (ANDERSON et al., 2001).

Como mencionado a principal função é feita no metabolismo da glicose, onde potencializa a ação da insulina ao facilitar a interação entre a glicose e o receptor. Esse mineral é absorvido no intestino delgado na porção do jejuno e a quantidade absorvida depende da porção fornecida via dieta (MOWAT, 1997; PECHOVA e PAVLATA, 2007). A quantidade medida no plasma é de 0,01 a 0,3 µg/L, esse cromo pode estar ligado ainda a transferrina e albumina. O cromo tem a capacidade de ser armazenado em vários tecidos do organismo, como fígado, rins, baço e epidídimo (ANDERSON, 2001). Entretanto, a quantidade absorvida é pequena mesmo quando fornecido na forma orgânica, o cromo orgânico apresenta taxa de absorção de 10 a 15% quando comparado ao inorgânico essa taxa diminui para 1 a 3% (CHANG e MOWAT, 1992).

Diante disso, são poucos estudos encontrados na literatura que avaliam a suplementação do cromo, atualmente devido ao apelo comercial pela inclusão do mineral na forma orgânica nas dietas essas pesquisas são mais frequentes. O primeiro estudo realizado com a suplementação de cromo a bovinos estressados observou-se aumento no ganho de peso, melhor eficiência alimentar e diminuição do cortisol plasmático circulante (CHANG e MOWAT, 1992). Grande parte das pesquisas se concentraram na avaliação do cromo, a animais em condições de estresse, pois os resultados são controversos quando avaliado o fornecimento em condições de conforto (GHORBANI et al., 2020).

Os animais quando são submetidos ao estresse, apresentam aumento na secreção do hormônio corticotrofina produzido pela glândula hipófise anterior, essa ação resulta no rápido

aumento do cortisol, que apresenta grande influência no metabolismo da glicose através da estimulação gliconeogênese (FAÇANHA et al., 2008). Assim, o cortisol sinaliza os tecidos que deve realizadar a conversão de aminoácidos em glicose, consequência disso, reduz as reservas proteicas, diminui a síntese de proteína e aumenta o catabolismo proteico intracelular (BURTON, 1995; ANDERSON, 2001).

Os benefícios da suplementação de cromo já foram verificados através de desempenho e ganho de peso em animais submetidos a estresse por transporte (CHANG e MOWAT, 1992), assim como em recém-desmamados e bezerros em condições de estresse por calor (YANCHEV et al., 2008; KARGAR et al., 2018). Fortalecimento do sistema imune de vacas em lactação também já foi reportado (BURTON et al., 1993), além de melhoria na resposta do fator de tolerância a glicose (ZANETTI et al., 2003) e diminuição do cortisol sérico (PECHOVA e PAVLATA, 2007; KUMAR et al., 2013).

Bezerros em condições de estresse por calor apresentar aumento de glicose sanguínea após desmame e diminuição de cortisol quando receberam 0,04 mg de Cr/kg de $PC^{0,75}$, além disso, observaram diminuição da GTF em relação a glicose, o que possibilitou os autores sugerirem uma melhoria na eficiência da insulina (YARI et al., 2010). Ao contrário desse estudo, Ghorbani et al. (2012) verificou os benefícios da suplementação de cromo-metionina via colostro e leite para bezerras durante o aleitamento, observaram que não houve diferença no desempenho para os animais suplementados, entretanto, notou-se menor concentração de β-hidroxibutirato quando suplementadas.

Recentemente, Kargar et al. (2018) avaliou a suplementação de cromo durante o aleitamento e pós-aleitamento de bezerros submetidos a estresse por calor, usando a fonte de cromo-metionina na dose de 0,05 mg de Cr/kg de $PC^{0,75}$. Visualizaram diferença no maior consumo de matéria seca e concentrado de animais suplementados, apesar da diminuição da frenquência de refeições, durante todo o período experimental foi observado melhor ganho de peso e menor taxa de respiração, resultados importantes para bezerros submetidos a condições de estresse.

Após o desmame a suplementação de cromo favorece para minimização dos impactos do estresse oxidativo, principalmente através do aumento da enzima catalase (MOUSAVI et al., 2018). A catalase é uma importante enzima ao sistema antioxidante, está presente no combate ao estresse oxidativo desde o desiquilíbrio inicial, transforma peroxido de hidrogênio

em duas moléculas de água (NELSON e KIESOW, 1972). Diante do apresentado, o cromo pode ter impacto sobre o desenvolvimento de bezerros e contribuir para diversas funções ao metabolismo, sistema imune e antioxidante importantes, entretanto, não se conhece qual a dose ideal para suplementação dessa categoria e a via de fornecimento indicada, ou seja, via dieta líquida ou sólida.

1.6 OBJETIVOS

1.6.1 OBJETIVO GERAL

Avaliar se a inclusão de cromo orgânico, curcumina e minerais injetáveis na dieta de bezerros leiteiros melhora o fortalecimento do sistema imunológico e desempenho durante o aleitamento e desaleitamento.

1.1 OBJETIVOS ESPECÍFICOS

- Verificar se a suplementação injetável de minerais é capaz de ativar resposta antioxidante e reduzir o estresse oxidativo de bezerras recém-nascidas;
- Acompanhar o desempenho de bezerros suplementados com curcumina durante o aleitamento de desaleitamento;
- Verificar se a curcumina apresenta a capacidade de redução de oocistos de *Eimeria* spp. nas fezes de bezerros infectados.
- Avaliar qual a melhor via de fornecimento de cromo na dieta, atráves de aumento da eficiência alimentar;
- Mensurar o nível de cromo no soro de bezerros suplementados.

2 - CAPÍTULO II
ARTIGOS e/ou MANUSCRITO

Os resultados desta dissertação são apresentados na forma de dois artigos e um manuscrito, com sua formatação de acordo com as orientações das revistas as quais foram submetidas:

Artigo I - **Mineralization in newborn calves contributes to health, improve the antioxidant system and reduces bacterial infections**

Publicado: Microbial Pathogenesis

Artigo II - **Dietary addition of curcumin favors weight gain and has antioxidant, antiinflammatory and anticoccidial action in dairy calves**

Publicado: Revista Colombiana de Ciencias Pecuarias

Manuscrito I - **Organic chromium (milk replacer and concentrate) dairy calf feed: benefits for growth performance, feed efficiency, digestibility and health**

Submetido: Animal Feed Science and tchnology

2.1 Artigo I

Mineralization in newborn calves contributes to health, improve the antioxidant system and reduces bacterial infections

Abstract. The first phase of life of dairy calves has elevated mortality indices linked with low immunity and sanitary challenges, mainly bacterial infections are involved in the pathogenesis of diarrhea, the leading cause of death. Also, other important problem is the nutritional deficiencies, such as the mineral deficiency. Thus, the aim of this study was to evaluate whether an intramuscular mineral supplementation based on selenium, copper, potassium, magnesium and phosphorus possess beneficial effects on health of dairy calves. For this, ten calves were divided in two groups: the group A was supplemented with injectable mineral, while the group B was used as control group (without mineral supplementation). The mineral complex was administrated via intramuscularly at dose of 3 mL/animal on days 2 and 14 post-birth. The total blood was collected on days 2, 10, 20 and 30 of life of animals in order to analyze the antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)), blood count and seric biochemistry linked with proteic, lipid and carbohydrate metabolism. Feces samples were also collected on days 10, 20 and 30 of life of animals to perform the total bacterial count, parasitological exam and fecal consistency score. Moreover, the weight and corporal temperature were also evaluated. The mineral supplementation presented beneficial properties to calves from birth to the 30th of life through the increase on activity of antioxidant enzymes, improvement of immunity, and avoiding

problems linked with diarrhea and anemia, can be considered an interesting approach to prevent these alterations linked with high mortality in the period of life.

Keywords: dairy calves; mineral supplementation, health, bacterial infection, pathogenesis.

INTRODUCTION

The increase on the production of milk and dairy products, in addition to the expansion on dairy farming rise the need of technification and advances on cattle management, breeding, genetic improvement, and balanced nutrition [1]. In consequence of this intensification on dairy farming to supply the demand, some problems associated with animal health are observed, such as elevated mortality rate caused by low immunity and sanitary deficiency especially on the first few weeks of life [2]. Also, nutrient-poor diets have been linked to the impairment of the immune system and high mortality. Thus, dietary supplementation has gained prominence as alternative to meet animal´s nutritional requirements, mainly those associated with mineral needs.

Nutritional supplementation, such as minerals, develops an essential role to animal metabolism, since they participate as enzymatic cofactors, contributing to proper cellular and tissue functions, and the improvement of the immune system, and consequently, increased animal resistance against infectious diseases and improved animal performance [3]. Some studies have demonstrated the importance of minerals to animal health, such as the use of commercial formulations based on zinc (Zn), selenium (Se), copper (Cu), potassium (K), magnesium (Mg) and phosphorus (P) [4]. The Se acts as a potent antioxidant and cofactor to the enzyme glutathione peroxidase (GPx), that is responsible to convert hydrogen peroxide on water, as well as it participates in the conversion of the hormones thyroxine (T4) and

triiodothyronine (T3) [5]. On the other hand, Cu plays important functions linked to bone tissue, blood circulation, cellular respiration, immune response, and protection against oxidative stress [6,7]. Similarly, Mg is cofactor of oxidative phosphorylation, mitochondrial regulation, and participates as an intermediary substance in the citric acid cycle [8], as well as the P that is essential to bone development [8]. Based on the importance of minerals to animal health, dietary supplementation can be considered an approach to improve animal health, especially on a critical phase of calf´s life where diarrhea is a frequent problem [9]. Since mineral supplementation also has been used successfully in cows [4,10]. Therefore, the aim of this study was to evaluate whether intramuscular mineral supplementation exerts any beneficial effect to the health of dairy calves, and consequently, prevents diarrhea.

MATERIAL AND METHODS

Mineral supplementation

Each calf received two intramuscular doses (3 mL) of a commercial mineral complex Fosfosal® (Virbac, France) on days 2 and 14 post calving. Each 100 mL of the product is composed of sodium glycerophosphate (14 g), monosodium phosphate (20.1 g), copper chloride (0.4 g), potassium chloride (0.6 g), magnesium chloride (2.5 g), sodium selenite (0.24 g) and sterile water. The used dose was based on manufacturers recommendation to adult cows, but adjusted for calves.

Animals and experimental design

This study was conducted in a commercial dairy farm located in Chapecó city, Santa Catarina State, Brazil. Ten Holstein calves born in intervals of 3 days were divided into two

groups: the group A was composed by five calves supplemented with mineral complex, while the group B was composed by five non-supplemented calves (the control group). Each animal was allocated in individual stalls and received 4L of integral cow milk daily. At 7 days of live, they also received protein concentrate, hay and water *ad libitum.* The animals were observed for 30 days.

Sample collection

Blood collection was performed on days 2, 10, 20 and 30 of life through the jugular vein using tubes containing EDTA 10 % (for blood count), sodium citrate (to evaluate the antioxidant enzymes) and without anticoagulant to obtain serum (for seric biochemistry). The serum was obtained through centrifugation (7000 rpm) during 10 min. Serum samples were stored at – 20 ºC.

To evaluate GPx activity, blood samples were centrifuged at 2500 rpm during 10 min. After centrifugation, platelets, leukocytes and plasma were discarded, and the blood cells were homogenized in PBS solution. This procedure was repeated three times. Finally, erythrocytes were stored at – 20 ºC until analyzis.

Fecal samples were collected directly from the rectal ampulla on days 10, 20 and 30 of the experiment to perform parasitological examinations using the centrifugal flotation technique. These samples were also used to determine the bacterial counts on days 10 and 30 of the experiment, according to the technique of serial dilutions followed by Petrifilm (3M) plating. Also, in order to evaluate the occurrence of diarrhea, fecal consistency was observed and analyzed according to the technique described by Ferreira et al. [11].

Body weight and body temperature were determined in the same sampling days.

Blood counts

The total count of erythrocytes and leucocytes, as well as the hemoglobin concentration were performed using a semi-automatic cell counter (CELM model CC530). The differential count was analyzed through blood smears stained with Romanowsky method and visualized under optical microscopy.

Seric biochemistry

Seric levels of urea, glucose, total proteins and albumin were measured using commercial kits (Analisa Golg) in a semi-automatic equipment (BioPlus 2000). Globulin levels were obtained through the mathematical formula: total protein – albumin.

Antioxidant enzymes

SOD activity

SOD activity in total blood was based on the inhibition of the radical superoxide reaction by adrenaline as described by McCord and Fridovich [12]. A unit of SOD is defined as the amount of enzyme that inhibits by 50% the speed of adrenalin oxidation. It leads to formation of the red-colored product, adrenochrome, which is detected by a spectrophotometer. SOD activity is determined by measuring the speed of adrenochrome formation, observed at 480 nm, in a reaction medium containing 50 mM glycine and NaOH, pH 10 and 1 mM adrenalin. The results were expressed as UI SOD/mg of protein.

CAT activity

CAT activity was measured in erythrocytes according to Nelson and Kiesov [19]. The buffer for the assay of CAT was the potassium phosphate buffer (TFK) 50 mM pH 7.5. CAT activity was determined by the decomposition of H_2O_2 at 240 nm. The enzymatic activity was expressed in nmol CAT/mg of protein.

GPx activity

GST activity was assayed spectrophotometrically at 340 nm by the method of Gunzler et al. [20]. The mixture contained an aliquot of erythrocytes, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH, and 100 mM CDNB, which was used as substrate. The enzymatic activity was expressed as Ug/Hg.

Statistical analyses

The data were evaluated firstly by descriptive statistics for contingency of the information and for further assumptions which are presented as descriptive for mean and standard deviation. All parameters: CAT, SOD, GPx, erythrocyte, hemoglobin, leukocyte, lymphocyte, neutrophil, monocyte, eosinophil, total protein, albumin, globulin, glucose, urea and average weight gain were evaluated. The data were tested for normality using the Shapiro-Wilk test, and the skewness, kurtosis and homogeneity by the Levene test. A Student's t-test was used to analyze all parameters that showed differences when comparing each group at each time period (days 1, 10, 20 and 30 of life), and bacterial counts (days 10 and 30). 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).

RESULTS

Clinical signs, body weight and temperature

All calves from the control group showed diarrhea during the of experiment, while supplemented calves were healthy. It is important to emphasize that all animals from the control group also showed pneumonia with seven days of life, and were treated with sulfa and trimethoprim (15 mg/kg). The occurrence of pneumonia can be explained by a wide temperature variation (9-25 ºC) in the same day, as well as excessive ventilation in wide open facilities. One animal from the control group died at 15 days of life due to pneumonia. None supplemented animal had pneumonia during the experimental period.

No difference was observed between groups regarding body weight, weight gain and body temperature (Table 1). It is important to emphasize that animals from the control group showed hyperthermia (higher than 39.3 ºC), that is, a higher temperature when compared to reference values for healthy animals. The average body temperature for animals of the groups A and B on days 10, 20 and 30 of life was 38.5 and 39 ºC, 38 and 39.5 ºC, e 38.2 e 39.8 ºC, respectively.

Blood count

The number of erythrocytes was significant lower for animals of the group B (the control group) in the end of the study (Figure 1-A), similarly to hemoglobin concentration on day 30 (Figure 1-B). On day 20 of the experiment, the number of total leukocytes, lymphocytes and eosinophils increased for animals of the group B (Figure 1-C-D-E) compared to animals of the group A (supplemented). The number of neutrophils and monocytes did not differ between groups throughout the experiment ($P>0.05$) (data not shown).

Seric biochemistry

Animals of the group B showed a drastic reduction on total protein levels on days 10 and 20 compared to the group A (Figure 2-A), similarly to globulin levels in the same periods ($P<0.05$; Figure 2-C). The albumin levels increased significantly in animals of the group B on days 10, 20 and 30 compared to the group A (Figure 2-B). Seric glucose levels were significantly higher in calves of the group A on days 10, 20 and 30 of the experiment compared to the group B (Figure 2-D). There was no significant difference between groups regarding seric urea levels (Table 1).

Antioxidant enzymes

In calves of the group A, there was significant ($P<0.05$) increase in CAT activity (Figure 3-A). Already, a significant decrease of CAT in the supplemented animals (group B) was observed on days 10, 20, 30 of age over time. The SOD activity (Figure 3-B) reduced in the group B on day 10, but increased on day 30. On the group A, the SOD increased on day 10, but showed a drastic reduction in the following periods. In animals of the group A, the GPx activity increased ($P<0.05$) on days 10, 20 and 30 of age, while in the animals of the group B, the GPx activity was lower compared to the group A (Figure 3-C), with a steady pattern.

Total bacteria count in feces and parasitological examination

There was no significant difference between groups regarding total bacterial counts (Figure 4). However, the number of bacteria was higher in animals of the control group, even

after antimicrobial treatment, as already mentioned in section 3.1. During the study, all the animals of both groups had *Giardia* spp. infection, with no statistical difference between groups ($P>0.05$) regarding the degree of infection by this parasite (most of the animals had mild infection). No correlation was observed between the occurrence of diarrhea and the degree of bacterial or parasitic infections ($P>0.05$).

DISCUSSION

Overall, we found that the mineral supplementation was beneficial to the health of dairy calves. This effect can be explained due to the properties of each mineral present in the commercial mineral complex used in this study, such as the antioxidant and immunological effects of Se [15] and Cu [16], as well as the essential role of Cu in the erythropoiesis [17]. The most important finding was that mineral supplementation stimulated erythropoiesis, improved the antioxidant system, prevented diarrhea and reduced bacterial counts in dairy cows.

In this present study, we observed that supplemented animals had higher erythrocyte and hemoglobin counts compared to the control group, similarly to what was observed by Soldá et al. [4] in dairy cows during the transitional period. This can be explained by the essential role of Cu to erythropoiesis, since this mineral is involved with red cell synthesis and is an essential component of adult red cells, but the manner whereby copper influences erythropoiesis is obscure [18]. Also, it is important to emphasize that Cu is an essential component of mature red cells, and when Cu concentration inside the erythrocyte is below a critical level, the survival time of the cells is shortened. Thus, the Cu supplementation can increase survival time of erythrocytes in the circulation, which explains the levels of

erythrocytes and hemoglobin found in those supplemented dairy cows [19]. On the other hand, the total number of leukocytes, lymphocytes and eosinophils were lower in the supplemented calves compared to the control group, which demonstrates the beneficial effect of mineral supplementation on the immune system [20]. It is important to emphasize that animals from the control group showed pneumonia during the experiment, and higher counts of leukocytes, lymphocytes and eosinophils can be explained due to the inflammatory response caused by disease [21], which corroborates to the hypothesis that mineral supplementation improves the immune system, as well as prevented the occurrence of pneumonia. Thus, the supplementation with minerals stimulates erythropoiesis and improves the immune system, and can be considered an approach to prevent anemia or infectious diseases on the first phase of life of dairy cows.

A positive effect of mineral supplementation is also linked with total protein and globulin levels, that is, the minerals prevented the reduction in these variables in dairy calves, in accordance to what was observed by Soldá et al. [4] in dairy cows during the transitional period. The decrease on seric protein levels is associated with low immunity [22], since the reduction on total protein levels is associated with the reduction of globulin levels, an important protein for the development of an effective immune response [23]. Thus, the use of mineral supplementation can contribute to prevent the impairment on immune system, such as the presence of Se, an important mineral with anti-inflammatory effects [24]. On the other hand, we observed an increase on seric glucose and albumin levels in the supplemented calves, that can indicate physiological stress and possible hepatic alterations linked with excessive amounts of one or more minerals. According to Barton and Iwama [25], an increase in blood glucose occurs as a response to a stressor, in order to provide most of the energy demand to

cope with stress. In this sense, a study conducted by Uriu-Adams and Keen [26] demonstrated that excessive Cu levels can induce stress observed by augmentation on glucose levels. Also, the decrease on seric levels of albumin can be considered an indicative of hepatic alteration, since 100 % of albumin is synthetized in the liver [27]. In this sense, evidences have demonstrated that excessive Cu [28] and zinc [29] levels induce hepatic damage, corroborating to our findings. Thus, the use of mineral supplementation to dairy calves can improve immune responses, but the excessive presence of one or more compounds can induce hepatic alterations.

The CAT, SOD and GPx activities were higher in supplemented dairy calves when compared to the control group, which demonstrates that mineral supplementation was able to stimulate the antioxidant defense system, as observed by Soldá et al. [4] in dairy cows supplemented with minerals during the transitional period. The increase on these antioxidant enzymes can be explained because the Cu, Zn, Mg and Se are cofactors of these antioxidant enzymes [30,31]. Recently, several studies have demonstrated the protective effects of Se [32], Cu [33], Mg [34] and Zn [35] against oxidative stress through the improvement on the antioxidant defense system, as observed in this study. As an example, the increase on CAT and GPx activities can contribute to convert more rapidly the excessive levels of hydrogen peroxide (H_2O_2), a molecule with oxidant properties [36]. Thus, the use of mineral supplementation improves the antioxidant defense system in dairy calves in the first phase of life.

In the study, we observed that mineral supplementation prevented the occurrence of diarrhea in dairy calves, in accordance to Thomaz et al. [37] in a diet for piglets containing organic trace mineral. Evidence has suggested that Cu and Zn supplementation exert an

important role in the reduction of acute diarrhea [38,39]. According to these authors, Zn supplementation exhibits therapeutic action by facilitating the transport of water and electrolytes across the intestinal mucosa, and consequently, reduces or prevents the occurrence of diarrhea. Thus, the use of mineral supplementation can contribute to prevent or reduce the occurrence of diarrhea in dairy calves.

Based on these evidences, the injectable mineral supplementation with Se, Mg, Cu, K and P exerted beneficial effects on animal health through the stimulation of erythropoiesis, improvement of the immune system, activation of antioxidant defense system, and on the prevention of diarrhea. Therefore, the supplementation possesses beneficial effects on the health of dairy calves on the first 30 days of age.

Ethics Committee

This study was approved by the Ethics Committee on Animal Use (CEUA) of the Universidade do Estado de Santa Catarina (UDESC), under protocol number 8075050716.

Conflict of interest

The authors declare no conflict of interest.

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Table 1: Mean and standard deviation of body weight, average weight gain and urea levels on 1, 10, 20 and 30 days of age.

Variable	**Days post calving**	**Mean ± standard deviation**		
		Group A	Group B	**p-value**
Body weight (kg)	1	37.0 (3.2)	37.1 (3.0)	0.89
	10	38.0 (2.9)	37.9 (3.1)	0.82
	20	39.8 (4.0)	37.2 (4.3)	0.34
	30	40.0 (4.1)	39.3 (3.9)	0.72
Average weight gain (kg)	1	-	-	-
	10	1 (2.8)	0.8 (1.3)	0.89
	20	2.8 (3.3)	0.1 (3.1)	0.24
	30	3.0 (2.2)	2.2 (1.5)	0.52
Urea (mg/dL)	1	26.0 (9.7)	27.4 (10.7)	0.83
	10	30.8 (3.2)	32.0 (13.0)	0.85
	20	27.2 (5.4)	32.5 (10.4)	0.40
	30	32.6 (7.1)	28.7 (7.0)	0.44

Note: The group A (mineral supplemented); the group B (the control group).

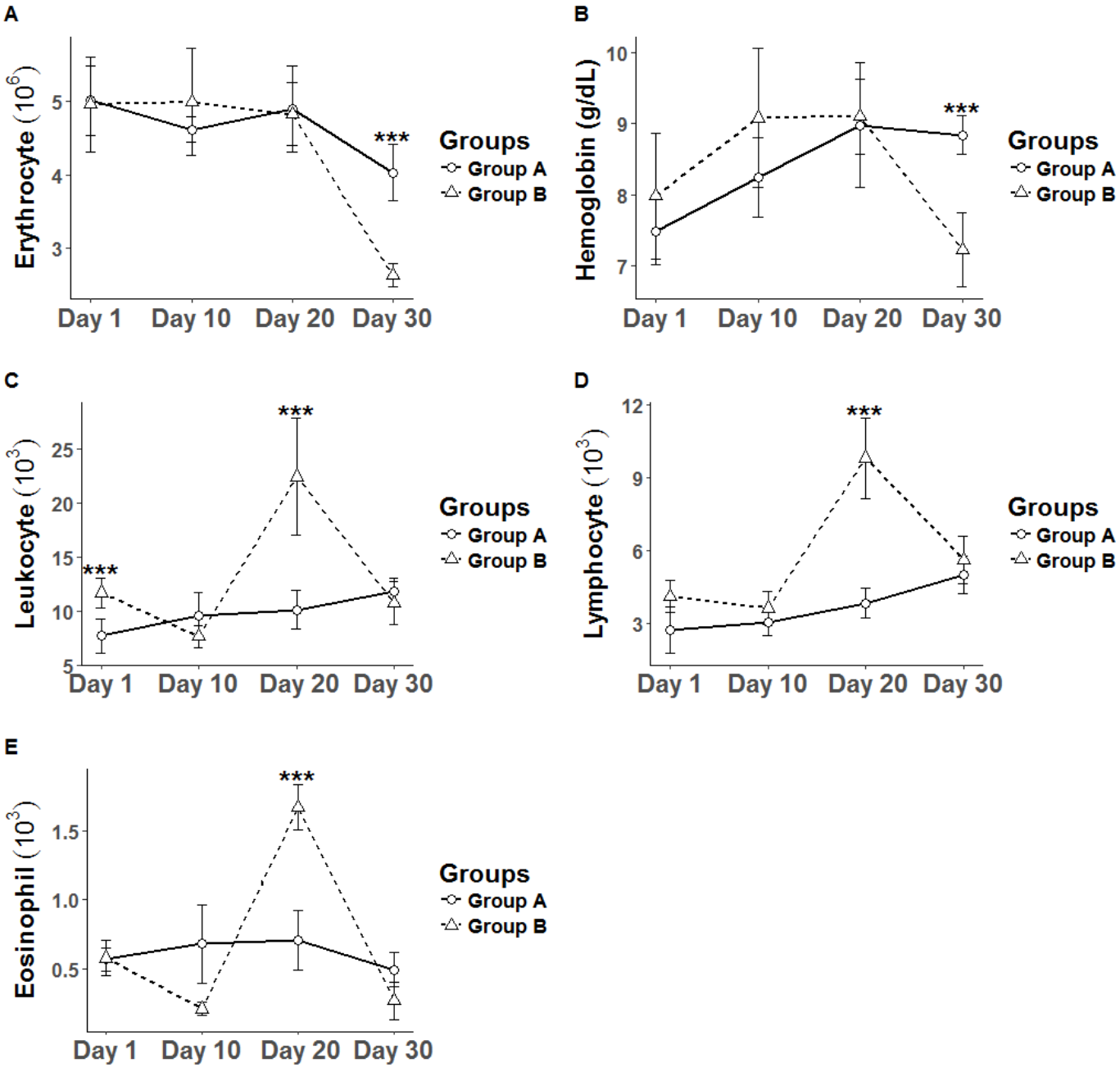

Figure 1: Erythrocytes, hemoglobin, leukocytes, lymphocytes and eosinophils counts in dairy calves supplemented with minerals on days 1, 10, 20 and 30 of age. * Indicates significant difference between groups ($P<0.05$). Note: the group A (mineral supplemented); the group B (the control group).

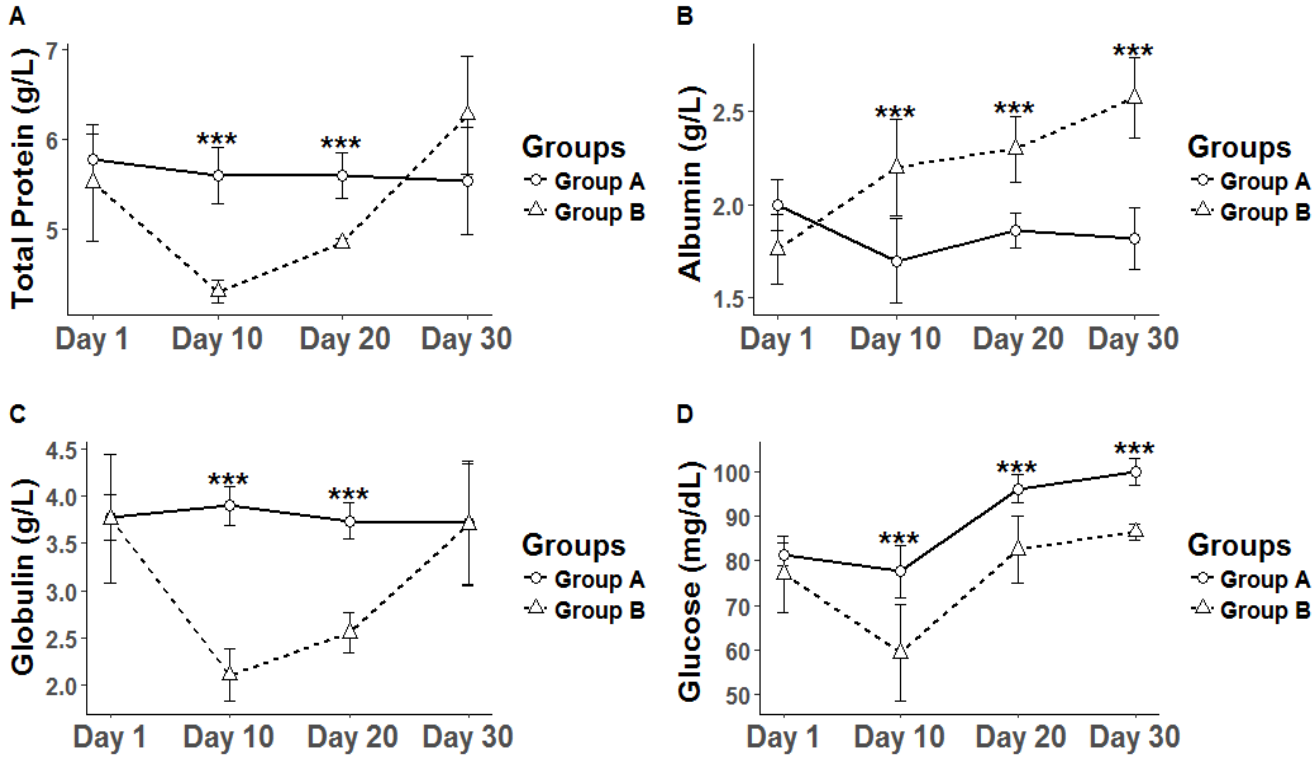

Figure 2: Seric levels of total protein [A], albumin [B], globulin [C] and glucose [D] in dairy calves supplemented with minerals on days 1, 10, 20 and 30 of age. * Indicates significant difference between groups ($P<0.05$). Note: The group A (mineral supplemented); the group B (the control group).

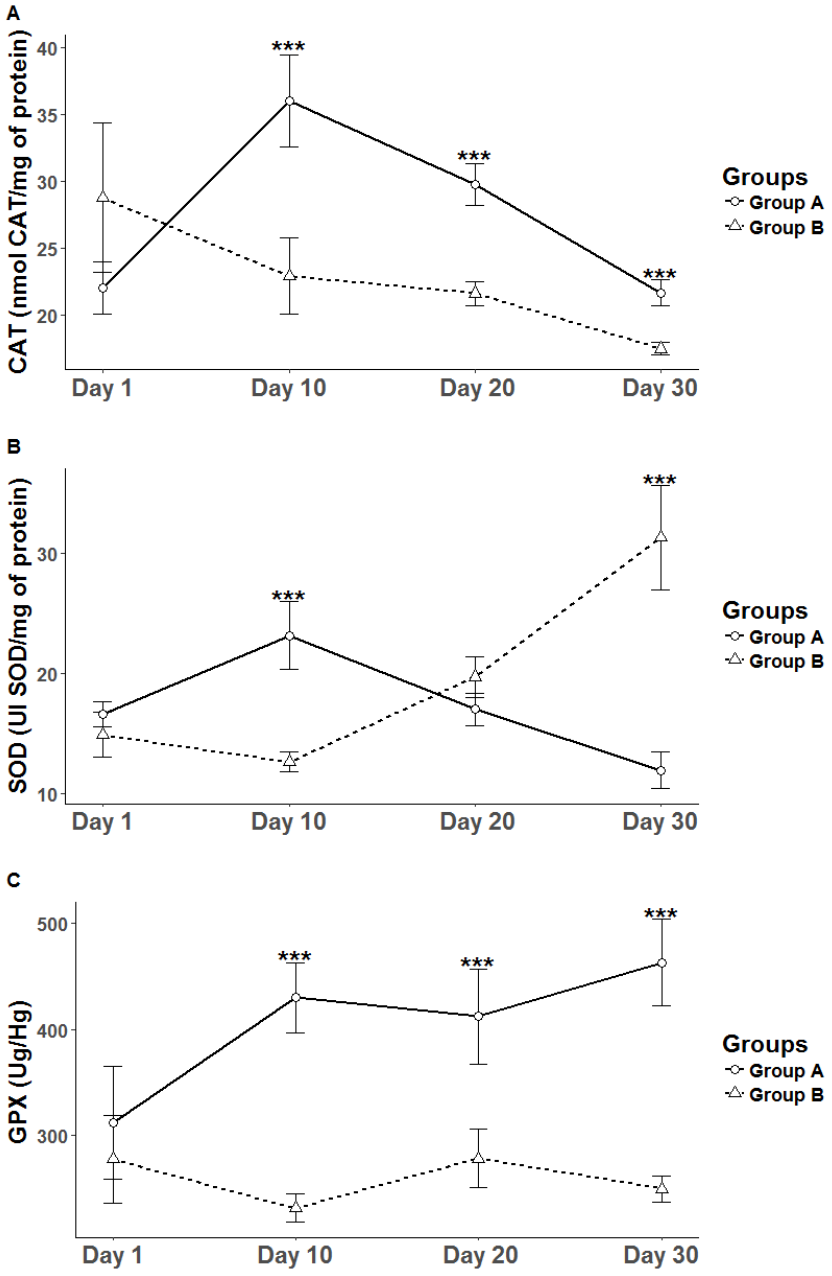

Figure 3: Catalase (CAT) [A], superoxide dismutase (SOD) [B] and glutathione peroxidase (GPx) [C] activities in dairy calves supplemented with minerals on days 1, 10, 20 and 30 of age. *Indicates significant difference between groups ($P<0.05$). Note: the group A (mineral supplemented); the group B (the control group).

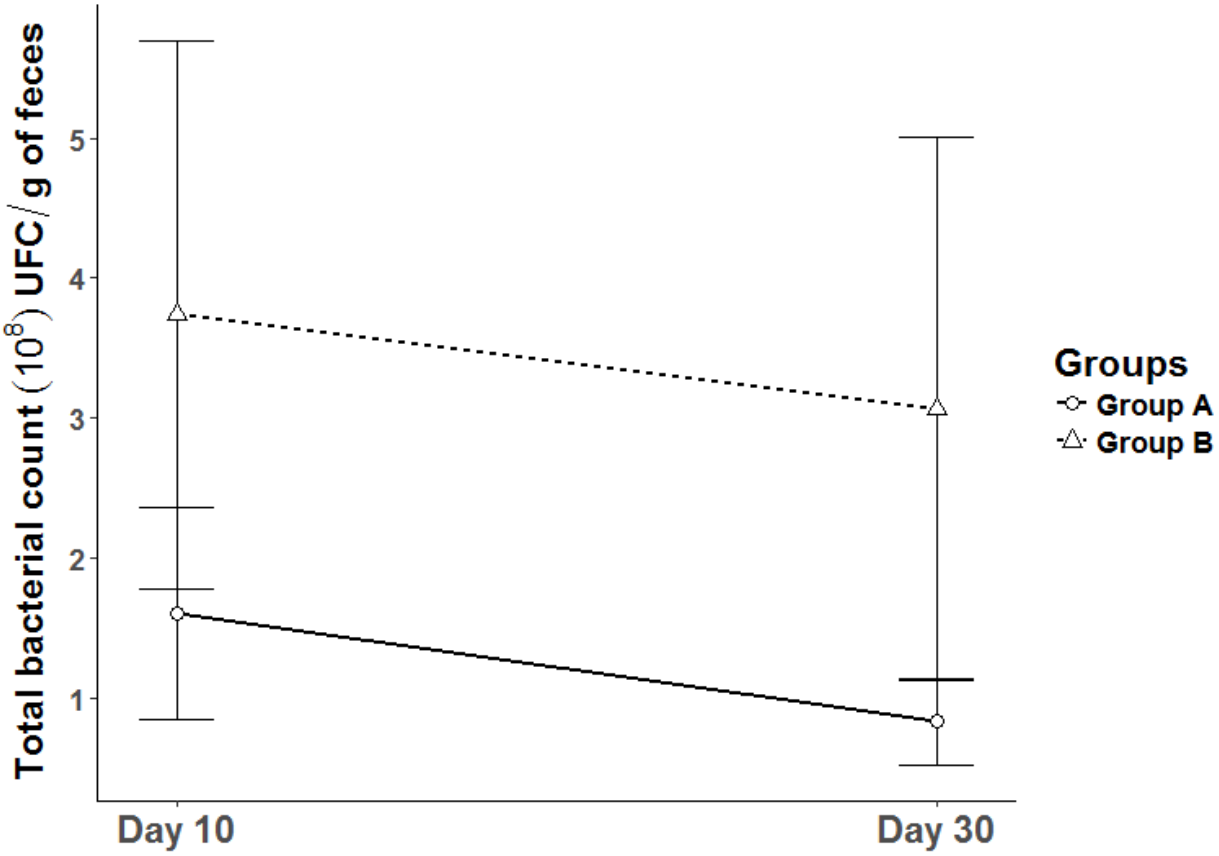

Figure 4: Bacterial counts numerically higher in the control group despite antibiotic treatment, but without statistical difference between groups on both tested moments (P>0.05). Note: the group A (mineral supplemented); the group B (the control group).

2.2 ARTIGO II

Addition of curcumin in dairy calf diet favors weight gain and has antioxidant, anti-inflammatory and anticoccidial action

La adición de curcumina en la dieta de los terneros lecheros favorece el aumento de peso y tiene acción antioxidante, antiinflamatoria y anticoccidial

A adição de curcumina na dieta dos bezerros leiteiros favorece o aumento de peso e tem ação antioxidante, anti-inflamatória e anticoccidiostática

Abstract

Objective: The objective of this study was to evaluate whether the addition of curcumin to the diet of calves at different stages (pre- and post-weaning) has a positive effect on metabolic profiles, performance, and anti-coccidian action. **Methods:** We selected 33 Holstein calves at various stages of development: experiment 1 (E1: n = 10) 18 ± 7 (preweaning), experiment 2 (E2: n = 11) 64 ± 4 (preweaning) and experiment 3 (E3: n = 12) 95 ± 8 (post-weaning) days of life. Were separated the calves in three groups according to phase of development. For each experiment, were divided animals into two sub-groups: control and curcumin. The curcumin groups received at 200 mg of additive per animal/day in milk (pre-weaning) or in concentrate (post-weaning). Fecal collections were performed on days 0, 10 and 15 of the experiment in order to count *Eimeria* oocysts per gram of feces and to perform fecal score analysis. Were measured complete blood counts, oxidant and antioxidant profiles, protein metabolism markers, lipid levels, glucose levels, and animal weights. Were also performed an analysis of the digestibility and composition of the diet offered to the animals in Experiment 3 (post-weaning). **Results:** Independent of phase, animals that received curcumin had greater weight gain of day 0 to 15 (E1, E2 and E3 p-values 0.04, 0.001 and 0.001, respectively), probably due to increased digestibility to hay and concentrate at 72 h (p=0.03 and 0.02, respectively). In the supplemented calves, we observed

on days 0, 10 and 15 of the experiment in order to count *Eimeria* oocysts per gram of feces and to perform fecal score analysis. Were measured complete blood counts, oxidant and antioxidant profiles, protein metabolism markers, lipid levels, glucose levels, and animal weights. Were also performed an analysis of the digestibility and composition of the diet offered to the animals in Experiment 3 (post-weaning). **Results:** Independent of phase, animals that received curcumin had greater weight gain of day 0 to 15 (E1, E2 and E3 p-values 0.04, 0.001 and 0.001, respectively), probably due to increased digestibility to hay and concentrate at 72 h (p=0.03 and 0.02, respectively). In the supplemented calves, we observed a lower level of oxidants (TBARS and ROS); that is, serum levels of free radicals and lipid peroxidation were lower. This was probably due to enzymatic antioxidants (GST (E1, E2 and E3 p-values 0.001, 0.001 and 0.02 respectively), CAT (E1 p-value 0.001) and SOD (E3 p-value 0.001) increasing in these treated animals at day 15. Furthermore, calves receiving curcumin had lower levels of *Eimeria* infection during the experimental period and significant in day 15 (E1 and E2 p-values 0.02, and 0.001 respectively). **Conclusions:** Supplementation of curcumin has coccidiostatic potential, favoring weight gain.

Keywords: *animal stress, calves, cattle growth, Eimeria, parasitology, supplementation.*

Resumen

Objetivo: El objetivo fue evaluar si la adición de curcumina en la alimentación de terneras en diferentes fases (pre y post-destete) presenta efecto positivo sobre perfil metabólico y desempeño de estos animales, y acción anti-coccidéos. **Métodos:** Para esto, se seleccionaron 33 terneros Holstein en varias etapas de desarrollo: experimento 1 (E1: n = 10) 18 ± 7 (pre-destete), experimento 2 (E2: n = 11) 64 ± 4 (pre-destete) y experimento 3 (E3: n = 12) 95 ± 8 (post-destete) días de vida. Para todos los experimentos el período experimental fue de 15 días, así como fueron delineados con dos grupos: control y tratados con curcumina. Los grupos curcumina recibieron la dosis de 200 mg de aditivo por animal/día en la leche (pre-destete) y en el concentrado (post-destete). Las colectas de heces y sangre fueron realizadas en los días 0, 10 y 15 de experimento para conteo de ooquistes de *Eimeria* por gramo de heces y análisis de puntaje fecal. De la sangre tomada se realizó el hemograma, perfil oxidante y antioxidante, metabolismo proteico, lipídico, glucosa sanguínea y pesaje de los animales.

También se realizó análisis de digestibilidad de la dieta total ofrecida de los animales del experimento 3 (post-destete). **Resultados:** Encontramos que, independientemente de la fase, los animales que recibieron curcumina tuvieron una mayor ganancia de peso en los días 0 a 15 (valores p de E1, E2 y E3 de 0.04, 0.001 y 0.001, respectivamente), probablemente debido al aumento de la digestibilidad al heno y al concentrado a las 72 h. (p=0.03 y 0.02, respectivamente). En los terneros suplementados, observamos un nivel más bajo de oxidantes (TBARS y ROS), es decir, los niveles séricos de radicales libres y la peroxidación lipídica fueron más bajos. Esto se debió probablemente a los antioxidantes enzimáticos (GST (E1, E2 y E3 p-valores 0,001, 0,001 y 0,02 respectivamente), CAT (E1 p-valor 0.001) y SOD (E3 p-valor 0.001) que aumentaron en estos animales tratados al día. 15. Además, los terneros que recibieron curcumina tuvieron niveles más bajos de infección por Eimeria durante el período experimental y fueron significativos en el día 15 (valores p de E1 y E2 de 0,02 y 0,001, respectivamente). **Conclusión:** La suplementación de curcumina tiene el potencial coccidiostático, favorece la ganancia de peso.

Palabras clave: *crecimiento de ganado, Eimeria, estrés animal, parasitología, suplementación, terneros.*

Resumo

Objetivo: O objetivo foi avaliar se a adição de curcumina na alimentação de bezerras em diferentes fases (pré e pós-desmame) apresenta efeito positivo sobre perfil metabólico, desempenho e ação anti-coccidéo. **Métodos:** Para isso, 33 bezerros holandeses foram selecionados em vários estágios de desenvolvimento: experimento 1 (E1: n=10) 18 ± 7 (pré-desmame), experimento 2 (E2: n=11) 64 ± 4 (pré-desmame) e experimento 3 (E3: n=12) 95 ± 8 (pós-desmame) dias de vida. Para todos os experimentos o período experimental foi de 15 dias, assim como foram delineados com dois grupos: controle e tratados com curcumina. Os grupos de curcumina receberam 200 mg do aditivo por animal/dia no leite (pré-desmame) ou em concentrado (pós-desmame). Coletas de fezes e sangue foram realizadas nos dias 0, 10 e 15 de experimento para contagem de oocistos de Eimeria por grama de fezes e análise de escore fecal. Do sangue colhido foram realizados o hemograma, perfil oxidante e antioxidante, metabolismo proteico, lipídico, glicose, além da pesagem dos animais. Também foi realizado

análise de digestibilidade da dieta total ofertada aos animais do experimento 3 (pós-desmame). **Resultados:** Independentemente da fase, os animais que receberam curcumina tiveram maior ganho de peso do dia 0 a 15 (valor de p para E1, E2 e E3 foi 0,04, 0,001 e 0,001, respectivamente), provavelmente devido ao aumento da digestibilidade ao feno e concentrado após 72h (p=0,03 e 0,02, respectivamente). Nos bezerros suplementados, observou-se menor nível de oxidantes (TBARS e ROS), ou seja, os níveis séricos de radicais livres e a peroxidação lipídica foram menores. Isto foi provavelmente devido a antioxidantes enzimáticos (GST (valores de p para E1, E2 e E3 foi 0,001, 0,001 e 0,02, respectivamente), CAT (valor de p para E1 foi 0,001) e SOD (valor de p para E3 foi 0,001) aumentando nestes animais tratados no dia 15. Além disso, bezerros recebendo curcumina tiveram menores níveis de infecção por *Eimeria* durante o período experimental e significativos no dia 15 (valores de p de E1 e E2 foi 0,02 e 0,001, respectivamente). **Conclusão:** A suplementação de curcumina aumenta o potencial coccidiostático e favorece o ganho de peso.

Palavras-chave: *bezerros, crescimento do gado, Eimeria, estresse animal, parasitologia, suplementação.*

Introduction

The production phase in calves is a challenging period, mainly due to the fact that the animal is born without antibodies and depends on the fast and efficient administration of colostrum (Santos *et al.,* 2010). Extrinsic factors such as the quality of the facilities affect the comfort of the calf, with potential to damage health. There may also be an associated lack of hygiene that favors the growth of disease-causing pathogens (Mota *et al.,* 2000). Thus, calves suffer high rates of diarrhea, pneumonia and poor performance, resulting in high morbidity and mortality in severe cases (Butler *et al.,* 2000).

In recent years, several drugs have been used to overcome or minimize these problems in calf rearing. However, when they are used excessively, cases of microorganism resistance occur (Obaidat *et al.,* 2018). Generally, treatments performed at this stage are costly, require more labor, and risk harm or death of the calf due to drug inefficiency. Faced with these challenges,

alternatives are sought for prevention, control, and cure of diseases of early life. Supplementation with plant extracts can be justified if they augment immune responses and antioxidant levels, leading to better animal performance (Busquet *et al.,* 2000). In recent years, an additive known as curcumin has been studied in poultry, showing promise in terms of improved meat quality and chicken performance (Fascina *et al.,* 2012; Kim *et al.,* 2013; Zhang *et al.,* 2015).

Curcumin is extracted from *Curcuma longa* L., a plant belonging to the family Zingiberaceae, popularly known as saffron (Maia *et al.,* 1995). The substance is found in the rhizome of the plant. The active compound is thought to be diphenylmethane, a hydrophobic polyphenol that confers color and aromatic characteristics (Bezerra *et al.,* 2013). The beneficial effect of curcumin is related to its structure, *i.e.* the aromatic ring hydroxyls, the double bonds in the alkene, and the diketone portion. These confer anti-inflammatory, antibacterial, antiviral, anticancer, antioxidant, and coccidiostatic activities (Almeida, 2006; Pelícia *et al.,* 2015). Although it has known benefits in poultry, it is unclear as to whether curcuminmay be useful in calves. Therefore, the objective of this study was to evaluate whether the addition of curcumin in the diet of calves at various stages (pre- and post-weaning) has positive effects on metabolic profiles, coccidiostatic activities and animal performance.

2 Materials and Methods

Ethical Considerations

The procedure was approved by the Animal Welfare Committee of Universidade do Estado de Santa Catarina (UDESC), under protocol number 3067300717.

Curcumin

Curcumin was purchased from Shaanxi Jiahe Phytochem Ltd/China (99% pure).

Experimental location

The study was carried out on a commercial farm located in the municipality of Chapecó, Santa Catarina, Brazil. The experimental period was 15 days and included animals at different stages

(pre- and post-weaning). Pre-weaning calves remained in individual pens, receiving four liters of milk per day, with access to concentrate, hay and water ad libitum, while the post-weaning calves were housed in collective pens with six animals. The diet offered was based on hay and concentrate with *ad libitum* access to water.

Experimental design

First, fecal collection and analysis was performed to quantify the number of *Eimeria* oocysts per gram of feces to identify and divide the groups. Three groups of Holstein breed calves were divided according to phase of life, as follows: Experiment 1 (E1) included 10 animals at the beginning of nursing, average age 18 ± 7 days (control group (n = 5) and treated group (n = 5)); Experiment 2 (E2) included 11 calves who were close to weaning, average age 64 ± 4 days (treated group (n = 6) and a control group (n = 5)); and Experiment 3 (E3) included 12 weaned animals, age 95 ± 8 days (treated group (n = 6) and a control group (n = 6)). The oocyte number of coccidian and age of the animals was used to divide the animals randomly into control and the experimental groups.

Soon after birth, calves received 4 liters of colostrum immediately (first 24 hours), followed by transitional milk (days 2 to 5), and subsequently post-day 6 animals were fed with 4 liters of pasteurized milk, divided twice a day, provided orally with the aid of a bottle (Experiments 1 and 2). The animals in the treated group received 50 mg of curcumin per liter of milk, totaling 200 mg per day. Experimental 3 animals ingested 100 mg of curcumin/kg of concentrate supplied (2 kg of concentrate per day were given to each calf), giving approximately 200 mg of curcumin per day. In the three experiments, the animals fed individually.

This is the first study to provide curcumin in calf diets. Nevertheless, other studies have reported curcumin in diets of dairy sheep (80 mg/animal/day) and lambs (52, 100 and 200 mg/kg feed) (Cervantes-Valencia e*t al.*, 2016; Jaguezeski *et al.*, 2018; Molosse *et al.*, 2019). There is no established dose of curcumin in the ruminant diet; nevertheless, based on these previous experiments, we chose 200 mg/animal/day.

Sample collection

Blood samples were taken on days 0, 10 and 15 of the experiment, via jugular venipuncture. Blood was collected in vacuum tubes either containing anticoagulants (sodium citrate and 100% EDTA) or without anticoagulants. Samples were stored in isothermal ice boxes at 10°C for transport to the laboratory. Samples collected in tubes without anticoagulant were centrifuged at 7000 rpm for 10 minutes to obtain serum.

Feces were collected on days 0, 10 and 15 of the experiment, directly from the rectal ampulla. Samples were stored on ice for transport to the laboratory, where they were maintained at 5°C until analysis.

Body weight

At the collection dates, the animals were also weighed using a commercial tape measure, calculated using the correlation of the thoracic perimeter with the weight. (Reis *et al.*, 2008). This tape is sold commercially, with the animals in the station anatomical position, in which the animal finds itself with the four limbs resting on the ground and the head and looking forward.

Hemogram

Hemogram was performed within 2 hours of sample collection. The erythrocyte count, total leukocyte and hemoglobin counts were performed using a semi-automatic blood cell counter (model CELM CC530). Hematocrit was determined using capillary tubes, centrifuged for 5 minutes at 10,000 rpm. The leukocyte differential was performed by staining the blood smear with a *Panótico Rápido* kit.

Serum biochemistry

Serum levels of total proteins, albumin, globulin, urea, cholesterol, glucose, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and creatinine were evaluated using a commercial kit (ANALISA®) on semi-automatic equipment (BIO PLUS 2000®). Globulin levels were obtained by subtraction (total proteins – albumin).

Antioxidant enzymes

In whole blood, catalase (CAT) enzyme activity was analyzed according to the technique described by Nelson and Kiesow (1972), where the enzyme activity is determined as hydrogen peroxidation and measured with absorbance at 240 nm. Results are expressed in U CAT/mL. Superoxide dismutase (SOD) was determined in whole blood according to a method described by McCord and Fridovich (1969). Results are expressed as U SOD/mL. Activity of the antioxidant enzyme glutathione S-transferase (GST) was measured using the method described by Habig *et al.* (1974). Results are expressed as nmol/mg protein.

Oxidants

In plasma, levels of reactive oxygen species (ROS) were measured according to a method described by Ali *et al.* (1992). Absorbance was 488 nm, and emission was 525 nm, measured in an LS-50 spectrophotometer. Free radical formation was quantified from a standard DCF curve in methanol (0.05-1 μM), the results were expressed as U DCF/mL.

Lipid peroxidation was determined by the levels of thiobarbituric acid reactive substances (TBARS) according to a method described by Jentzsch *et al.* (1996). The results were expressed as nmol malondialdehyde/ml.

Stool parasitology

Fecal samples were used to determine the number of *Eimeria* oocysts per gram of feces, using a technique adapted from Faust and collaborators (Monteiro, 2010).

Fecal score

The fecal score determination is a simple methodology for determining the condition of the animals' stools. The fecal scoring system we used was described by Larson *et al.* (1977), where the scale for fluidity was as follows: 1, normal; 2, soft; 3, watery; and 4, fluid. For consistency, the score was as follows: 1, normal; 2, frothy, 3, mucous; 4, sticky; and 5: very firm. Color was scored as follows: 1, white; 2, gray; 3, yellow; 4, brown; 5, red (blood present); 6, green; and 7, very dark. Finally, the odor score was as follows: 1, normal; 2, mildly malodorous; and 3, highly malodorous.

In vitro digestibility

For determination of the apparent digestibility *in vitro*, samples of oat hay and commercial concentrate were prepared to simulate the total diet supplied to the calves. We used ruminal fermenter DAISY®, and feces from calves served as the inoculum. The feces were collected directly from the rectal ampulla, yielding approximately 800 g. The incubation was performed according to a method described by Alcalde *et al.* (2001), in two stages of fermentation (48 h ruminal fermentation followed by 24 for acid and enzymatic digestion). Half of the replicates were removed at 48 h of incubation, and the other half were digested for 72 h.

The samples were ground in a knife-type mill with a 1 mm grid. Afterwards, 250 mg of each food sample was incubated in TNT bags (Lopes *et al*., 2014), in quadruplicate. In two vials of the fermenter, curcumin was added at a dose of 0.0125 mg for each 250 mg food sample, simulating 250 mg per kilogram of ingested concentrate per animal.

Dietary analysis

Samples were collected from the total diet provided (concentrate and hay) for all calves in the three experiments (Table 1). From these, dry matter, mineral matter, ether extract and crude protein were measured, following a method described by Silva and Queiroz (2006). In addition, neutral detergent fiber and acid detergent fiber were analyzed followed the methodology described by Van Soest (1994).

Table 1. Nutritional composition of oat hay and concentrate supplied to calves.

Composition	**Hay**	**Concentrate**
Dry Matter (%) in dry matter	95.5	89.0
Mineral Protein (%) in dry matter	7.5	7.3
Crude Protein (%) in dry matter	12,0	16,0
Ethereal Extract (%) in dry matter	1.4	2.8
Neutral Detergent Fiber (%) in dry matter	61,0	49.4
Acid Detergent Fiber (%) in dry matter	36.2	10.8
TDN (%) in dry matter		

Statistical analysis

Data were analyzed using descriptive statistics for contingency of information and for further assumptions that were presented as descriptive (mean and standard deviation) for weight, weight gain, oocyst number, biochemistry and blood cell parameters. Tabulated data were submitted to the normality test (Shapiro), and data were not normal (weight, OOPG, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, CAT, ROS, SOD, GST, glucose and cholesterol). Therefore, they were transformed to logarithm for the purpose of normalization. Then, the comparison between groups was made using the Student test (t-test), considered significant when $P < 0.05$. One-way ANOVA was performed using repeated measurements to test for differences in the parameters over time (considering blocks of control and treated group). The feces scores were analyzed using a Chi-square independence test, aimed to evaluate the presence or absence of the score. Statistical manipulations were performed using R-language, v.3.1 (R Development Core Team 2012).

We calculated the post-hoc power for the groups in three experiments, considering GST as output; because there were many, this make was difficult; we were trying to access not only blood cells. If we use the mean of each group and SD with an alpha of 0.05, we end up with reasonable powers of 92.2% (Experiment 1), 90.3% (Experiment 2) and 88.4% (Experiment 3).

RESULTS

Body weight and weight gain

Body weight did not differ between groups ($p > 0.05$) in the three experiments (E1, E2 and E3), or three time periods (days 0, 10 and 15) (Table 2). Over time, no difference in body weight of the animals from the three experiments was observed ($p > 0.05$; Table 2). Regardless of the age when curcumin was added to the diet, the supplement increased calf weight gain at days 0 to 15 (E1, E2 and E3 p-values 0.04, 0.001 and 0.001, respectively, Table 2). The large individual weight variation at the beginning of the experiments could be the cause of the differences in weight gain observed.

Fecal score and coccidial infection

On fecal scoring, no difference was observed between groups (for fluidity, consistency, color, and odor) in the three experiments ($p < 0.05$). However, the number of coccidian oocysts in the animals of Experiment 1 at day 15 was lower in the treated group, compared with that of the control group. In Experiment 2, a similar result was also observed at day 15, that is, reduced oocyst count in feces (Table 2). Over time, there was a decrease in OOPG of calves that received curcumin in the diet (E1 and E2) ($p < 0.05$; Table 2). Importantly, animal showed no clinical signs of disease during the experiment, and therefore there was no need for drug treatment.

Table 2. Mean values and standard deviation for weight, weight gain and *Eimeria* spp. oocysts per gram of feces (OOPG) found on days 0, 10 and 15 of the experiment for animals of the control and treated groups.

Variables	Day	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
		CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]
Weight* (kg)	0	46.8±6.3	50.6±6.2	0.85	72.8±4.7	70.8±10	0.89	114.3±11.4	110±12.1	0.85
	10	49±6.9	54.4±7.3	0.74	74.6±7.3	77.6±8.5	0.67	118.9±9.6	116±11	0.92
	15	49.8±7.7	55.6±6.7	0.79	77.6±7.5	80.8±8.7	0.91	122±11	123±10	0.94
	P[2]	0.45	0.21		0.35	0.30		0.42	0.19	
Weight gain* (kg)	0-10	2.2±1.6	3.8±2.1	0.13	1.8±1.5	7±3.4	**0.001**	4.6±2.4	6±3.3	0.21
	0-15	3.0±1.4	5.0±1.8	**0.04**	4.8±2.7	10±4.1	**0.001**	7.7±2.8	13±4.5	**0.001**
OOPG*	0	159.9±180.7	280±196.7[a]	0.84	140±210	158±228[a]	0.76	66.8±87.5	33.3±51	0.74
	10	473.6±623.0	28±62.6[b]	0.09	144±202	3.2±7.1[b]	0.07	128±292.4	0±0	0.26
	15	305.8±281.7	15.3±34.3[b]	**0.02**	68.5±59.7	0.0±0.0[b]	**0.001**	69.6±79.4	0±0	0.08
	P[2]	0.57	**0.04**		0.60	**0.001**		0.46	0.12	

Note: *Transformed values for logarithm; Experiment 1: heifers with 18 ± 7 days of life; Experiment 2: heifers with 64 ± 4 days of life; Experiment 3: heifers with 95 ± 8 days of life. Animals from experiment 1 and 2 were in the pre-weaning phase, and experiment 3 was in the post-weaning phase. In the three experiments we had two independent groups, that is, one control group and one treated group (supplemented with curcumin in the diet).

Note: [1] P<0.05 shows difference between groups.

[2] P<0.05 shows difference over time in each group (day 0 vs 10, day 0 vs 15, day 10 vs 15). When P<0.05 the difference between periods (day) was identified by different letters in the same column.

Hemogram

Erythrocyte count, hemoglobin concentration, hematocrit, neutrophil, monocyte, and eosinophil numbers did not vary between groups in all experiments (p>0.05). Leukocyte and lymphocyte count also did not differ between groups in E1 and E2.

In Experiment 3 (E3), leukocytes counts were lower in the treated group compared with the control group at day 15 (Table 3). This lower in total leukocytes number is related to the lower of lymphocytes in the same period (Table 3). However, when these variables were evaluated over time, we noticed that there was an increase in the number of total leukocytes as a consequence of the increase of lymphocytes only in the animals of the control group.

Oxidant and antioxidant profiles

In the post-weaning phase, animals who received curcumin for 15 days had a reduction in ROS and TBARS levels compared with control animals (Table 5). In general, the activity of antioxidant enzymes (SOD, GST and CAT) of curcumin-fed calves increased (p <0.05) compared with control (Table 5). It is important to note that the enzyme activities did not all change concomitantly, nor did we observe effects in all experiments. However, in general we observed an antioxidant effect of curcumin.

Table 3. Mean values and standard deviation for erythrocyte, hemoglobin, hematocrit, leukocyte, lymphocyte, neutrophil, monocyte and eosinophil found on days 0, 10 and 15 of the experiment for control and treated animals

VARIABLES	DAY	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
		CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]
Erythrocytes (x10^6/µL)	0	4.7±1.2	4.4±0.7	0.89	3.9±0.26	3.9±0.9	0.94	4.87±0.24	4.8±0.8	0.96
	10	3.9±0.83	4.8±0.94	0.45	4.6±0.68	4.35±0.77	0.86	5.05±0.72	4.4±0.5	0.73
	15	4±0.84	4.6±0.86	0.54	4.9±0.64	4.9±1	0.95	5.1±0.68	5.29±0.7	0.92
	P[2]	0.43	0.75		0.12	0.36		0.74	0.31	
Hemoglobin (g/dL)	0	9.1±0.9	8.6±0.81	0.88	9.1±0.7	8.8±0.6	0.87	9.5±0.67	9±0.97	0.89
	10	8.7±0.6	8.6±0.64	0.97	9.5±0.69	8.8±0.5	0.23	9.2±3.6	8.6±4	0.70
	15	8.8±0.7	8.7±0.87	0.90	9.8±0.9	9.9±0.4	0.91	9.2±0.5	9.4±1.7	0.93
	P[2]	0.81	0.90		0.62	0.09		0.92	0.70	
Hematocrit (%)	0	34.6±2.8	33.2±2.5	0.95	33±3.1	32.8±1.9	0.88	36.1±3.7	34.3±3.5	0.88
	10	34.7±2.6	32.3±2.4	0.89	35.6±1.8	34.8±1.7	0.85	35.8±2.8	32±2.5	0.75
	15	33.4±2.7	32±3.1	0.96	36.6±4	35.1±4.8	0.90	32±3.1	29±1.7	0.19
	P[2]	0.94	0.95		0.75	0.81		0.63	0.06	
Leukocytes* (x10^3/µL)	0	8.7±4	9.4±0.9	0.84	8.2±1.98	8±1.99	0.92	6.8±2[b]	7.6±1.7	0.68
	10	9.73±3.3	9.57±1.5	0.95	7.68±1.82	7.55±1.2	0.90	7.2±1.36[b]	7.4±1.2	0.79
	15	7.9±1.9	6.5±2.3	0.56	7.9±0.7	8.76±2.7	0.74	10.4±1[a]	7.5±1.1	**0.001**
	P[2]	0.55	0.71		0.68	0.73		**0.01**	0.81	
Lymphocytes* (x10^3/µL)	0	4.5±2	4.8±0.9	0.78	3.9±0.8	4.6±1.65	0.67	3.56±1.4[b]	3.5±0.8	0.96
	10	3.9±1.7	4.7±1.5	0.45	4.2±1.5	3.5±0.8	0.36	4±1.2[b]	4.1±1.1	0.93
	15	4.6±1.6	4.0±2.3	0.56	5.2±1.1	5.9±1.8	0.44	6.98±1.1[a]	4.7±1.2	**0.01**
	P[2]	0.59	0.33		0.24	0.11		**0.001**	0.20	
Neutrophils* (x10^3/µL)	0	3.4±1.9	4.3±0.4	0.36	3.8±1.2	3±0.64	0.71	2.97±1	3.58±0.9	0.45
	10	5.1±2.3	4.2±3.7	0.40	3±0.7	3.4±0.62	0.36	2.98±0.98	3.16±1	0.72
	15	2.9±1.2	2.3±1.5	0.69	2.4±0.8	2.5±1.4	0.92	3±0.7	2.9±1.77	0.96
	P[2]	0.18	0.07		0.09	0.25		0.79	0.72	
Monocytes* (x10^3/µL)	0	0.73±0.6	0.26±0.16	0.74	0.27±0.18	0.21±0.15	0.77	0.21±0.18	0.32±0.19	0.66
	10	0.53±2.3	0.4±0.4	0.84	0.31±0.25	0.37±0.3	0.85	0.2±0.27	0.21±0.16	0.95
	15	0.36±0.2	0.2±0.16	0.90	0.23±0.11	0.26±0.3	0.86	0.35±0.1	0.21±0.13	0.11
	P[2]	0.32	0.48		0.67	0.63		0.59	0.53	
Eosinophils* (x10^3/µL)	0	0.06±0.08	0.08±0.14	0.90	0.14±0.15	0.08±0.09	0.87	0.08±0.15	0.15±0.15	0.93
	10	0.20±0.25	0.27±0.35	0.85	0.07±0.11	0.17±0.18	0.60	0.07±0.11	0.0±0.0	0.74
	15	0.02±0.04	0.03±0.06	0.95	0.08±0.18	0.14±0.69	0.68	0.02±0.04	0.01±0.02	0.88
	P[2]	0.51	0.46		0.62	0.54		0.65	0.47	

Note: *Transformed values for logarithm; Experiment 1: heifers with 18 ± 7 days of life; Experiment 2: heifers with 64 ± 4 days of life; Experiment 3: heifers with 95 ± 8 days of life. Animals from experiment 1 and 2 were in the pre-weaning phase, and experiment 3 was in the post-weaning phase. In the three experiments we had two independent groups, that is, one control group and one treated group (supplemented with curcumin in the diet).
Note: [1] P<0.05 shows difference between groups;
[2] P<0.05 shows difference over time in each group (day 0 vs 10, day 0 vs 15, day 10 vs 15). When P<0.05 the difference between periods (day) was identified by different letters in the same column.

In both groups of E1 and E3, a reduction in TBARS levels was observed over time (Table 5). ROS levels also decreased in the animals in the treated group (only E2). GST activity increased over time in the treated groups of all experiments (E1, E2, E3). Likewise, SOD activity increased in the treated group animals from E2 and E3. CAT activity did not differ over time (Table 5).

Table 5. Mean values and standard deviation for lipid peroxidation (TBARS), oxygen reactive species (ROS), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) found on days 0, 10 and 15 of the experiment for animals of the control and treated groups.

Variables	Day	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
		CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]	CONTROL	TREATED	P[1]
TBARS (nmol/ml)	0	12.5±5.2 [a]	10.7±3.4 [a]	0.88	9.8±4	8.2±6	0.69	10.2±4 [a]	9.5±2.1 [a]	0.87
	10	6±1.8 [ab]	5.2±1 [b]	0.90	5.5±1.2	5.4±1.2	0.95	5.2±0.5 [ab]	5±0.6 [b]	0.92
	15	5.5±0.6 [b]	4.9±0.4 [b]	0.86	5.1±0.7	4.8±0.4	0.85	4.1±1 [b]	2.5±0.1 [b]	**0.001**
	P[2]	**0.01**	**0.001**		0.07	0.18		**0.001**	**0.001**	
ROS (U DCFA/µL)	0	13.4±10.1	10.7±4.4	0.84	6.4±2.5	9.2±5.4 [a]	0.38	7.5±1.5 [ab]	6.8±0.9	0.90
	10	14.3±0.8	13.95±0.8	0.96	4±0.5	3.4±0.4 [b]	0.68	4.7±1.8 [b]	5.1±1.1	0.82
	15	12.8±6	12.7±3.7	0.97	4.2±2.3	6.5±4.5 [ab]	0.42	14.6±7.5 [a]	5.7±1.7	**0.001**
	P[2]	0.80	0.62		0.08	**0.001**		**0.02**	0.67	
GST (µmol/Cdnb/ min)	0	113.4±43.3	106±50[c]	0.74	130.6±50	109.2±43 [b]	0.45	98.4±36	117±26.6 [b]	0.22
	10	149.4±59	179.5±26 [b]	0.65	167.2±28	179.6±17 [ab]	0.76	103±38.2	134±28 [ab]	0.34
	15	148.7±33	270.3±25 [a]	**0.001**	146±21	214.2±34.2[a]	**0.001**	110.4±36.1	148.9±12.7[a]	**0.02**
	P[2]	0.25	**0.001**		0.36	**0.001**		0.58	**0.05**	
SOD (UI/mg protein)	0	9.5±2	9.2±3.3	0.87	9.3±2.5	7.18±2.5 [ab]	0.78	5.8±3.6	4.21±1.3 [b]	0.54
	10	9.4±1	12.2±3.3	0.13	7±5.2	5.3±1.7 [b]	0.74	7.18±3.0	6.3±3.8 [ab]	0.71

	15	13.3±2.8	12.3±3.8	0.89	11.6±3.6	10.5±2.7 [a]	0.92	5.4±1.2	7.9±1.2 [a]	**0.04**
	P[2]	0.06	0.29		0.35	**0.001**		0.44	**0.001**	
CAT	0	5±3.4	4.1±1.2	0.85	3.88±0.7	4.64±2.3	0.65	5.8±22	4.8±1.4	0.42
(nmol/mg	10	3.4±0.8	4.3±2.6	0.83	4.75±2.2	3.4±0.6	0.71	5.1±2	5.14±1.7	0.89
protein)	15	2.9±1	4.4±0.8	**0.001**	5.39±3.6	4.6±1.4	0.47	5.1±1.7	5±2	0.87
	P[2]	0.21	0.75		0.23	0.55		0.46	0.78	

Note: *Transformed values for logarithm; Experiment 1: heifers with 18 ± 7 days of life; Experiment 2: heifers with 64 ± 4 days of life; Experiment 3: heifers with 95 ± 8 days of life. Animals from experiment 1 and 2 were in the pre-weaning phase, and experiment 3 was in the post-weaning phase. In the three experiments we had two independent groups, that is, one control group and one treated group (supplemented with curcumin in the diet).

Note: **[1]** P<0.05 shows difference between groups;

[2] P<0.05 shows difference over time in each group (day 0 vs 10, day 0 vs 15, day 10 vs 15). When P<0.05 the difference between periods (day) was identified by different letters in the same column.

Digestibility

After incubation of 48 h (simulating ruminal digestion), no differences were found between treatments (p >0.05) between groups (without and with curcumin) for digestibility in hay or concentrate (Figure 1). However, curcumin combined with hay and concentrate at 72 hours increased the digestibility 18.1% and 29.0%, respectively (p <0.05).

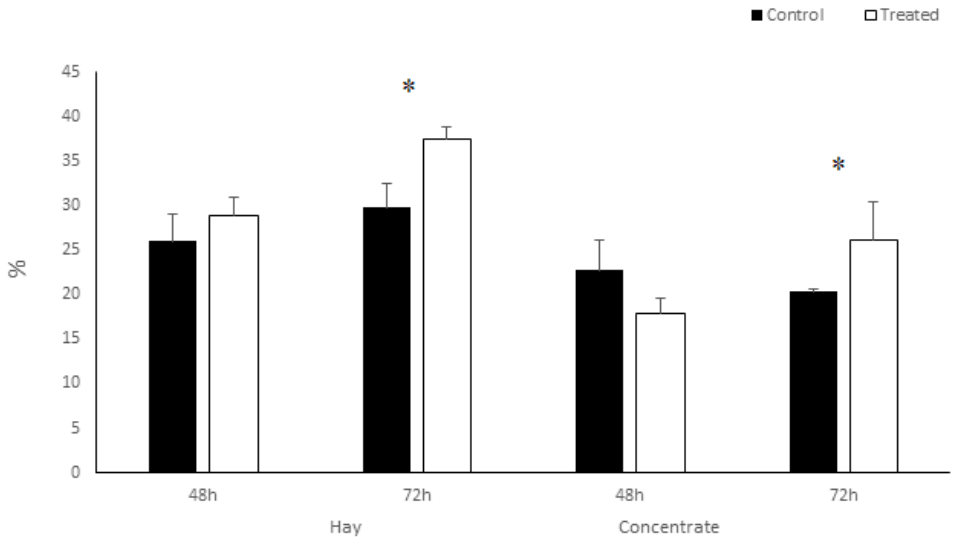

Figure 1. *In vitro* digestibility using curcumin associated with hay and with concentrate supplied to the calves during the experiments at 48 and 72h (* p <0.05).

Discussion

When analyzing calf weight gain, we showed that curcumin gave an average weight gain of 4.4, 8.65 and 10.35 Kg more than those of the control group in Experiments 1, 2, and 3, respectively. This result may be due to curcumin potentiating the digestibility of the diet (Rahmani *et al.*, 2017), as verified by the *in vitro* digestibility test. Consequently, the absorption of nutrients that favor weight gain indirectly improved, that is, the coccidiostatic response presented by curcumin reduced the number of intestinal lesions caused by coccidiosis. The antimicrobial activity of curcumin is already known (Gunes *et al.*, 2013), and according to researchers, feeding with curcumin changes the ruminal flora and nitrogen metabolism in beef cattle (Vorlaphim *et al.*, 2011), leading to ruminal fermentation modulation, consequently increasing weight gain in the calves in this study.

Curcumin has been shown to reduce the damage caused by coccidiosis in chickens (Kim *et al.*, 2013; Pelícia *et al.*, 2015; Peek *et al.*, 2013). In this study, calves receiving curcumin 200 mg/animal/day had a reduction in oocyst excretion, similar to that observed recently in lambs receiving oral doses of *C. longa* (Cervantes-Valencia *et al.*, 2016). This coccidiostatic property is due to induction of cellular apoptosis caused by precipitation in the sporozoite, consequently affecting viability, adhesion capacity and morphology of oocysts (Khalafalla *et al.*, 2011).

In the three experiments, we did not observe an effect of the curcumin diet on the number of red cells, hemoglobin concentration and hematocrit, similar to those observed in sheep supplemented with curcumin in diet (Jaguezeski *et al.*, 2018). However, in E3, we observed a lower in the number of leukocytes as a consequence of the lower of lymphocytes in calves that consumed curcumin in the concentrate; this was not observed in the animals that ingested curcumin in milk. However, the statistical analysis over time showed that curcumin supplementation did not reduce the number of these cells; however, it prevented them from increasing, as observed in the control animals. The discrepancy of leukogram results between experiments may be related to several factors, among them age, developing immunity, ruminal morphometry and the form of curcumin (concentrate or milk) intake. It is important to emphasize that because the calves were fed with a bottle (animals of E1 and E2), there was formation of esophageal gutter, and therefore the milk with curcumin did not pass through the rumen, as opposed to the situation with E3 calves, where curcumin was added to the concentrate. Our hypothesis is that, in young animals, the inflammatory response was stronger

due to first contact with microorganisms; consequently, the dose of curcumin ingested by calves (E1 and E2) was not sufficient to reduce the number of leukocytes due to the known anti-inflammatory action, recently describe in dairy sheep (Jaguezeski *et al.*, 2018).

In ruminants, the results may be recent (Cervantes-Valencia e*t al.,* 2016; Jaguezeski *et al.*, 2018), but curcumin is well-known to have anti-inflammatory properties (Fattori *et al.*, 2015; Kim *et al.,* 2013). However, the calves in the treated group in the pre-weaning phase of E1 had their immune response activated. That is to say there was an increase in globulin levels. This increase of globulins in calves of E1 may be a beneficial effect of curcumin, because in the first days of life, these animals are exposed to various microorganisms. In E1, the animals still have strong immune responses from colostrum that is rich in immunoglobulins (Ig), one of the globulin fractions that was increased in this study. The inhibitory action of curcumin on direct Ig is not known, allowing us to suggest that in some way as yet unknown, curcumin stimulated or maintained Ig levels in the blood of these calves. The mechanisms of how this occurred will be the focus of future study from our research group.

However, in non-nursing calves (E3), the anti-inflammatory effect of curcumin given in the diet was clear because there was a reduction in the number of lymphocytes and globulins. This anti-inflammatory action of curcumin is attributed to the ability to block the activation of nuclear factor kappa B (NF-Kb), and to reduce the growth of granulomas in response to various inflammatory stimuli, without causing toxicity to the organism (Araujo and Leon, 2001). In dairy sheep and lambs, the anti-inflammatory response of curcumin in the diet was also confirmed by inhibition of pro-inflammatory cytokines and increase of anti-inflammatory cytokines (Cervantes-Valencia e*t al.,* 2016; Jaguezeski *et al.*, 2018).

In weaned calves, the reduction of oxidants and increase in antioxidants was expected, as the antioxidant properties of curcumin are well-known (Rajput *et al.*, 2014). Lambs supplemented with curcumin also had reduction of lipid peroxidation and nitrite levels (Cervantes-Valencia *et al.,* 2016). This is also a well-described effect in meat and blood in chickens supplemented with curcumin (Zhang *et al.,* 2015b). The interaction of curcumin with enzymes and genes responsible for the oxidative profile lowers lipid peroxidation and free radical levels (Duan *et al.,* 2012). We believe there was a positive interaction between curcumin and antioxidant enzymes that act to repair and protect injured cells, as well as to eliminate and convert free radicals such as hydrogen peroxide, hydroxyl and superoxide radicals into water and oxygen

(Vincent *et al.*, 2007; El-Bahr, 2015). In our study, these effects likely contributed to better calf performance.

In the current study, the increase of hay and concentrate digestibility in the presence of curcumin was observed in vitro. In vivo study has confirmed our results, showing that curcumin in the diet can alter digestibility in ruminants. This was shown in a study with dairy sheep receiving 80 mg/animal/day, resulting in higher digestibility of neutral detergent fiber and consequently increasing milk production (Jaguezeski et al., 2018). Vorlaphim et al. (2011) found that curcumin-bred cattle had decreased apparent digestibility of detergent fiber. These results related to digestibility help to explain the best performance of the calves that received curcumin in their diets.

Curcumin given as a feed additive for nursing and non-nursing calves modulates the immune response, depending on the age of the animals. Curcumin prevented and controlled coccidian infection and reduced oxidative stress in calves. In addition, the addition of curcumin contributed to weight gain of calves at various ages, since curcumin enhances digestibility of feed. Curcumin in the diet of calves also did not alter metabolism of carbohydrates and lipids, protein catabolism, neither did it have hepatotoxic or nephrotoxic effects. The use of curcumin in the diet of calves can be a viable alternative, with beneficial effects on the health and performance of the animals. It is important to emphasize that in our preliminary experiments, curcumin had beneficial effects to the animals at this dose and experimental period. However, to ensure the safety of curcumin in the diet of calves more studies are accurate, exploring a longer experimental period, with different doses, and as well as greater number of animals per group.

Conflict of interest

The authors declare that they have no conflict of interest.

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2.3 MANUSCRITO I

Organic chromium (milk replacer and concentrate) dairy calf feed: benefits for growth performance, feed efficiency, digestibility and health

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ABSTRACT

Chromium (Cr) is a mineral that helps animals subjected to stressful conditions The suckling period is characterized by several stressful episodes, particularly at the first hours after birth and at weaning. There is little consumption of concentrate by calves in the first weeks of life; consequently, consumption of any supplement when added to feed would be small as well. Thus, we hypothesized that if Cr is consumed in milk replacer instead, the calves could consumed it earlier. Therefore, the aim of our study was to determine whether the inclusion of organic chromium in calf feed (via milk replacer or concentrate) during the suckling phase would improve calf health and growth performance. We used 24 dairy calves with an average age of 8 ± 4 days and 39.8 ± 6.9 kg average body weight. They were randomly divided into three groups: SC (n = 8), receiving 4 mg Cr/animal/day via milk replacer during the 60 experimental days of suckling; CC (n = 8), receiving 4 mg Cr/animal/day via concentrate; and C (n = 8), animals that did not receive chromium. The experiment lasted 75 days, divided into two well-defined stages: suckling (1–60 days) and weaning (61–75 days). During the experiment, body weight, feed consumption and blood collection were measured. At the end of the experiment, feed efficiency and digestibility analysis were conducted. We observed that the inclusion of organic chromium (regardless of route) increased body weight gain and feed efficiency. We found that protein digestibility was greater for the SC group. The consumption of chromium increased in the Cr levels un serum of the calves, higher in the first week in the animals in the SC group. This did not happen for the CC group; however, with higher consumption of concentrate, Cr levels increased and remained high until the end of the experiment. Glucose levels were higher in the groups that consumed chromium. Total protein levels were higher in the SC and CC groups than in group C. We conclude that the addition of Cr in calf feed improves their health and indirectly favors growth performance as well as increasing protein digestibility.

Keywords: chromium, calves, feed efficiency, digestibility, glucose

Introduction

The suckling period is marked by great challenges as in any other breeding stage. Among these challenges is the consumption of colostrum, supply of liquid and solid diets of adequate quality and quantity, sanitary challenges, maintenance of appropriate facilities and management, and comfortable environments, among others (Santos et al., 2010). In recent years, young animals began to be treated as future assets on farms, as opposed to their previous consideration as animals that provided no economic return. This is because researchers found that problems in the breeding and rearing phases affected the animal's productive life into adulthood (Dado-Senn et al., 2020; King et al., 2019).

In view of the intensification of rearing practices, we aimed to study the benefits of nutraceutical diets. In particular, among the most studied nutrients are micro-minerals, which, according to the literature, are important for bolstering health and consequently improving performance (Horst et al., 2018; Chang et al., 2020). Adequate mineral supplementation is associated with better animal development (Solaiman et al., 2006). Chromium has recently been included in ruminant diets, despite the fact that the functions of this mineral in the animal organism were not known, and in spite of its low bioavailability in inorganic form (Kegley et al., 1997).

It is now known that chromium is an essential element. In ruminants, it participates in the metabolism of glucose and protein; it can prevent cases of ketosis and reduce the deposition of tissue fat, in addition to the benefits to the immune system (Giometti et al., 2006; Mirzaei et al., 2011). Chromium enhances the activation of insulin receptors and allows better use of circulating glucose by cells mediated by chromodulin (Vicent, 2015; Mertz, 1993). In general, the effects of chromium depend on the source, because when inorganic sources of minerals are available, their bioavailability is less than 15%, while in organic form, it is approximately 90% (Goff, 2018). When we refer to inorganic sources of chromium, bioavailability is less than 3%; therefore, only the organic form is appropriate for inclusion in concentrate formulations (Kozloski et al., 2006).

There are few studies that evaluate chromium supplementation in calves during suckling and weaning, and those that exist presented conflicting results. Chromium in the diet is believed to improve the response of animals under stress conditions (Kargar et al., 2018; Mousavi et al., 2018). Among the stresses in calves are the change from a liquid diet to a solid

diet, in addition to changes in behavior and well-being, due to changes in metabolism, via their differing absorption pathways. In newborn calves, the consumption of chromium via concentrate is small, as the intake of this feed is absent or reduced during the first days of life. As a result, and due to the importance of this element in building the immune system, alternatives to chromium intake may strengthen health and consequently stimulate animal growth. With this in mind, on farms that use milk replacer in calf feeding, the inclusion of chromium in the formulation of the milk replacer can be a way of compensating for the deficiency of this essential mineral in the body of the calves. Therefore, our hypothesis was that the supply of organic chromium through the liquid diet would improve immune and antioxidant responses and thus the performance of dairy calves.

Therefore, the objective of our study was to determine whether the inclusion of organic chromium in calf feed (via milk replacer or concentrate) during the suckling phase had positive effects on calf health and growth performance.

Material and methods

2.1. The experimental site

This study was carried out in the experimental section of the Experimental Farm of the State University of Santa Catarina (UDESC), located in the municipality of Guatambú, Santa Catarina, Brazil. The section measured 20 m x 10 m, divided into 24 individual pens of 4.5 m²/animal with dimensions of 3 m x 1.5 m. In each stall, a feeder and drinker were available, as well as shavings for the bed area for resting the animals. The site was cleaned daily, with an emphasis on removing feces, washing floor areas and applying lime and shavings in wet areas. During the experimental period, the air temperature and relative humidity were recorded daily using the Data Logger Hobo U12-001 equipment.

At the beginning of the experimental period, calves were challenged daily by replacement of milk with milk replacer. This feeding transition, associated with low immunity, triggered diarrhea in all calves, as well as pneumonia in some calves. All animals with diarrhea and four have respiratory problems were medicated using doxycycline hydrochloride (4.520 g) and bezetimide hydrochloride (0.0165 g) at 1 mL for every 10 kg of body weight for 3 days to control diarrhea. When this treatment was not effective, we used the combination of sulfadoxine (20 g) and trimethoprim (4 g) in a single dose of 3 mL per 50 kg of body weight.

In cases of pneumonia, marbofloxacin (20 g) at a 1 ml per 25 kg of body weight was used (single dose) combined with anti-inflammatory and analgesic (meloxicam: 2 g) in a dose of 2.5 ml each 100 kg for up to 3 days. All medications used followed the manufacturer's recommendations, under veterinary supervision.

2.2. Animals

Holstein male calves were donated from dairy farms located in the western region of Santa Catarina. Before transporting the animals to the experimental farm, the calves were fed colostrum and/or milk. In this study, 24 calves were used at an average age of 8 ± 4 days and an average weight of 39.81 ± 6.9 kg. The calves arrived at the farm 5 days before the beginning of the experiment; therefore, on day 1 of the experimental period, blood was taken to measure levels of total protein in the serum; because according to researchers this method is important in this phase to verify the efficiency of colostrum. (Wilm et al., 2018). Protein levels varied widely among animals used in the research, received via donation from several dairy farms, which suggests that not all male calves received the same care regarding colostrum. This information was used to form the treatments; so we highlight that the mean protein value obtained for group C was 5.86 ± 1.74 g/dL; for SC group it was 5.84 ± 1.91 g/dL, and for RC it was 5.61 ± 1.37 g/dL.

2.3. Experimental design and diets

2.3.1. Stage I – Suckling

The calves were randomly divided according to body weight into three groups. The SC group (n = 8) received 4 mg Cr/animal/day as a milk replacer during the 60 experimental days of suckling; The RC group (n = 8) received 4 mg Cr/animal/day via concentrate; Group C (n = 8), animals did not receive chromium.

The organic chromium included in feed was chromium 10 (10% chromium and 90% non-essential amino acids). The dose of 4 mg/animal/day was based on previous studies (Kargar et al. 2018; Vincent 2015), which showed positive results in animal feed.

The milk replacer used for the experiment was a commercial product (Natural Health and Animal Nutrition), which, during the production process, was supplemented with organic chromium at 1 mg/kg of milk replacer. The concentrate was produced from ground corn, soybean meal, wheat bran and vitamin-mineral core (Table 1 and 2). Approximately 80 kg of

concentrate was supplemented with organic chromium (4 mg/100 g of concentrate). The two feeds used in this study followed the recommendations of the NRC (2001) for suckling calves.

In the first four days of the experiment, the animals went through a period of adaptation to the milk replacer, and in the first two days, 50% of feed was milk replacer and another 50% was cow's milk; on the third and fourth day, the volume of oil was only 25% and milk replacer became part of 75% of the food supplied; finally, from the fifth day of the study, the animals were fed only with milk replacer. The milk replacer supply followed the manufacturer's recommendations, that is, 100 g of the food diluted in 900 mL of water. The milk replacer was supplied by 4 liters of per animal per day, at 37 ºC, and divided into two meals (8:00 am and 5:00 pm).

The concentrate was offered ad libitum to the calves in the three treatments. However, at the beginning of the experiment, the consumption of concentrate was low (less than the quantity supplied), so we fixed the quantity of 100 g of concentrate/animal/day. However, when these animals started to consume larger amounts of concentrate, the amount this feed was increased (feeder always had feed). Feeding the animals in the CC group with concentrate was carried out as follows: First, all animals received 100 g of concentrate containing 4 mg Cr; then, chromium-free concentrate (the same consumed by the other two treatments) was made available ad libitum. In this manner, we were able to control the quantity of chromium in the feed.

2.3.2. Stage II – After weaning

Age of the calves and consumption of high concentrate were criteria for weaning. At 61 days of the experiment, gradual weaning (feed transition) was performed, that is, the 1st and 2nd weaning days provided 2 L of milk replacer/animal/day divided into two feedings (8:00 am and 5:00 pm); on the 3rd and the 4th day, we provided a total of 1 L of milk replacer/animal/day also divided into two feedings; on the 5th and 6th day, we provided 0.5 L of milk replacer/animal/day in just one feeding (8 am). During the food transition process (between days 61 to 66), there was a limited supply of 85 Tifton hay (1 kg/animal/day) in a specific feeder for this roughage. At this stage, the consumption of concentrate was also limited to 2 kg/animal/day, divided into two meals (8:00 am and 5:00 pm).

In this period of 15 days after weaning, the SC group received chromium via concentrate (4 mg/animal/day), similar to the calves in the RC group. It is important to note that, in the first six days of this phase, the amount of chromium via concentrate was calculated considering the quantity consumed via milk replacer in order to ensure that the consumption of milk replacer in the two test groups was only 4 mg/animal/day.

2.4. Animal weighing and consumption assessment

The calves were weighed weekly and individually using a Universal Line digital scale. Weighing was performed in the morning, after fasting of 12 hours. The consumption of the concentrate was evaluated daily by weighing the quantity supplied and orts. The consumption of milk replacer supplied did not differ during the entire experimental period; neither did the amount of hay that was consumed in its entirety. Based on this information, feed efficiency (%) was calculated using the equation daily weight gain (kg)/ingestion of dry matter (kg) x 100.

2.5. Sample collection

Blood samples were collected on days 0, 20, 40, 60 and 75 of the experiment. The samples were collected from the jugular vein using vacuum tubes. The material was allocated in two tubes (without and with anticoagulant). The antioxidant EDTA (ethylenediamine tetraacetic acid) was used. The samples were stored at 10 ºC during transport to the laboratory in a thermal box. The samples from the tubes without anticoagulant were centrifuged at 7000 rpm for 10 minutes to obtain serum. Anticoagulant samples were used for blood count analysis described below.

In the last five days of phase 1 of the experiment (between days 56 to 60), total collection of feces from the calves was performed daily. Samples were homogenized, weighed and a sample was taken for feed digestibility analysis.

Feed samples (concentrate, and hay) were collected and frozen (–20 ºC) until analysis in a specialized laboratory.

2.6. Laboratory analyses

2.6.1. Feed analysis

The feed supplied (concentrate and hay) was analyzed using near infrared reflectance spectroscopy (Shankar, 2015). To analyze the milk replacer, we used the chemical method described by Thompson (1990).

2.6.2. Hemogram

Counting of erythrocytes, leukocytes and hemoglobin was performed using a semi-automatic blood cell counter (model CELM CC530). Subsequently, the leukocyte differential was calculated by reading the blood smear stained with a Rapid Panotic kit. Hematocrit was measured with capillary tubes centrifuged at 1400 rpm for 1 minute.

2.6.3. Serum biochemistries

In serum, the levels of total proteins, albumin, urea, cholesterol, triglycerides and glucose were evaluated using a commercial kit (ANALISA®) and semiautomatic equipment (BIO PLUS 2000®) according to the manufacturers' recommendations. Globulin levels were obtained using the equation globulin = total proteins – albumin.

2.6.4. Oxidant/antioxidant status

The levels of oxygen reactive species (ROS) in serum were analyzed using the method described by Ali et al. (1992). The method consists of incubating 10 µL of serum with 12 µL of dichlorofluorescein (DFC) per mL for 1 hour in the dark at 37 ºC. The fluorescence results were determined using 488 nm excitation and 520 nm emission, the results were expressed in U DCF/mg protein.

The levels of TBARS (substances reactive to thiobarbituric acid) were evaluated to determine the levels of lipid peroxidation, for this purpose the method described by Jentzsch et al. (1996). TBARS results were obtained by spectrophotometer at 535 nm and the results are expressed in ηmol MDA/mL.

The serum glutathione S-transferase (GST) enzyme was determined by spectrophotometry at 340 nm using the modified methodology by Habig et al. (1974), for this, 20 µL of serum mixed with 0.1 M of potassium phosphate buffer, 100 mM of glutathione

(GSH) and 100 nM of 2,4-dinitrochlorobenzene (CDNB) was used, as substrate. The results were expressed in μmol CDNB/min/mL.

In addition, the levels of non-enzymatic antioxidants in serum (non-protein Thiols) were evaluated, following the methodology described by Sedlak and Lindsay (1968) recently described by Maltez et al. (2018). Results are expressed in mMol NPSH/mL

2.6.5. Proteinogram

For protein separation, polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE) was performed according to a technique described by Fagliari et al. (1998) using mini-gels (10 x 10 cm). The gels were stained with Coomassie blue and photographed to identify and quantify protein fractions using Labimage 1D 40 software (Loccus Biotechnology). Patterns containing fractions with molecular weights between 10 and 250 kDa (Kaleidoscope, BioRad) were used as a reference for the identification of protein fractions.

2.6.6. Serum chromium levels

The determination of the chromium levels in the serum was performed first by the sample digestion process with the addition of nitric, acetic and perchloric acid followed by constant heating. After this process, we added hydrochloric acid and subsequently determined the levels of chromium using mass spectrometry (Heard et al., 2007). Results were expressed as nM.

2.7 Digestibility

Between the 55^{th} and 60^{th} day of the experimental period, total feces produced by the calves were collected, with weighing of total food supplied and the total feces. In this manner we calculated the dry matter digestibility coefficient and crude protein according to the following equation: ((Ingestion (g) – Excretion in feces (g))/Ingestion (g) x 100).

2.8 Statistical analysis

All data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA), with the Satterthwaite approximation to determine the denominator's

degrees of freedom for the test of fixed effects. The results of body, weight gain, average daily gain, feed intake, feed efficiency, digestibility and blood variables on d 75 (Period 2 – after weaning) were tested for fixed effects of treatment and using animal (treatment) as random variable. All blood variables collected before weaning (d 1, d 20, d 40 and d 60) were analyzed as repeated measures and tested for fixed effects of treatment, day, and treatment × day, and using animal (treatment) as random variable. All results obtained on d 1 for each variable, were also included as covariates; however, covariates were removed from the model when $P > 0.10$. The covariance structures were selected according to the lowest Akaike information criterion. The compound symmetric covariance structure was selected for serum concentration of glucose and ROS. The Toeplitz covariance structure was selected for serum concentration of total protein, hematocrit, and eosinophils. First order autoregressive covariance structure was selected for all other variables. A simple Pearson correlation was evaluated among the serum concentration of chromium × others blood variables using the CORR procedure of SAS (only significant correlations were presented). Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

3. Results

3.1. Performance

The results of weight, total weight gain, daily weight gain, consumption of concentrate and milk replacer, feeding efficiency and digestibility during the suckling period (phase 1) are described in Table 3. Calves that consumed chromium regardless of the supply way presented trend of greater weight on days 21 and 28 ($p = 0.10$ and $p = 0.08$, respectively). However, in the weighing from the 35^{th} to the 60^{th} day, a significant difference was observed between the treatments, that is, the SC and RC groups presented similar weights to one another that were higher than those of group C ($p = 0.01$). For total weight gain and average daily gain, we also noticed the same differences.

Regarding consumption of concentrate and total food, there was a difference between the groups throughout the experimental period, with the SC and RC calves showing higher consumption. The supply of milk replacer was fixed and we not considered it on statistical analysis. There was no significant difference for digestibility coefficient of dry matter;

however, for protein, amounts were greater in group SC than in group C; group RC was similar to both ($p = 0.05$). Regarding feeding efficiency during suckling (1–60), there was a significant difference ($p = 0.03$) with the SC group different from the RC and C groups, which did not differ from one another.

Post-weaning (phase 2) results are displayed in Table 4. There was a difference in weights on day 75 ($p < 0.01$), with calves that consumed chromium having higher final weights. Total weight gain and average daily gain were higher in the groups that received organic chromium. Calves supplemented with Cr consumed more food (concentrate and milk replacer) throughout the experimental period (1–75 days; $p < 0.01$) and after weaning (61–75 days; $p < 0.01$). Regarding feed efficiency post-weaning (60–75 days) and during the total experimental period (1–75 days), the SC and RC groups were similar to one another and different from C.

3.2. Serum biochemistry

For the albumin and triglyceride parameters, there were no differences among results during stage 1 of the experiment. For globulin, there was no treatment effect. During the day, however, there was a tendency for an effect on the interaction (TxD), and on day 60, the levels of globulin were higher in the groups that consumed Cr (Table 5). The levels of total proteins were such that there was an effect of treatment and interaction (TxD). On day 60, the SC and RC groups differed from C.

The glucose variable showed effects of day, treatment and interaction (Table 5). The groups that consumed Cr (SC and RC) had higher levels of glucose than did group C. In addition, all treatments showed fluctuations in glucose levels during the experimental period. On days 20, 40, and 60 of the experiment, we observed higher levels of glucose in the animals that consumed Cr. The urea results demonstrated a day effect, that is, during the experiment, there were fluctuations in the values. Cholesterol levels also fluctuated during the experiment; however, on day 20, there was an interaction effect, with the RC and C groups being similar to each other and greater than the SC.

After weaning, there was a difference between treatments in terms of urea levels, where the RC group showed higher levels than the SC and C groups ($p = 0.04$). For glucose, the SC and RC groups were similar to one another, but higher than C (Table 8).

3.3. Hemogram

Table 6 describes the results for hemoglobin, erythrocytes, neutrophils, lymphocytes, eosinophils, and monocytes, with no significant differences among groups. In terms of hematocrit, there was a difference between the days of collection of the experiment and a trend for interaction (TxD) on day 40; in addition, the results of total leukocytes also showed a trend on day 40, with SC calves showing higher levels than the other treatments.

3.4. Serum antioxidants

The parameters for assessing the antioxidant and oxidant status during the suckling phase did not differ for treatment and interaction. However, for the day, there were variations for GST, total thiols and TBARS (Table 7).

3.5. Serum concentration of chromium

Figure 1 shows the results of serum chromium. On day 20, Cr levels were higher in SC calves than in RC and C calves, who showed similar results ($p < 0.01$). However, on days 40 and 60, Cr levels were higher in groups SC and RC than in group C ($p < 0.01$). After weaning (day 75), the RC group showed higher values than did the SC or C groups (table 8).

3.6. Proteinogram

Table 6 shows the results of a proteinogram during the nursing period. Levels of ceruloplasmin, IgG light chain, and IgG heavy chain showed no interactions between treatment and day; however, there was variation in results over time common for growing animals. For IgA, there was an interaction between treatment and day. On days 40 and 60, the calves that received Cr (SC and RC) showed higher concentrations of immunoglobulin. After weaning, we observed a tendency ($p < 0.06$) for the SC and RC groups to have higher concentrations of IgA (Table 9).

3.7. Pearson correlations

Pearson's correlations were classified as positive and negative and were stratified as follows: 0 to 0.3 = negligible; 0.3 to 0.5 = weak; 0.5 to 0.7 = moderate; 0.7 to 0.9 = strong;

and above 0.9 = very strong. The correlations between chromium and the analyzed variables are displayed in Table 9. For albumin, total protein, hematocrit, hemoglobin and lymphocytes, the correlations were positive and negligible; urea and GST were classified as positive and weak; glucose showed a positive and strong correlation. The neutrophil and erythrocyte correlations were negative and negligible; for TBARS and eosinophils, the classifications were negative and moderate.

4. Discussion

Cr can have a great influence on the performance of animals; nevertheless, it remains difficult to determine whether that influence is positive or negative. Previous studies generated conflicting results. Previous trials observed the benefits of Cr on performance; however, currently its importance is seen in animals under stress conditions (Ghorbani et al., 2012; Yari et al., 2010; Pechova and Pavlata, 2007). Another difficulty is determining the appropriate dose for supplementation. For example, according to the NRC, the requirement of Cr for calves is not described, and supplementation must be done using only organic sources due to fact that the inorganic form is unable to be absorbed (NRC, 2001; Kozloski et al., 2006).

Our experiment was carried out with a maximum temperature of 35.075 ºC and a minimum of 6.077 ºC, a maximum relative humidity of 99.33% and a minimum of 31.105%. Thermal comfort of calves is defined by temperatures in the range of 15 ºC to 25 ºC and THI below 78. At indices higher than that, calves present alterations in cortisol levels, increased heart rate, respiratory rate and rectal temperature (Kovács et al., 2019; Yari et al., 2010).

In our study, calves from the SC and RC groups performed better when compared to C. This improvement was attributed to higher weight gain. Supplemented animals consumed more concentrate and had better feeding efficiency during suckling and after weaning. In a recent study that provided 0.05 mg Cr/kg of $PC^{0.75}$ to suckling calves under heat stress conditions, they did not observe differences in weight gain; however, a difference in the consumption of concentrate was seen, and the authors noted that supplemented calves had a higher visit to the feeder (Kargar et al., 2018; Mousavi et al., 2018). Cr is believed to present better physiological responses when provided under stress conditions, as studies by Yari et al. (2010) and Kumar et al. (2015) found no difference in the supply of Cr during suckling in the absence thermal stress.

For calves, even under adequate environmental conditions, stress occurs. Some episodes are inevitably very stressful, including birth and the transition from liquid to solid diet (Pechova and Pavlata, 2007). Therefore, Cr should be included in essential minerals for calves, as this change was shown to be important because it deals with growing animals and growth under stress conditions. The improvement in performance is believed to be related to glucose metabolism because Cr is involved in the active molecule of chromodulin. This occurs by the mineral when circulating in the serum it binds to apochromodulin (pro-enzyme); in this manner, chromodulin potentiates the action of insulin by facilitating the interaction between the hormone and receptors (Anderson, 1998; Zanetti et al., 2003).

The digestibility of protein was higher in the SC group. We believe that this is due to the route proposed in this study, as providing better use of the protein fraction of the food to the growing animal is very important. We assume that this occurred because the mineral molecule is linked to a group of amino acids, and the association between mineral and organic molecules provides greater stability to the complex, reducing antagonism and improving utilization by the animal (Spears 1996). Cr has different destinations in the animal; through the liquid diet, it becomes more available for absorption in the jejunum (Chen et al., 1973). Liquid diet does not pass through the ruminant's pre-stomach compartments; this is due to the presence of esophageal leak in suckling animals; so, the liquid food is directed to the abomasum for chemical digestion. The provision of a solid diet to calves aims to stimulate rumen development. Therefore, the portion that is consumed passes through the compartments of the pre-stomach and undergoes degradation by microorganisms present in the environment (Lima et al., 2013).

This improvement in the protein digestibility coefficient resulted in an increase in the protein levels of calves supplemented with Cr during the experiment. Insulin that is potentiated by Cr, as previously seen, also influences protein metabolism through the absorption of amino acids in tissues (Pechova and Pavlata, 2007). Yari et al. (2010) did not observe a change in total protein levels when the calves were supplemented with Cr under conditions of thermal stress. During the experiment, we found variations in some variables for day. These oscillations are normal due to the development of the animal.

Among the parameters evaluated in the serum, glucose was the one that showed the greatest variation, as expected. The SC and RC groups had higher concentrations of the

molecule. This increase augmented use by tissues due to the potentiated effect of Cr (Zanetti et al., 2003). Ghorbani et al. (2012), Kargar et al. (2018) and Mousavi et al. (2018) showed no differences in glucose levels when Cr was supplemented. The glucose levels found in our study (64.03, 61.93, and 53.22 mg/dL in SC, RC, and C, respectively) were lower than those measured in the previously mentioned studies.

The difference in our study with those carried out recently was the evaluation of Cr bioavailability through analysis in serum (Kargar et al., 2018; Mousavi et al., 2019). We observed that the supply of Cr showed greater absorption in the calves of the SC group and RC than in controls. There was only a difference between the groups supplemented on the 20^{th}, and the SC group showed better use when compared to the CR group. We believe that this occurred due to the low consumption of concentrate in the first month of life; therefore, in this initial period, it would recommend to supply Cr via liquid diet so as to guarantee the desired consumption. A study carried out with buffalo calves under conditions of heat stress also showed an increase in plasma Cr levels; the authors used doses of 0.5, 1.0 and 1.5 mg Cr/kg DM (Kumar et al., 2015).

Cr has effects on metabolism as well as an influence on the immune system; this improvement in practice reduces the occurrence of infectious diseases (Burton, 1995). In a recent study that evaluated the health response of calves supplemented with Cr, there were fewer days of clinical signs of pneumonia and fewer days of medications; however, there was no change in the frequency of diarrhea (Mousavi et al., 2019). In our study, the greatest challenge was during the first month of life, where there were major changes in the calves' lives, including change of installation, diet, and handlers. Furthermore, several animals from various farms were brought together.

5. Conclusion

The results indicate that organic chromium supplementation improved performance, feed efficiency and increased glucose levels in calves supplemented during suckling and weaning. However, among the groups that received chromium by different routes, we recommend supplementation via liquid diet because it provided greater protein digestibility.

Ethics committee

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the University of the State of Santa Catarina (UDESC) under protocol number 2877070619.

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Table 1: Feed (green matter) supplied to calves during the suckling phase (Stage I: 1–60 days) and post-weaning (Stage II: 61–75 days).

Feed	Quantity (g/animal/d)
Stage I	
Milk replacer[1]	400
Concentrate[2]	Ad libitum
Stage II	
Milk replacer[1]	Six-day transition period: provided only on days 61 and 62 (200 g), 63 and 64 (100 g) and 65 and 66 (50 g).
Concentrate[2]	Ad libitum
Hay[3]	Ad libitum

Note 1: The ingredients used to produce the commercial milk replacer (Sucêdaneo Lácteo, Natural® Saúde e Nutrição Animal, Chapecó) were whey, milk permeate, milk protein concentrate, micronized soy flour, coconut oil, milk replacer kernel. At the time of production, 1 third of the milk replacer used in our study was supplemented with organic chromium (mineral chromium 10%, Aminogel®, São Paulo) at a concentration of 1 mg Cr/kg of milk replacer (intake of 4 mg Cr/calf) /day). Warranty levels: dry matter: 95,4%, TDN tabulated: 63,84, ether extract: 10,98%, lactose: 27,6%, crude protein: 23,04%, calcium: 6,34%, phosphor: 0,88%, magnesium: 0,63%, cobalt: 15 ppm, copper: 124,16 ppm, iron: 215 ppm, iodine: 12,36 ppm, manganese: 107,4, selenium: 6,47 ppm, zinc: 259,1 ppm, vitamin A: 20 kUI/kg, vitamin D 4 kUI/kg, vitamin E 103,7 UI/kg, biotin: 5 mg, folic acid: 12 mg/kg, pantothenic acid: 25 mg/kg, choline: 1.020 mg, niacin: 97 ppm, vitamin B1: 25,48 mg/kg, vitamin B12: 2 mg/kg, vitamin B2: 12 mg/kg, vitamin B6: 45 mg/kg.

Note 2: The ingredients used in the production of the concentrate were ground corn (410 g/kg), soybean meal (410 g/kg), wheat bran (140 g/kg) and vitamin-mineral core (40 g/kg). The core composition was calcite limestone, bicalcium phosphate, sodium selenite, iron sulfate, ventilated sulfur, magnesium oxide, manganese oxide, sodium chloride, calcium iodate, zinc sulfate, copper sulfate, kaolin, vitamin A/D3, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin K3, cobalt sulfate, monensin sodium, antioxidant, flavoring, choline chloride, niacin, biotin, pantothenic acid, folic acid, zinc chelate. At the time of

production, the concentrate was supplemented with organic chromium (mineral chromium 10%, Aminogel®, São Paulo) at a concentration of 4 mg Cr/100 g of concentrate.

Note 3: The Tifton 85 hay used in this study was purchased from a rural product in the western region of Santa Catarina, Brazil.

Table 2: Chemical composition analyzed and calculated energy of the food consumed by the calves during the experiment.

Feed	Without organic chromium supplementation	With organic chromium supplementation
Milk replacer		
Dry matter (g/kg)	959	962
Moisture (g/kg)	41.0	38.0
Crude protein (g/kg)	247	238
Ether extract (g/kg)	112	105
Lactose (g/kg)	262	270
Free glucose (g/kg)	2.10	2.0
Free galactose (g/kg)	3.60	3.50
Ash (g/kg)	52.3	55.5
TDN	65.8	64.5
Concentrate		
Dry matter (g/kg)	902	916
Crude protein (g/kg)	248	244
Ether extract (g/kg)	29.5	27.7
Starch (g/kg)	292	296
Starch - CNF (g/kg)	687	692
NDF (g/kg)	185	193
ADF (g/kg)	97.7	104
Mineral material (g/kg)	46.4	44.5
TDN	77.9	76.8
NE Mcal/kg	1.79	1.76
NEm Mcal/kg	2.04	2.01
Hay		
Dry matter (g/kg)	875	875
Crude protein (g/kg)	134	134
Ether extract (g/kg)	16.6	16.6
NDF (g/kg)	364	364
ADF (g/kg)	76.0	76.0
Starch (g/kg)	35.3	35.3
Mineral material (g/kg)	94.4	94.4

Note: TDN= Total digestible nutrientes; NFC= Non fiber carbohydrates; NDF= Neutral detergent fiber; ADF= Acid detergent fiber; NE= Net energy; NEm= Net energy maintenance;

Table 3. Growth performance of calves supplemented with chromium mixed in the milk replacer or concentrate during Stage I - suckling

Variables	Treatments[1]			SEM	*P*-value
	C	SC	RC		
Body weight, kg					
d 1	38.94	40.62	39.87	2.57	0.90
d 7	37.44	40.25	39.06	2.65	0.60
d 14	37.36	41.64	41.62	3.00	0.51
d 21	40.69^{y}	46.89^{x}	46.23^{x}	2.09	0.10
d 28	45.25^{y}	53.97^{x}	51.71^{x}	3.17	0.08
d 35	49.77^{y}	57.50^{x}	57.86^{x}	2.57	**0.03**
d 42	54.49^{y}	63.63^{x}	63.25^{x}	2.97	**0.01**
d 49	68.48^{y}	73.91^{x}	75.27^{x}	2.70	**<0.01**
d 60	74.14^{y}	80.11^{x}	83.67^{x}	2.77	**<0.01**
Weight gain, kg					
d 1 to 60	34.24^{y}	40.47^{x}	41.81^{x}	2.25	**<0.01**
Average daily gain, kg/d					
d 1 to 60	0.57^{y}	0.67^{x}	0.70^{x}	0.05	**<0.01**
Concentrate intake					
1st week, kg of DM/d	0.067^{y}	0.220^{x}	0.182^{x}	0.04	**0.05**
2nd week, kg of DM/d	0.141^{y}	0.307^{x}	0.227^{xy}	0.04	**0.05**
3rd week, kg of DM/d	0.232^{z}	0.411^{x}	0.318^{y}	0.03	**<0.01**
4th week, kg of DM/d	0.247^{z}	0.444^{x}	0.354^{y}	0.03	**<0.01**
5th week, kg of DM/d	0.264^{z}	0.513^{x}	0.404^{y}	0.03	**<0.01**
6th week, kg of DM/d	0.359^{y}	0.582^{x}	0.516^{x}	0.04	**<0.01**
7th week, kg of DM/d	0.539^{y}	0.718^{x}	0.709^{x}	0.04	**<0.01**
8th week, kg of DM/d	0.642^{z}	0.849^{y}	1^{x}	0.04	**<0.01**
9th week, kg of DM/d	0.949^{z}	1.164^{y}	1.278^{x}	0.05	**<0.01**
d 1 to 60, kg of DM	24.09^{y}	32.268^{x}	$31,69^{x}$	1.56	**<0.01**
d 1 to 60, kg of DM	$21,43^{y}$	$29,086^{x}$	$28,21^{x}$	1.39	**<0.01**
Milk replacer intake					

d 1 to 60, kg of DM/d	0.368	0.368	0.368	-	-
d 1 to 60, total kg of DM	22.08	22.08	22.08	-	-
Total feed intake					
d 1 to 60, kg of DM	44.44^{y}	52.41^{x}	51.41^{x}	1.64	**<0.01**
Feed efficiency					
d 1 to 60, WG/DMI	77.18^{y}	81.33^{x}	76.66^{y}	2.28	**0.03**
Digestibility					
DM, %	79.95	81.01	81.20	0.56	0.13
Crude protein, %	44.38y	52.03x	47.25xy	2.32	**0.05**

[1]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate).

[x-z]Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

Table 4. Growth performance of calves supplemented with chromium mixed in the milk or concentrate after weaning (**Stage II**)

Variables	Treatments[1]			SEM	*P*-value
	C	SC	RC		
Body weight, kg					
d 75	79.08^{y}	87.54^{x}	90.62^{x}	3.26	**<0.01**
Weight gain, kg					
d 60 to 75	4.94^{y}	7.43^{x}	6.94^{x}	1.85	**0.03**
d 1 to 75	39.18^{y}	47.90^{x}	48.76^{x}	4.20	**<0.01**
Average daily gain, kg/d					
d 60 to 75	0.329^{y}	0.495^{x}	0.463^{x}	0.08	**0.03**
d 1 to 75	0.522^{y}	0.639^{x}	0.650^{x}	0.03	**<0.01**
Concentrate intake					
d 60 to 75, kg of DM/d	1.4^{y}	1.68^{x}	1.78^{x}	0.09	**0.02**
d 60 to 75, kg of DM	15.88^{y}	19,25^{x}	20,68^{x}	0.82	**<0.01**
d 1 to 75, kg of DM	37,32^{y}	48,34^{x}	48,89^{x}	2.46	**<0.01**
Hay intake, kg of DM	11,41	11,41	11,41	-	-
Milk replacer intake					
d 61 to 66, kg of DM	7.0	7.0	7.0	-	-
Total feed intake					
d 60 to 75, kg of DM/d	31.08^{y}	34.60^{x}	36.02^{x}	0.83	**<0.01**
d 1 to 75, kg of DM/d	75.52^{y}	87.01^{x}	87.43^{x}	2.46	**<0.01**
Feed efficiency					
d 60 to 75, WG/DMI	15.85^{y}	21.46^{x}	21.07^{x}	2.66	**<0.01**
d 1 to 75 WG/DMI	51.82^{y}	54.75^{x}	55.66^{x}	2.05	**<0.01**

[1]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate).

$^{x\text{-}z}$Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

Table 5. Serum biochemistry of calves supplemented with chromium mixed in the milk or concentrate (**Stage I – before weaning**).

Variables	Treatments[1]			SEM	*P* – values[2]		
	C	SC	RC		Treat	Day	Treat × Day
Albumin (g/dL)					0.79	0.26	0.26
d 1	2.62	2.65	3.19	0.27			
d 20	3.18	3.24	3.21	0.29			
d 40	2.99	3.56	2.61	0.29			
d 60	3.23	3.19	3.38	0.29			
Average	3.01	3.16	3.09	0.17			
Globulin (g/dL)					0.23	0.61	0.08
d 1	3.27	3.22	3.38	0.32			
d 20	3.27	3.19	3.64	0.37			
d 40	3.08	3.31	3.39	0.37			
d 60	2.69^{y}	4.62^{x}	3.98^{x}	0.37			
Average	3.08	3.58	3.60	0.19			
Total protein (g/dL)					**0.05**	0.10	**0.03**
d 1	5.86	5.84^{C}	6.61^{AB}	0.39			
d 20	6.44	6.44^{B}	6.89^{AB}	0.41			
d 40	6.07	6.85^{AB}	6.04^{B}	0.41			
d 60	5.93^{y}	7.80^{Ax}	7.40^{Ax}	0.41			
Average	6.07^{y}	6.73^{x}	6.73^{x}	0.19			
Urea (mg/dL)					0.82	**<0.01**	0.95
d 1	33.62^{C}	33.33^{C}	31.75^{D}	1.83			
d 20	40.46^{B}	40.64^{B}	41.63^{B}	2.07			
d 40	52.98^{A}	52.60^{A}	51.37^{A}	2.07			
d 60	35.14^{BC}	38.23^{B}	36.30^{C}	2.08			
Average	40.55	41.20	40.26	1.22			
Cholesterol (mg/dL)					0.58	**<0.01**	**<0.01**

d 1	50.94	50.48^{B}	43.84^{B}	2.81			
d 20	50.81^{x}	38.65^{Cy}	51.84^{Ax}	2.81			
d 40	56.93	60.36^{A}	52.46^{A}	2.81			
d 60	54.89	54.24^{AB}	57.56^{A}	2.98			
Average	53.39	50.93	51.43	1.64			
Triglycerides (mg/dL)					0.85	0.11	0.16
d 1	52.98	23.88	26.11	9.45			
d 20	13.29	17.03	22.98	10.92			
d 40	10.42	24.57	24.86	10.96			
d 60	4.73	23.84	22.79	13.49			
Average	20.35	22.33	24.18	5.20			
Glucose (mg/dL)					**<0.01**	**<0.01**	**<0.01**
d 1	51.76^{B}	49.30^{B}	51.57^{C}	1.76			
d 20	49.55^{Bz}	69.90^{Ax}	60.94^{By}	1.99			
d 40	54.35^{ABy}	68.75^{Ax}	66.94^{Ax}	1.99			
d 60	57.25^{Ay}	68.18^{Ax}	68.29^{Ax}	1.99			
Average	53.22^{y}	64.03^{x}	61.93^{x}	0.90			

[1]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate).

[x-z]Within a row, without a common superscript differ ($P \leq 0.05$).

[A-C]Within treatment, without a common superscript differ ($P \leq 0.05$).

Table 6. Serum proteinogram of calves supplemented with chromium mixed in the milk or concentrate (Stage I – sucking).

Variables[1]	Treatments[2]			SEM	*P* – values		
	C	SC	RC		Treat	Day	Treat × Day
IgA (g/dL)					0.40	<0.01	<0.01
d 1	0.68^{A}	0.67^{B}	0.78^{A}	0.06			
d 20	0.55^{B}	0.55^{BC}	0.54^{B}	0.06			
d 40	0.43^{Cy}	0.69^{Ax}	0.41^{Cy}	0.06			
d 60	0.43^{Cy}	0.41^{Cy}	0.60^{Bx}	0.06			
Average	0.52	0.58	0.58	0.03			
Ceruloplasmin (g/dL)					0.54	0.11	0.39
d 1	0.70	0.60	0.68	0.11			
d 20	0.41	0.49	0.41	0.11			
d 40	0.59	0.59	0.46	0.11			
d 60	0.63	0.45	0.79	0.11			
Average	0.58	0.53	0.58	0.04			
IgG light chain (g/dL)					0.63	<0.01	0.85
d 1	0.53	0.48^{AB}	0.54^{A}	0.09			
d 20	0.50	0.52^{AB}	0.54^{A}	0.09			
d 40	0.35	0.41^{B}	0.32^{B}	0.09			
d 60	0.54	0.65^{A}	0.74^{A}	0.09			
Average	0.48	0.52	0.53	0.04			
IgG heavy chain (g/dL)					0.12	<0.01	0.10
d 1	0.95^{A}	0.96^{A}	0.91^{A}	0.06			
d 20	0.63^{B}	0.51^{B}	0.61^{C}	0.06			
d 40	0.35^{Cy}	0.49^{Bx}	0.54^{Cx}	0.06			
d 60	0.50^{Bx}	$0.63B^{xy}$	0.76^{By}	0.06			
Average	0.61	0.64	0.71	0.04			

[1]IgA (Immunoglobulin A), IgG light chain (Immunoglobulin G light chain), IgG heavy chain (Immunoglobulin G heavy chain).

[2]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate).

[x-z]Within a row, without a common superscript differ ($P \leq 0.05$).

[A-C]Within treatment, without a common superscript differ ($P \leq 0.05$).

Table 7. Hemogram of calves supplemented with chromium mixed in the milk or concentrate (**Stage 1 – before weaning).**

Variables	Treatments[1]			SEM	*P* – values[2]		
	C	SC	RC		Treat	Day	Treat × Day
Hematocrit (%)					0.70	**<0.01**	0.10
d 1	30.99^{C}	30.16^{B}	31.83^{B}	1.29			
d 20	31.39^{C}	29.82^{B}	28.58^{C}	1.46			
d 40	27.59^{By}	31.85^{Bx}	27.83^{Cy}	1.48			
d 60	36.45^{A}	36.72^{A}	36.10^{A}	1.78			
Average	31.60	32.14	31.08	0.95			
Hemoglobin (g/dL)					0.59	**<0.01**	0.17
d 1	9.25^{B}	9.02^{BC}	9.50^{B}	0.38			
d 20	8.98^{BC}	8.48^{C}	8.10^{C}	0.44			
d 40	8.11^{C}	9.46^{B}	8.40^{C}	0.44			
d 60	10.53^{A}	10.99^{A}	10.68^{A}	0.53			
Average	9.21	9.49	9.17	0.25			
Erythrocytes (x10^{6}µL)					0.51	**<0.01**	0.92
d 1	7.19^{A}	7.02^{A}	7.26^{A}	0.40			
d 20	6.24^{AB}	6.57^{AB}	5.90^{B}	0.46			
d 40	5.10^{B}	5.76^{B}	5.43^{B}	0.46			
d 60	5.23^{B}	5.63^{B}	5.06^{B}	0.46			
Average	5.94	6.25	5.92	0.24			
Leukocytes (x10^{3} µL)					0.85	0.53	0.09
d 1	10.82	10.44	10.32	0.89			
d 20	10.40	9.01	9.40	0.89			
d 40	8.67y	11.43x	9.00y	0.89			
d 60	10.90	8.87	10.13	0.95			
Average	10.20	9.94	9.71	0.63			
Neutrophils (x10^{3}/µL)					0.95	**<0.01**	0.87
d 1	4.72^{A}	4.43^{A}	4.34^{A}	0.54			

d 20	2.48^{B}	1.85^{B}	2.69^{B}	0.65			
d 40	1.92^{B}	2.55^{B}	1.98^{B}	0.61			
d 60	4.01^{A}	3.84^{A}	4.25^{A}	0.61			
Average	3.28	3.17	3.32	0.39			
Lymphocytes (x10^{3}/µL)					0.44	**<0.01**	0.19
d 1	4.63	4.47	4.37	0.62			
d 20	6.58	5.75	5.48	0.62			
d 40	5.84	7.66	5.75	0.62			
d 60	5.77	5.08	4.60	0.65			
Average	5.71	5.74	5.05	0.46			
Eosinophils (x10^{3}/µL)					0.35	**<0.01**	0.66
d 1	0.67^{A}	0.57^{A}	0.50^{A}	0.10			
d 20	0.65^{A}	0.31^{AB}	0.38^{A}	0.12			
d 40	0.02^{B}	0.05^{B}	0.06^{B}	0.12			
d 60	0.18^{B}	0.12^{B}	0.26^{AB}	0.12			
Average	0.38	0.26	0.30	0.06			
Monocytes (x10^{3}/µL)					0.69	0.14	0.97
d 1	1.04	0.90	0.93	0.18			
d 20	0.87	0.69	0.67	0.21			
d 40	1.03	1.15	1.04	0.21			
d 60	1.19	0.91	1.00	0.26			
Average	1.03	0.91	0.91	0.12			

[1]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate).
[x-z]Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
[A-C]Within treatment, without a common superscript differ ($P \leq 0.05$).

Table 8. Serum antioxidant response of calves supplemented with chromium mixed in the milk or concentrate **(Stage 1 – before weaning).**

Variables[1]	Treatments[2]			SEM	*P* – values		
	C	SC	RC		Treat	Day	Treat × Day
GST ((μmol CDNB/min))					0.96	**<0.01**	0.41
d 1	28.36^{B}	33.37^{B}	41.39^{AB}	10.86			
d 20	34.53^{B}	38.48^{B}	57.63^{A}	12.55			
d 40	39.91^{B}	39.52^{B}	31.18^{B}	12.55			
d 60	82.71^{A}	67.61^{A}	57.07^{A}	11.65			
Average	46.38	44.74	46.82	5.96			
Total thiols (mMol NPSH/mL)					0.57	**<0.01**	0.44
d 1	0.21^{B}	0.20^{BC}	0.17^{B}	0.06			
d 20	0.15^{B}	0.15^{C}	0.16^{B}	0.06			
d 40	0.26^{B}	0.33^{AB}	0.33^{A}	0.06			
d 60	0.47^{A}	0.37^{A}	0.26^{AB}	0.06			
Average	0.27	0.26	0.23	0.03			
ROS (U DCFA/μL)					0.34	0.11	0.70
d 1	3871.26	4473.20	4379.56	3400.39			
d 20	3171.87	3584.99	3585.73	3400.39			
d 40	6545.44	26895.00	6717.63	3400.39			
d 60	7481.88	7359.20	7888.27	3400.39			
Average	5267.61	10578	5642.80	1857.73			
TBARS (mMol MDA/mL)					0.87	**<0.01**	0.33
d 1	16.97^{A}	15.81^{A}	15.12^{A}	0.80			
d 20	11.12^{B}	11.34^{B}	11.85^{B}	0.92			
d 40	10.70^{B}	11.93^{B}	11.13^{B}	0.92			

d 60	9.20^{B}	9.24^{C}	11.01^{B}	0.85
Average	11.99	12.08	12.28	0.40

[1] GST (Glutathione S-transferase), Total thiols (non-enzymatic antioxidants:), ROS (Reactive oxygen species), TBARS (thiobarbituric acid reactive substances).
[2]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate)
[x-z]Within a row, without a common superscript differ ($P \leq 0.05$).
[A-B]Within treatment, without a common superscript differ ($P \leq 0.05$).

Table 9. Serum biochemistry, hemogram, antioxidant variables and serum concentration of chromium of calves supplemented with chromium mixed in the milk or concentrate (**Stage 2 – after weaning**)

Variables	Treatments[1]			SEM	*P* – value
	C	SC	RC		
Serum biochemistry (d 75)					
Albumin (g/dL)	3.48	3.86	3.67	0.35	0.69
Globulin (g/dL)	4.70	4.49	4.64	0.59	0.96
Total protein (g/dL)	8.14	8.45	8.22	0.34	0.75
Urea (mg/dL)	37.00^{y}	36.13^{y}	41.43^{x}	1.33	**0.04**
Cholesterol (mg/dL)	63.00	59.75	55.71	3.70	0.33
Triglycerides (mg/dL)	27.12	25.27	28.18	1.83	0.35
Glucose (mg/dL)	50.80^{y}	68.57^{x}	69.25^{x}	2.68	**<0.01**
Proteinogram (d 75)					
IgA (g/dL)	0.47^{y}	0.73^{x}	0.62^{xy}	0.08	0.06
Ceruloplasmin (g/dL)	0.61	0.73	0.64	0.09	0.67
IgG heavy chain (g/dL)	0.86	0.90	0.84	0.02	0.11
IgG light chain (g/dL)	0.99	1.10	1.17	0.22	0.84
Hemogram (d 75)					
Hematocrit (%)	35.60	36.25	36.14	1.52	0.94
Hemoglobin (g/dL)	10.28	10.77	11.07	0.35	0.25
Erythrocytes ($x10^{6}$µL)	6.85	6.51	6.79	0.32	0.65
Leukocytes ($x10^{3}$ µL)	10.50	12.40	10.05	1.66	0.45
Neutrophils ($x10^{3}$/µL)	3.93	3.08	3.34	0.68	0.63
Lymphocytes ($x10^{3}$/µL)	5.84	8.08	5.47	0.98	0.19
Eosinophils ($x10^{3}$/µL)	0.19	0.32	0.35	0.12	0.62
Monocytes ($x10^{3}$/µL)	0.74	0.97	0.87	0.19	0.64
Antioxidant variables (d 75)					
GST ((µmol CDNB/min))	134.25	129.80	125.93	7.99	0.76
Total thiols (mMol NPSH/mL)	0.40	0.43	0.57	0.11	0.55
ROS (U DCFA/µL)	5290.49	5559.20	5742.33	473.84	0.80

TBARS (Mmol MDA/mL)	12.30	10.70	13.93	1.17	0.15
Serum chromium (nM; d 75)	237.25^{y}	247.17^{y}	260.67^{x}	4.19	**0.01**

[1]The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate)

$^{x\text{-}z}$Within a row, without a common superscript differ ($P \leq 0.05$).

Table 10. Pearson correlation coefficients among serum concentration of chromium and others blood variables.

Variables[1]	**Pearson correlation coefficients**	***P* - value**
Chromium × Albumin	0.24	**0.03**
Chromium × Total protein	0.28	**0.01**
Chromium × Urea	0.41	**<0.01**
Chromium × Glucose	0.74	**<0.01**
Chromium × Hematocrit	0.23	**0.04**
Chromium × Hemoglobin	0.22	**0.04**
Chromium × Erythrocytes	-0.23	**0.04**
Chromium × Neutrophils	-0.27	**0.02**
Chromium × Lymphocytes	0.28	**0.01**
Chromium × Eosinophils	-0.41	**<0.01**
Chromium × GST	0.36	**<0.01**
Chromium × TBARS	-0.50	**<0.01**

[1]Glutathione transferase (GST) and thiobarbituric acid reactive substances (TBARS). Variables that did not have significative (P > 0.05) or tended to did not have significative (P > 0.10) correlation were not presented.

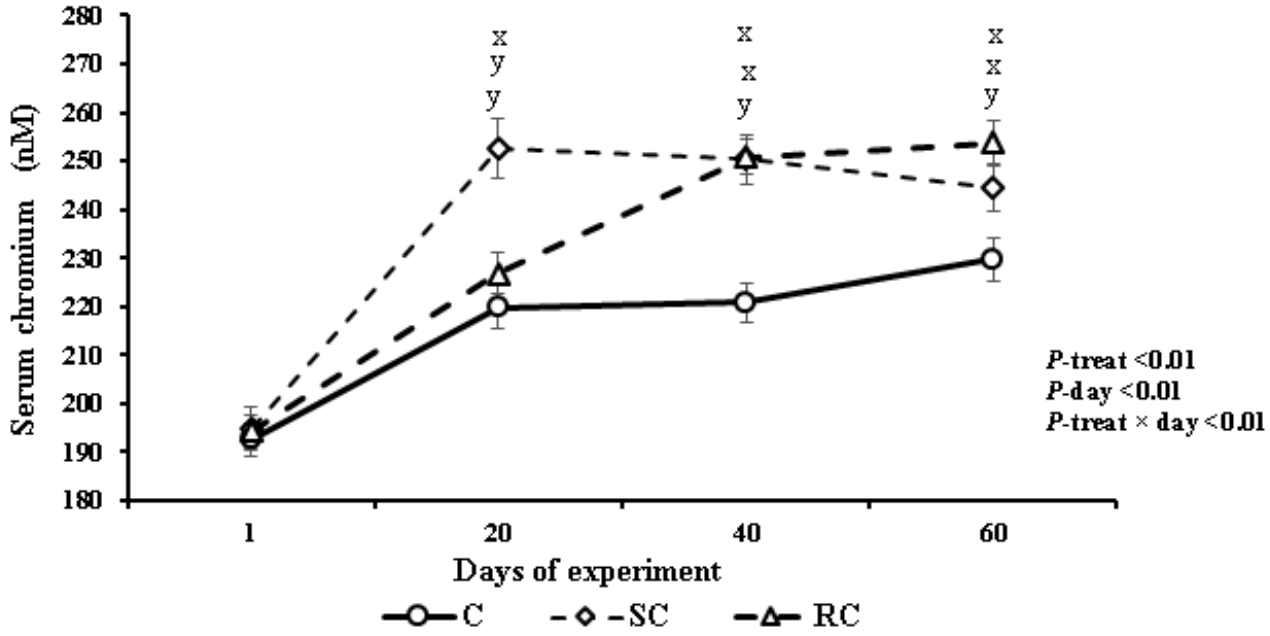

Figure 1. Serum concentration of chromium of calves supplemented with chromium mixed in the milk or concentrate (period 1 – before weaning). The treatments C (control), SC (4 mg Cr/animal/day over milk replacer) and RC (4 mg Cr /animal/day over concentrate). The results of chromium levels are expressed in nM.
$^{x\text{-}y}$Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM.

3. CONSIDERAÇOES FINAIS

A criação de bezerras era e ainda é negligenciada em muitas fazendas, pois é conhecida como a fase sem retorno econômico imediato. Entretanto, já é sabido que a criação da bezerra adequadamente vai ter efeito positivo na fase produtiva desse animal. Portanto, fornecer as condições ideias de criação para o animal conseguir expressar seu potencial genético, e além de avaliar alternativas que gerem maiores resultados durante a fase de aleitamento são importantes; assim como foi foco da nossa pesquisa e mostrou que a suplementação de cromo orgânico é uma excelente alternativa para potenilizar a criação de bezerros leiteiros; assim como uso injetável de minerais e inclusão de curcumina na dieta dessa categoria animal.

No artigo I nós avaliamos a estratégia de aplicação de um complexo de mineral a base de Se, Mg, Cu, K e P pela via intramuscular em bezerras no primeiro mês de vida. Nesse período ocorre a transição da imunidade inata para adquirida durante esse processo é visualizado um grande desafio. Verificamos que os minerais podem minimizar os impactos dessa fase inicial através do aumento de enzimas antioxidantes que combatem o estresse oxidativo e também melhorou o fortalecimento do sistema imunológico e redução de casos de diarreia.

Adição de curcumina na dieta de bezerras durante 3 períodos diferentes de criação relatado no artigo II. Ficou evidenciado o aumento no ganho de peso, estimulação do sistema imune, redução do estresse oxidantivo e controle infecções por coccídeo. Além disso, proporcionou aumento da digestibilidade *in vitro* do concentrando. A principal desvantagem da inclusão da curcumina a dieta dos animais é ao elevado custo, pois com pureza elevada, o produto deve ser importado, elevando os custos. Uma alternativa que deve ser avaliada é inclusão de extrato de *Curcuma longa* a dieta dos animais, planta da onde é extraído a curcumina; no entanto, a desvantagem de usar o extrato é a baixa concentração de curcumina (oscila entre 3 a 8%).

Por fim, estudamos a inclusão de cromo orgânico na dieta de bezerros por duas vias (concentrado e sucedâneo). Devido ser uma tecnologia disponível ao mercado recentemente, que ainda precisa estabelecer os níveis de exigência para bezerros leiteiros, nosso estudo apresenta riquíssimos dados. Verificamos que a inclusão de Cr na dieta aumentou a

digestibilidade da proteína dos bezerros que consumiram o cromo via dieta líquida; além disso, notou-se melhor desempenho e eficiência alimentar quando os bezerros consumiram cromo na dieta, independenmente da via de administração.

Com base nos três estudos, podemos concluir que as estratégias para potencializar saúde e desempenho dos bezerros tiveram efeitos positivos; e mostram-se como uma alternativa viável e rentável no sistema de produção de bezerros leiteiros; principalmente se conseguir reduzir mortalidade de bezerras, categoria animal com preço de venda e compra elevado nos últimos anos.

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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 "Suplementação injetável de selênio, magnésio, cobre, potássio e fósforo em bezerras leiteiras", protocolada sob o CEUA nº 8075050716 (ID 000179), 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 03/08/2016.

We certify that the proposal "Injectable supplementation of selenium, magnesium, copper, potassium and phosphorus in dairy calves", utilizing 20 Bovines (20 females), protocol number CEUA 8075050716 (ID 000179), 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 08/03/2016.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2016 a 10/2016 Área: Zootecnia

Origem:	Não aplicável biotério				
Espécie:	Bovinos	sexo: Fêmeas	idade:	2 a 40 dias	N: 20
Linhagem:	holandez		Peso:	25 a 60 kg	

Local do experimento: Esta pesquisa será realizada em propriedade localizada no interior do munícipio de Chapecó ? Oeste de SC, sendo que a principal renda da propriedade é a produção leiteira O rebanho é constituído por aproximadamente 180 vacas em lactação, sendo animais confinados da fase inicial de bezerras até a fase de lactação. O sistema de produção implantado é intensivo (Freestall), onde é realizado duas ordenhas diárias.

Lages, 11 de setembro de 2020

Ubirajara Maciel da Costa
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

em aberto
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

ANEXO II

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 "Bezerras suplementadas com diferentes doses de curcumina no período de amamentação", protocolada sob o CEUA nº 3067300717 (ID 000431), 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 21/08/2017.

We certify that the proposal "Heifers supplemented with different doses of curcumin during breastfeeding", utilizing 30 Bovines (30 females), protocol number CEUA 3067300717 (ID 000431), 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 08/21/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 08/2017 a 11/2017 Área: Zootecnia

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

Local do experimento: O presente estudo será realizado em propriedade localizada no munícipio de Chapecó-SC, sendo a principal atividade desenvolvida é a produção leiteira.

Lages, 11 de setembro de 2020

Ubirajara Maciel da Costa
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

em aberto
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

ANEXO III

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 "Cromo quelatado na dieta de bezerras lactantes e seus efeitos sobre desempenho e perfil metabólico", protocolada sob o CEUA nº 2877070619 (ID 000971), 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 18/07/2019.

We certify that the proposal "Chromium chelated in the diet of lactating heifers and their effects on performance and metabolic profile", utilizing 30 Bovines (30 females), protocol number CEUA 2877070619 (ID 000971), 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 07/18/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 06/2019 a 05/2020 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	1 a 60 dias	N:	30
Linhagem:	Holandês			Peso:	30 a 70 kg		

Local do experimento: Esse estudo será realizado em propriedade localizada no município de Chapecó [] SC, parceira em outros projetos já finalizados e publicados.

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ANEXO IV

Accepted Manuscript

Mineralization in newborn calves contributes to health, improve the antioxidant system and reduces bacterial infections

Patrícia Glombowsky, Aleksandro S. da Silva, Natan M. Soldá, Gabriela M. Galli, Angelisa H. Biazus, Gabriela Campigotto, Nathieli B. Bottari, Rejane S. Sousa, Maiara C. Brisola, Lenita M. Stefani, Matheus D. Baldissera, Marta L.R. Leal, Vera M. Morsch, Maria Rosa C. Schetinger, Gustavo Machado

PII: S0882-4010(17)31516-4

DOI: 10.1016/j.micpath.2017.12.012

Reference: YMPAT 2648

To appear in: *Microbial Pathogenesis*

Received Date: 16 November 2017

Revised Date: 5 December 2017

Accepted Date: 5 December 2017

Please cite this article as: Glombowsky Patrí, da Silva AS, Soldá NM, Galli GM, Biazus AH, Campigotto G, Bottari NB, Sousa RS, Brisola MC, Stefani LM, Baldissera MD, Leal MLR, Morsch VM, Schetinger MRC, Machado G, Mineralization in newborn calves contributes to health, improve the antioxidant system and reduces bacterial infections, *Microbial Pathogenesis* (2018), doi: 10.1016/j.micpath.2017.12.012.

ANEXO V

Dietary addition of curcumin favors weight gain and has antioxidant, anti-inflammatory and anticoccidial action in dairy calves

La adición de curcumina en la dieta de los terneros lecheros favorece el aumento de peso y tiene acción antioxidante, antiinflamatoria y anticoccidial

A adição de curcumina na dieta dos bezerros leiteiros favorece o aumento de peso e tem ação antioxidante, anti-inflamatória e anticoccidiostática

Patrícia Glombowsky[1]; Andreia Volpato[1], Gabriela Campigotto[1]; Natan M Soldá[1]; Daiane-da S dos-Santos[2]; Nathieli B Bottari[2]; Maria-Rosa C Schetinger[3]; Vera M Morsch[3]; Fernanda Rigon[2]; Ana-Luiza B Schogor[1, 2]; Aleksandro S Da-Silva[1, 2, 3*].

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Received: February 28, 2018; accepted: August 9, 2019

To cite this article:
Glombowsky P, Volpato A, Campigotto G, Soldá NM, dos-Santos DdS, Bottari NB, Schetinger MRC, Morsch VM, Rigon F, Schogor ALB, Da-Silva AS. Dietary addition of curcumin favors weight gain and has antioxidant, anti-inflammatory and anticoccidial action in dairy calves. Rev Colomb Cienc Pecu 2020; 33(1): 16-31
DOI: https://doi.org/10.17533/udea.rccp.v33n1a02