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EFEITO METAFILÁTICO DE MINERAIS SOBRE SAÚDE, DESEMPENHO, COMPOSIÇÃO QUÍMICO-FISICA E QUALIDADE DA CARNE DE CORDEIROS

CHRYSTIAN JASSANA CAZAROTTO

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

Orientador: Aleksandro Schafer da Silva

Co-orientador: Julcemar Dias Kessler

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Elaborada por
Chrystian Jassanã Cazarotto

como requisito parcial para obtenção do grau de Mestre em Zootecnia

Comissão Examinadora:

Dr. Aleksandro Schafer da Silva - Orientador (UDESC-Oeste)

Dra. Margarete Dulce Bagatini (UFFS)

Dr. Fernando Hentz (GH Consultoria Agropecuária)

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RESUMO

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

EFEITO METAFILÁTICO DE MINERAIS SOBRE SAÚDE, DESEMPENHO, COMPOSIÇÃO QUÍMICO-FISICA E QUALIDADE DA CARNE DE CORDEIROS

AUTOR: Chrystian Jassanã Cazarotto
ORIENTADOR: Aleksandro Schafer da Silva
Chapecó, 16 de Julho de 2018.

O objetivo desse estudo foi avaliar se existe um efeito metafilático de minerais (zinco, cobre, selênio e manganês) sobre desempenho, qualidade de carne, respostas imunológicas e antioxidantes, bem como se aplicação do mineral é capaz de prevenir a coccidiose. Cento e dez cordeiros da raça Lacaune recém-nascidos foram divididos em dois grupos: controle (não tratados – n=55) e tratados (n=55) com três doses de 0,33 ml/kg de complexo mineral nos dias de vida 1, 30 e 60. A dieta oferecida aos animais foi a mesma e o consumo não diferiram entre os grupos (P> 0,05). Todos os animais foram pesados no dia de vida 1, 30, 60, 90 e 150. Foram coletadas amostras de sangue para a avaliação da resposta imune, níveis de antioxidantes e parâmetros bioquímicos séricos (modulação hepática, lipídica e proteica). Concomitante às coletas de sangue foram realizadas coletas de fezes dos animais para pesquisa de oocistos de parasitos gastrointestinais a fim mensurar o nível de infecção dos animais. Os cordeiros tratados foram mais pesados (P<0,05) nos dias 15, 45, 60 e 90 de experimento. No dia 30 não houve diferença no peso, período que coincidiu com surto de coccidiose que foi mais severo nos animais do grupo controle. A atividade da catalase não diferiu entre os grupos (10/grupo), enquanto as atividades de superóxido dismutase e xantina oxidase foram maiores (P<0,05) nos cordeiros tratados em comparação ao controle. Os níveis séricos de proteína total e globulinas foram maiores (P<0,05) nos animais suplementados nos dias 15, 30 e 45, o que configura uma estimulação da resposta imunologica. Essa hipótese foi confirmada com aumento de linfócitos (dia 45), bem como níveis séricos de imunoglobulinas (IgM e IgG) nos animais tratados (dias 15 e 30) comparado ao controle. Níveis séricos de glicose foram maiores no grupo tratado no dia 15 e 30. A mortalidade dos animais tratados foi 7,27% menor que a do controle. Aos 150 dias de vida, 12 animais foram abatidos para análise físico-química da carne, status oxidante e antioxidante, e para análise alométrica. Houve um aumento na espessura de gordura (P<0,004), assim como o pH (P<0,002) foi menor na carne dos animais do grupo tratado comparado ao controle. No grupo controle, a carne foi mais clara (de acordo com a luminosidade (cor L)) (P<0,04), assim como houve maior perda de peso por cozimento (P<0,004). A força de cisalhamento foi menor na carne de cordeiros tratados (P<0,008), sugerindo que a aplicação de minerais foi associada ao aumento da maciez da carne. Além disso, as atividades de catalase e superóxido dismutase foram maiores (P<0,01) em animais do grupo tratado, associadas a uma redução nos níveis de espécies reativas de oxigênio (P<0,01) e produtos de peroxidação lipídica (P=0,02). Concluímos que o efeito metafilático de minerais é benéfico a saúde do cordeiro, aumentando as defesas antioxidantes e imunológicas, refletidas pelo maior ganho de peso e minimizando a coccidiose. Além disso, esses dados sugerem que os minerais tiveram um efeito nutraceutico devido modular o status oxidante e antioxidante, refletindo melhor qualidade da carne.

Palavras-chave: cordeiros, efeito nutraceutico; qualidade da carne; desempenho zootecnico.

ABSTRACT

Master's Dissertation
Programa de Pós-Graduação em Zootecnia
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METAFILATIC EFFECT OF MINERALS ON HEALTH, PERFORMANCE, MEAT CHEMICAL-PHYSICAL COMPOSITION AND QUALITY OF LAMBS

AUTHOR: Chrystian Jassanã Cazarotto
ADVISOR: Aleksandro Schafer da Silva
Chapecó 16 of July of 2018

The objective of this study was to evaluate if there is a metaphylactic effect of minerals (zinc, copper, selenium and manganese) on performance, meat quality, immune responses and antioxidants, as well as if mineral application is able to prevent coccidiosis. One hundred and ten newborn Lacaune lambs were divided into two groups: control (untreated - n = 55) and treated (n = 55) with three doses of 0.33 ml/kg of mineral complex in days of life 1, 30 and 60. The diet offered to the animals was the same and the consumption did not differ between groups (P > 0.05). All animals were weighed at day 1, 30, 60, 90 and 150. Blood samples were collected for the evaluation of immune response, antioxidant levels and serum biochemical parameters (hepatic, lipid and protein modulation). Concomitant to the blood collections were collected from animals feces to investigate oocysts of gastrointestinal parasites in order to measure the level of infection of the animals. The treated lambs were heavier (P < 0.05) on days 15, 45, 60 and 90 of the experiment. On day 30 there was no difference in weight, a period that coincided with a coccidiosis outbreak that was more severe in the control group. Catalase activity did not differ between groups (10/group), while superoxide dismutase and xanthine oxidase activities were higher (P < 0.05) in treated lambs compared to control. Serum levels of total protein and globulins were higher (P < 0.05) in animals supplemented on days 15, 30 and 45, which constitutes a stimulation of the immune response. This hypothesis was confirmed by increased lymphocytes (day 45) as well as serum levels of immunoglobulins (IgM and IgG) in treated animals (days 15 and 30) compared to control. Serum glucose levels were higher in the treated group on day 15 and 30. The mortality of treated animals was 7.27% lower than that of the control. At 150 days of life, 12 animals were slaughtered for physical-chemical analysis of meat, oxidant and antioxidant status, and for allometric analysis. There was an increase in the fat thickness (P <0.004), as well as the pH (P < 0.002) was lower in the meat of the treated group than in the control group. In the control group, the meat was lighter (according to the luminosity (color L)) (P < 0.04), as there was greater weight loss per cooking (P < 0.004). The shear force was lower in the meat of treated lambs (P < 0.008), suggesting that the application of minerals was associated with an increase in meat tenderness. In addition, catalase and superoxide dismutase activity were higher (P < 0.01) in animals in the treated group, associated with a reduction in the levels of reactive oxygen species (P < 0.01) and lipid peroxidation products (P = 0.02). We conclude that the metaphoric effect of minerals is beneficial to the health of the lamb, increasing the antioxidant and immune defenses, reflected by greater weight gain and minimizing coccidiosis. Furthermore, these data suggest that the minerals had a nutraceutical effect due to modulating oxidant and antioxidant status, reflecting better meat quality.

Key words: lambs, nutraceutical effect; quality of meat; zootechnical performance.

SUMÁRIO

CAPÍTULO I
REVISÃO DE LITERATURA

1. PANORAMA DA OVINOCULTURA

Diante de um mercado promissor, a criação ovina para diversos fins vem crescendo no Brasil, sendo que em 2015 atingiu um número próximo a 18,41 milhões de cabeças, com um aumento de 4,5% em relação a 2014 (IBGE, 2016). A Região Nordeste se destaca na criação de ovinos e concentra cerca de 60,6% do rebanho nacional. A Região Sul, representa 26,5% do efetivo da espécie no país, acompanhada pelas Regiões Centro-Oeste (5,6%), Sudeste (3,8%) e Norte (3,6%). O estado de Santa Catarina possui um pequeno percentual da produção nacional, isto é, 350 mil ovinos, sendo que a maior parte é destinado a carne (IBGE, 2016).

O ovino é considerado uma espécie de grande importância, difundindo-se por diversos países do mundo, podendo ser manejado de diferentes formas e sob diferentes condições ambientais. Devido à crescente demanda pela carne ovina, a ovinocultura de corte está se tornando uma atividade cada vez mais atraente para a diversificação da produção agropecuária (RIBEIRO et al., 2013).

O mercado consumidor tem demonstrado elevado potencial de crescimento (Madruga et al., 2008). Esse aumento na produtividade tem sido observado após a década de 1990, onde ocorreu a grande crise na lã. Madruga et al. (2005) já observavam que a ovinocultura vem se apresentado como uma atividade promissora no agronegócio brasileiro, por dispor dos requisitos necessários, como: extensão territorial para pecuária, clima tropical, muito verde, mão-de-obra barata, produzindo animais a baixo custo.

O Brasil apresenta potencial para competir com os maiores produtores de carne ovina no mundo, isto é, Austrália e Nova Zelândia. Entretanto, o Brasil ainda importa carne ovina de país como por exemplo, Uruguai por não atender a demanda interna. Mesmo com o crescimento desse agronegocio, necessitamos importar carne ovina, o que dificulta o desenvolvimento da atividade, pois reduz incentivos nacionais para investimentos.

Segundo Pilar et al. (2002), existe atualmente uma crescente procura e aceitação de produtos oriundos da ovinocultura (leite, carne, pele e lã), tanto no mercado interno, quanto no mercado externo, levando como consequência as mudanças nos segmentos de produção e comercialização. No entanto, um dos entraves para o desenvolvimento da ovinocultura no Brasil é o baixo desempenho produtivo, ocasionado principalmente pelo manejo inadequado nos rebanhos. Conforme Ribeiro et al. (2013) o desempenho dos rebanhos pode ser expresso pela quantidade de quilogramas de cordeiro desmamado por ovelha, o que é afetado pela combinação entre fatores como a nutrição, sanidade, manejo e genética.

De acordo com literatura, a produção e o consumo de carne ovina no Brasil têm crescido e estimulado a realização de pesquisas (MACMANUS et al., 2003). Esses dados têm contribuído para melhorar os sistemas de produção mediante práticas de manejo, alimentação, cruzamentos e seleção dos indivíduos ou raças, a fim de oferecer um produto de melhor qualidade a um mercado exigente e crescente.

2. OVINOCULTURA LEITEIRA E O CORDEIRO COMO SUBPRODUTO

Segundo Boyazoglu e Morand-fehr (2001) a região mediterrânea é a mais tradicional e representativa produtora de leite e de queijos de ovelhas. Praticamente todas as cabras e 60% das ovelhas são ordenhadas total ou parcialmente, e cerca de 95% de seu leite são transformados em derivados lácteos com conotação de qualidade típica local, tais como os queijos Feta, Roquefort, Pecorino Romano e Manchego, entre outros. A carne dos animais é considerada subproduto da atividade leiteira.

A produção leiteira ainda não foi devidamente explorada no Brasil, mas é um produto nobre do ponto de vista da fabricação de queijos, pois está fortemente ligada à industrialização de produtos lácteos. Consequentemente, é um leite de alto valor, destinado à fabricação de queijos finos, de elevado valor de mercado (SELAIVE e OSÓRIO, 2014). A exploração da atividade leiteira ovina em escala industrial é recente no país, mais precisamente com a introdução da raça Lacaune no Rio Grande do Sul, pela Cabanha Dedo Verde no ano de 1992 (BOYAZOGLU e MORAND-FEHR, 2001). Segundo Selaive e Osório (2014), a exploração de leite ovino tem sido vista como alternativa sustentável de baixo investimento inicial e fácil adoção pela mão de obra familiar, melhorando, desta forma, a qualidade de vida do pequeno e do médio produtor rural. No entanto, a carne das animais raças leiteiras vem como um subptoduto para valorizar ainda mais a atividade.

Alguns trabalhos revelam que a recria dos cordeiros tem sido a causa de maior custo dentro do sistema de produção de leite, pois o aleitamento artificial com leite substituto é caro, além de não existir no mercado sucedâneos que atendam às exigências do jovem cordeiro

(MCKUSICK et al. 2001). No entanto, THOMAS et al. (2001) observaram um aumento de 61% na produção de leite quando os cordeiros foram criados com sucedâneo, quando comparados àqueles desmamados aos 30 dias de idade. De acordo com Mckusick et al. (2001), ovelhas em sistema misto de produção de leite produzem menos leite comercial durante o período de amamentação, comparado às ovelhas em sistema de desmama precoce. Estes pesquisadores avaliaram a produção de leite em três diferentes sistemas de desmama, com médias de 261 ± 10 e 236 ± 10 kg/ovelha para o sistema sem cordeiro e sistema misto (ordenha + cordeiro) respectivamente. Para o terceiro sistema, em que as ovelhas foram ordenhadas após o desmame realizado aos 30 dias de idade, a média de produção foi de 172 ± 10 kg/ovelha. Tratando-se de produção de leite para a fabricação de queijos e outros produtos industrializados, como a carne, a escolha do sistema de produção passa a ser de suma importância, visando máximo rendimento leiteiro e econômico.

3. DESAFIO NA PRODUÇÃO DE CORDEIROS

Por serem considerados animais de ciclo rápido e metabolismo acelerado, os ovinos são propensos à diversas enfermidades, as quais acabam comprometendo todo o ciclo produtivo, a rentabilidade da produção e a depreciação do produto final, isto é, a carne (GARCIA et al., 2003).

Barros (2010), afirma que, os ovinos são suscetíveis a uma série de doenças, algumas delas características da própria espécie, e se não corretamente controladas através de um adequado manejo sanitário, afetam a eficiência reprodutiva do rebanho, prejudicando o desenvolvimento dos animais e provocando principalmente altos índices de mortalidade. Segundo Martin (1993), o retardo no desenvolvimento e o alto índice de mortalidade em cordeiros nas primeiras semanas de vida são os fatores que mais influenciam negativamente, gerando perdas econômicas que podem estar associadas ao mau manejo dos animais, precariedade das instalações no momento do nascimento até o desmame e a ausência de alimentação adequada.

A incidência de cada doença varia com o sistema de criação, isto é, geralmente sistema intensivos, com instalações inadequadas ou sem um manejo sanitário correro, são mais propensos a doenças coletivas causadas por agentes infeciosos. A eimeriose é uma doença frequente na criação em confinamento e principalmente em animais jovens, sendo de menor incidência em animais em pastejo, sistema extensivo. Segundo Benavides et al. (2015) o manejo incorreto afeta principalmente a fase dos recém-nascidos, pois é nesta época é quando os animais ficam mais susceptíveis a doenças bacterianas, virais e por parasitos.

De acordo com Nunes (2006), um dos principais fatores que afetam os ganhos na produção de ovinos é a mortalidade neonatal de cordeiros, sendo que a percentagem de mortalidade é afetada por uma variedade de fatores relacionados ao manejo e a doenças conforme já mencionado. Segundo Christley et al. (2003), o peso do cordeiro ao nascer, concentração sérica de imunoglobulinas, tipo de parto (simples ou múltiplo), ordem de parto da ovelha e seu comportamento, são fatores que afetam a sobrevivência do cordeiro durante o período neonatal. Christley et al. (2003), explica ainda que, a aquisição de imunidade passiva advinda do colostro materno está associada fortemente com a proteção contra doenças infecciosas e consequentemente contra a morte de cordeiros na fase inicial de vida, mas é importante lembrar que geralmente a morte do cordeiro ocorre por uma combinação de duas ou mais causas. Nunes (2006), em estudo realizado no Norte de Minas Gerais com cordeiros da raça Santa Inês (puros e mestiços), relatou taxa de mortalidade neonatal de 27,8%, com maior frequência nas duas primeiras semanas de vida, sendo as principais causas de morte até 90 dias de idade (desmama), devido falhas no manejo e doenças infecciosas.

A alimentação é um dos principais fatores que influenciarão a produção de ovinos. Cada fase de vida dos animais apresenta diferentes necessidades fisiológicas, por isso devemos ter muito cuidado ao oferecer alimentação, que deve estar balanceada (Rassu et al., 2002). Conforme já mencionado, os manejos logo após ao nascimento também devem ser controlados pois esse cordeiro será o produto final da produção. Manejos como ingestão do colostro, logo após o nascimento, cura de umbigo, pesagem, identificação e gestão de dados, são alguns dos fatores necessários para obter lucratividade do plantel. Uma das formas de aumentar o ganho de peso à desmama e minimizar os índices de mortalidade, é através do creep-feeding, isto é, a utilização de um cocho privativo, dentro de um cercado, ao qual só o cordeiro tem acesso (GARCIA et al., 2003). Estando o cordeiro, ainda em fase de aleitamento, ele recebe um reforço alimentar com uma ração concentrada balanceada, isso é necessária para estimular os animais a ingerir alimentos sólidos, pois em alguns sistemas de criação a desmama ocorre a partir de 45 dias de vida do animal. Pesquisas tem mostrado que muitos minerais adicionados a dieta ou aplicados na forma injetável tem sido benéfico para saúde dos animais, principalmente pela ativação da resposta imunológica e controle do estresse oxidativo (SILVA et al., 2016), o que vem estimumando a realização de novos estudos. Com base nessas pesquisas, empresas tem produzido complexos minerais e vitamínicos para serem usados de forma injetável.

A pesquisa recente tem mostrado que para a produção de cordeiros se tornar rentável, com animais saudáveis e com um melhor rendimento de carcaça, é necessário manejo adequado de criação, além da importância na suplementação desses animais. Dessa forma, os produtores terão uma desmama precoce, o tempo de engorda diminui e reduza os custos, aumentando assim a lucratividade e alavancando a produção de cordeiros no país.

4. SUPLEMENTAÇÃO MINERAIS

Primeiramente é importante ressaltar que suplementação e aplicação de minerais são duas coisas diferentes. O termo suplementação é o mais comum e usado a anos, pois esta relacionado a adição dos minerais na dieta dos animais. Já a aplicação de minerais pela via subcutânea e intramuscular é uma metodologia mais recente, que tem mostrado grande sucesso, e essa técnica tem sido caracterizada como efeito nutraceutico ou metafilatico dos minerais. No entanto, se consultarmos a literatura, mesmo quando aplicado os minerais pela via injetável, diversos artigos foram publicados como suplementação mineral.

Importante lembrar que de acordo com o nível de exigência, os minerais podem ser classificados em macro ou microminerais, ou seja, macrominerais são requeridos pelo organismo em grandes proporções (quantidades maiores que 100 ppm), e são usados principalmente para funções estruturais ou mantenção do balanço ácido-base. Microminerais por sua vez são exigidos em proporções menores que 100 ppm e atuam principalmente como co-fatores enzimáticos contribuindo de forma estrutural ou funcional para a atividade das enzimas, hormônios ou vitaminas e são representados pelo zinco (Zn), ferro (Fe), cobalto (Co), cobre (Cu), molibdênio (Mo), manganês (Mn), iodo (I), selênio (Se), flúor (F) e níquel (Ni) (MENDONÇA JUNIOR et al., 2011). É importante destacar que esses minerais são igualmente importantes para manutenção de funções fisiológica essencial a vida, e portanto, a falta de um deles limita o desempenho animal.

A mineralização dos ovinos é uma prática zootécnica viável do ponto de vista prático e econômico, principalmente quando deseja-se aumentar a produtividade desta espécie animal, alem, disso a correta mineralização do rebanho ovino é de grande importância para as funções vitais, pois a carências de minerais podem ocasionar grandes danos aos animais, pois gera uma queda na produtividade, ou seja, prejudica o desenvolvimento, o crescimento, o ganho-de-peso, facilitando o aparecimento de doenças, a queda da fertilidade (SILVA SOBRINHO, 2000). Aproximadamente, 5% do peso vivo do animal é constituído de minerais, podendo esta concentração variar em função da idade, espécie, raça e do próprio indivíduo

(MARTIN, 1993). Segundo LAMB et al. (2008) a adição dos microminerais em dietas está diretamente relacionada a algumas funções orgânicas como a melhora da imunidade e do desenvolvimento produtivo e reprodutivo de rebanhos. Devido à susceptibilidade dos cordeiros aos agentes patógenos do meio em que se encontram, a sua nutrição deve ser corretamente elaborada, pois ela permitirá ao animal em crescimento expressar o seu potencial genético e manterem-se saudáveis. Já em 1985, pesquisadores chamavam a atenção para importância dos minerais, pois estes agem como componente estrutural de órgãos e tecidos do corpo, como componentes de fluidos intra e extracelulares, no equilíbrio eletrolitico e ácido base e como componente estrutural de metaloenzimas de hormônios e vitaminas, e como co-fatores ou ativadores de sistemas enzimáticos (NRC, 2007).

Conforme descrito anteriormente, dietas em quantidade insuficiente de minerais ou rações desbalanceadas resultam em carência de um ou mais elementos e faz-se necessário a correção das mesmas ou a suplementação com o uso de complexos pré-formulados (PEIXOTO et al., 2005). A importância da suplementação da alimentação com produtos que forneçam suporte nesse período crítico, pode ser essa uma ótima opção. Dentre os elementos utilizados para este fim ressaltam-se os macro e micro minerais.

Complexos minerais são amplamente estudados em ruminantes. Recentemente, um teste de campo com vacas leiteiras avaliou o efeito de um produto comercial a base dos minerais Zn, Se, Mn e Cu em 230 e 260 dias de gestação, e 35 dias após o parto sobre características de produção, fertilidade e de saúde. De acordo com esses autores, a suplementação, embora sem efeitos significativos sobre a fertilidade e produção de leite, apresentou efeito positivo significativo sobre a saúde do úbere das vacas multíparas, diminuindo a contagem de células somáticas (CCS) e a incidência de mastite clínica (MACHADO et al., 2013). Estudos recentes do nosso grupo de pesquisa verificaram efeitos nutraceuticos benéficos de minerais (produto comercial – Fosfosal) aplicados em vacas leiteiras (WARKEN et al., 2018) e bezerras recém-nascidas (GLOMBOWSKY et al., 2018). No estudo com vacas observou-se redução de CCS no leite, assim como atividade antioxidante e estimulação do sistema imunológico com aumento de citocinas. Já nas bezerras o principal resultado foi a prevenção contra diarreia, isto é, nenhum dos animais precisou ser tratado com antibiótico devido a agravamento desse sinal clinico, diferente do que ocorreu nos animais do grupo controle. Efeito metafilático do cobre e zinco associado foi observado sobre o proteinograma de bezerras recém-nascidas, sendo que esses animais tiveram maiores níveis de ceruplasmina, imunoglobulinas de cadeia pesada e leve, entre outras proteinas de fase aguda em níveis superiores, e consequentemente reduzindo uso de antibiótico devido à infecção gastrointestinal (TOMASI et al., 2018).

Pouco se sabe sobre a ação de elementos minerais oriundos de suplementação em cordeiros na fase de desmame e pós-desmame, e muito menos sabe-se sobre efeitos nutraceuticos/metafilaticos desses minerais quando aplicados de forma injetável. Em bezerros de corte, pesquisadores concluíram quea aplicação subcutânea de um complexo mineral a base de Zn, Mn, Cu e Se levou a uma maior resposta humoral através de elevação de anticorpos, maior concentração de proteínas de fase aguda e elevada concentração dos minerais no tecido hepático (ARTHINGTON et al., 2014). Importante ressaltar, que esses microelementos foram os presentes no suplemento mineral usado nesse estudo, com a finalidade de contrução dessa dissertação.

O selênio é um micromineral de propriedades antioxidantes (UNDERWOOD e SUTLLE, 1999) que protege as membranas celulares da degeneração oxidativa fazendo parte de enzimas como por exemplo a Superóxido dismutase (SOD), impotante enzima antioxidante (MCDOWELL, 1996). O selênio é também um estimulante do sistema imunológico, influenciando a expressão de respostas não-específicas, humorais e celulares (COMINETTI e COZOLINO, 2009). Smith et al. (1986), demonstraram que a dieta suplementada com selênio ajuda a manter os mecanismos de defesa do organismo, incluindo a produção de anticorpos e proliferação celular. Outra função importante do selênio é na produção de hormônios da tireóide (fundamental pois seus hormônios Tiroxina (T3) e Triiodotironina (T4) garantem o funcionamento de todo o organismo) (MacDonald et al., 2002). Os ruminantes absorvem o selênio (em torno de 54%) menos eficientemente que os não-ruminantes (80%), pois o rúmen é um ambiente químico que favorece a redução (Ortolani, 2002). González & Silva (2003) comentam que na deficiência do elemento, há diminuição na atividade da glutationa-peroxidase. Esta determinação pode se realizada para auxiliar no diagnóstico da enfermidade. Na produção animal, o selenito de sódio destaca-se como a principal fonte de selênio usado nas dietas (GRILLI et al., 2013). Outro composto com atividade similar, o disseleneto de difenila $(PhSe)_2$, conserva as mesmas propriedades do selênio inorgânico (MEOTTI et al., 2004) e não é usado na alimentação provavelmente devido ao elevado custo. Em estudos realizados por Biazus et al. (2018), avaliaram a suplementação subcutânea de disseleneto de difenila $(PhSe)_2$, em ovinos leiteiros e observaram que a suplementação de ovelhas leiteiras de $(PhSe)_2$, ativou respostas antioxidantes e antiinflamatórias e aumentou o teor de gordura do leite. Além disso, esse protocolo aumentou o antioxidante e, consequentemente, reduziu a concentração de oxidantes no leite, o que é desejável para a qualidade do produto.

O zinco é componente de algumas metaloenzimas tais como superoxido-dismutase, anidrase carbônica, alcool-desidrogenase, carboxipeptidase, fosfatase alcalina, DNA e RNA polimerases, com efeitos nos metabolismos dos carboidratos, lipídios, proteínas e ácidos nucléicos (NRC, 2007). De acordo com McDowell (1992), entre os principais efeitos do zinco na produção e secreção de hormônios estão relacionados com a testosterona, insulina e corticóides da adrenal. A principal forma de armazenamento do zinco é como metalotioneína no fígado. Sua síntese é induzida pela presença do elemento no fígado (McDowell, 1992). Em estudos realizados com bovinos leiteiros, Pardo et al. (2004) observaram que a deficiência de zinco tem sido associada à pododermatite em bovinos, concluindo que o zinco favorece a integridade dos cascos, por acelerar a cicatrização das feridas, aumentar a velocidade de reparação do tecido epitelial e manter a integridade celular. A maioria dos sinais clínicos, apresentados pelos animais com deficiência de zinco estão intimamente relacionados com a função deste elemento nas DNA e RNA polimerases e seu papel na replicação e diferenciação celular. MacDonald et al. (2002) cita que os principais sinais em suínos, se caracterizam por crescimento retardado, diminuição do apetite, baixa conversão alimentar e paraqueratose. Em aves verifica-se problemas de crescimento, anormalidades nas pernas conhecidas por "Síndrome do jarrete inchado". Em bovinos, o NRC (2007) comenta sobre redução na ingestão de matéria seca e na taxa de crescimento, além de problemas de paraquaratose e pêlos quebradiços.

O excesso ou a falta de cobre, pode provocar intoxicações ou carência em ruminantes, em especial nos ovinos, pelo fato destes animais necessitarem de menores quantidades de cobre na sua alimentação variando de 3 a 14 ppm (NRC, 2007). O cobre presente em altos níveis na dieta se acumula, no decorrer de meses ou anos no fígado. Quando o limiar de saturação é atingido, existe uma súbita liberação de cobre livre. Além de causar dano hepático, o cobre penetra nas hemácias causando hemólise e liberação de hemoglobina, que produz insuficiência renal, levando freqüentemente os animais à morte (ORTOLANI, 2002). Por estar envolvido no mecanismo de oxidação, sua deficiência leva a transtornos no metabolismo oxidativo, podendo manifestar-se de múltiplas formas. (GONZÁLEZ e SILVA, 2003). O cobre pode ser relacionando a outras funções, como: regulação dos processos vitais de crescimento e diferenciação celular, respiração celular, sistema imunológico, reprodução, angiogênese, mielinização dos neurônios, além de ser essencial para a formação da hemoglobina (MCDOWELL, 2003). Diante das várias funções do cobre no organismo, justificam-se as pesquisas que mostram significativas melhoras no desenvolvimento do animal com a introdução de cobre na dieta, como Cheng et al. (2008) que mostraram que altos níveis de cobre na dieta podem interferir positivamente nas características da carcaça por afetar o metabolismo lipídico de cordeiros, bem como na melhora do sistema imunológico. Solaiman et al. (2006) observaram que níveis superiores aos valores basais de cobre na dieta podem melhorar o ganho de peso e a resposta imunológica, mostrando a importância do cobre na dieta de animais.

Segundo Moraes (2001), o manganês é necessário para a manutenção da estrutura óssea normal e o funcionamento adequado do sistema nervoso central. O manganês (Mn) se encontra em maiores concentrações nos ossos, rins, fígado, pâncreas e glândula pituitária, todos os tecidos ricos em mitocôndrias. Conforme relataram Underwood e Suttle (1999), as concentrações variam no corpo todo desde 0,5 a 3,9 mg/kg de matéria seca (MS) na carcaça de ovinos e bezerros. Segundo Scottá et al. (2014) o Mn é componente de enzimas (quinases, hidrolases, trasferases e descarboxilases) envolvidas no metabolismo dos carboidratos, dos lipídios e das proteínas, atuando como um co-fator na produção da enzima antioxidante superóxido dismutase. É componente dos mucopolissacarídeos na matriz orgânica dos ossos. Ainda Scottá et al. (2014) explica que, o Mn participa no desenvolvimento de cartilagens, e também propicia uma resistência contra a ação de agentes oxidantes e consequente formação de radicais livres nas células. Possui ainda função imune associando-se a macrófagos e neutrófilos. A absorção ocorre em toda a extensão do intestino delgado. Conforme Moraes (2001) os sintomas da deficiência desse elemento podem ser expressos por anomalias no esqueleto de animais jovens e recém-nascidos. O manganês é pouco absorvido pelos ruminantes (1% ou menos). Os fatores dietéticos que podem influenciar na biodisponibilidade do manganês têm recebido pouca atenção, provavelmente porque a deficiência de manganês não é considerada um grave problema na nutrição de ruminantes (SPEARS, 2003).

De maneira geral, animais de produção exigem algum tipo de suplementação dietética de minerais para que ocorra prevenção de deficiências (CHURCH, 1991). Tendo em vista os efeitos dos minerais cobre, zinco, selênio e manganês no metabolismo animal, torna-se possível sugerir que, quando há administração injetavel desses elementos em cordeiros, pode ocorrer uma diminuição dos compostos oxidantes ou aumento de antioxidantes, tanto no sangue quanto na carne desses animais, tornando-os mais resistentes à distúrbios patológicos e originando um produto de melhor qualidade, e de maior interesse para a indústria da carne com propriedades benéficas para os consumidores. Além disso, estes minerais podem ainda interferir na resposta imunológica e antioxidante dos animais.

5. QUALIDADE DA CARNE

Confome já descrito, a expansão do mercado da carne ovina no Brasil tem mostrado crescimento significativo nos últimos tempos, em decorrência a esse aumento, crescem também as exigências do mercado consumidor, fato que assinala para a necessidade de se conhecerem os fatores que interferem nas características físicas e químicas da carne, pois estas determinam sua qualidade e aceitabilidade (MARTÍNEZ-CEREZO et al., 2005). Segundo Bueno et al. (2000), as carcaças de boa qualidade devem ter uma elevada proporção de músculos, cobertura de gordura subcutânea uniforme e teor de gordura adequado para que seja aceita pelo mercado consumidor. A padronização das carcaças de cordeiros a ser colocadas no mercado também é necessária para valorizar o produto e atrair o consumidor. A textura, para os vários tipos de carnes, é o critério de qualidade mais importante. Bressan & Beraquet (2002), explicam que embora seja ampla a faixa de aceitação de maciez pelos consumidores, é certo que há vantagens para a carne mais macia quando os outros fatores são constantes.

A composição centesimal da carne sofre variações em função do tipo de músculo, da idade, da espécie animal, da nutrição, da raça, da condição sexual, do manejo pré-abate e pós-abate dos animais (FORREST, 1979). Para obter qualidade na carne de ovina, principalmente a carne de cordeiro, é necessária uma combinação dos atributos sabor, suculência, textura, maciez e aparência (SILVA-SOBRINHO, 2000). Então, é fundamental a implantação de novas alternativas ou tecnologias, visando maior produtividade e qualidade, para atender a um mercado consumidor (GARCIA et al., 2005). Segundo Truscott et al. (1984), os varejistas consideram a cor da carne fator de importância primária na aceitação pelos consumidores (que preferem a cor vermelho-vivo (oximioglobina) da carne fresca, preterindo a cor marrom (metamioglobina). Normalmente, as carnes escuras são rejeitadas pelo consumidor, pois associa a cor escura a carnes com maior tempo de prateleira ou oriundas de animais velhos, no entanto, essa relação nem sempre é verdadeira. Segundo Sainz, (1996) muitas vezes a carne de animais abatidos com pouca reserva de glicogênio não atinge valores de pH suficientemente baixos para produzir colorações normais, independentemente de sua idade. Conforme Garcia et al. (2005) a cor da carne pode ser medida pelo método objetivo, utilizando-se colorímetro, que determina os componentes de cor L* (luminosidade), a* (teor de vermelho) e b* (teor de amarelo). De acordo com literatura, o pH final na carne ovina varia de 5,4 a 5,8; porém, valores altos (6,0 ou acima) podem ser encontrados em casos de depleção dos depósitos de glicogênio muscular antes do abate o que pode influenciar na qualidade do produto final (GARCIA et al., 2005), conforme já descrevia Sainz na descada de 90 (SAINZ

1996). Segundo Sañudo e Sierra (1993), a capacidade de retenção de água é um parâmetro biofísicoquímico que pode ser definido como uma maior ou menor nível de fixação da água de composição do músculo nas cadeias de actina-miosina. É um parâmetro de grande importância econômica e sensorial. A composição química também influencia a qualidade da carne e varia de acordo com fatores como raça, sexo e alimentação (SAINZ, 1996). Pilar et al. (2002), explica que, o teor em proteínas com alto valor biológico é uma característica positiva da carne ovina, assim como o de lipídios, que, além de ter elevado valor energético, é composto por ácidos graxos essenciais e influência nas características sensoriais do produto. Como pode ser constatado, são muitos os fatores que podem afetar a composição químico-fisica e qualidade da carne, além disso os minerais também pode ter papel importante na produção de uma carne de maior aceitação e nutraceutica.

Segundo Oliveira et al. (2010), quando os animais são terminados a pasto a forragem apresenta baixos teores de proteína bruta e digestibilidade, além disso as quantidades minerais presente nessas pastagens, não são suficientes para suprir a necessididade fisiológica dos animais, o que geralmente ocorre durante a estação seca na maior parte do Brasil tropical, o desempenho pode não ser o desejável, faz-se necessário então a suplementação mineral. Segundo Sainz (1996), o nível nutricional dos animais está relacionado positivamente a quantidade de gordura na carcaça, sobretudo em animais alimentados com dietas balanceadas e com aportes minerais e vitaminicos, podendo influenciar na qualidade da carne, como pH, maciez, cor e perdas de peso ao cozimento, propriedades da carne que determinam atributos para a comercialização, como aparência e adaptabilidade aos processamentos industriais. Esses efeitos dos minerais sobre sistema antioxidante no sangue podem se estender para os músculos, contribuindo para aumento de antioxidantes enzimáticos e não enzimáticos que podem melhorar a qualidade da carne.

6. OBJETIVOS

6.1. OBJETIVO GERAL

O estudo tem como objetivo geral avaliar se a administração injetável de zinco, cobre, selênio e magnésio (produto comercial) é capaz de reduzir a mortalidade de cordeiros recém-nascidos, assim como ativar respostas imunológicas e antioxidantes e melhorar o desempenho desses animais. Além disso, avaliar o efeito mertafilático dos minerais na modulação do status oxidante/antioxidante na carne dos cordeiros de uma forma que melhore a sua qualidade.

6.2. OBJETIVOS ESPECÍFICOS

- Avaliar se a aplicação de minerais subcutâneo em cordeiros reduz mortalidade e melhora peso e ganho de peso.
- Avaliar se a aplicação de minerais subcutâneo alterá os níveis de glicose e o afeta positivamente o metabolismo proteico dos cordeiros.
- Avaliar se a aplicação de minerais subcutâneo ativa as enzimas antioxidantes (CAT e SOD) no sangue total e xantina oxidase no soro dos cordeiros.
- Avaliar se a aplicação de minerais subcutâneo aumenta os níveis de imunoglobulinas (IgG e IgM) no soro dos cordeiros.
- Mensurar se a aplicação de minerais subcutâneo modula os níveis de oxidantes (EROS e TBARS) e antioxidantes (CAT, SOD) na carne dos cordeiros.
- Avaliar se a aplicação de minerais subcutâneo melhorá a qualidade da carne dos cordeiros.

CAPÍTULO II
MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de dois manuscritos com suas formatações de acordo com as orientações das revistas as quais foram submetidos:

Manuscrito I- Metaphylactic effect of minerals on immunological and antioxidant response, weight gain and minimizing of coccidiosis in newborn lambs.

Submetido ao *Research Veterinary Science*

Manuscrito II – Nutraceutical effect of trace elements as injectable doses reduces mortality, modulates oxidant and antioxidant status, and improves the quality of lamb meat

Submetido ao *Biological Trace Element Research*

MANUSCRITO I

Metaphylactic effect of minerals on immunological and antioxidant responses, weight gain and minimization of coccidiosis of newborn lambs

Chrystian J. Cazarotto[1], Jhonatan. P. Boito[1], Roger R. Gebert[1], João H. Reis[1], Gustavo Machado[2], Nathieli B. Bottari[3], Vera M. Morsch[3], Maria R. C. Schetinger[3], Pedro H. Doleski[3], Marta L.R. Leal[4], Matheus D. Baldissera[5], Aleksandro S. Da Silva[1,3*]

[1] Graduate Program of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, Santa Catarina (SC), Brazil.

[2] Veterinary Population Medicine, University of Minnesota, Sait Paul, MN, United States of America.

[3] Post-Graduate Program in Toxicological Biochemical and Department of Biochemistry and Molecular Biology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

[4] Department of Large Animal, UFSM, Santa Maria, RS, Brazil.

[5] Department of Microbiology and Parasitology, UFSM, Santa Maria, RS, Brazil.

[*]Author for correspondence: Department of Animal Science, University of Santa Catarina State. 680D, Beloni Trombeta Zanin Street, Chapecó/SC, Brazil Zip: 89815-630, Phone: 55 49 3322-4202. Fax: 55 49 3311-9316. (E-mail: dasilva.aleksandro@gmail.com).

Abstract

The aim of this study was to evaluate the metaphylactic effect of minerals on immunological and antioxidant responses, as well as performance and prevent coccidiosis of newborn lambs. We divided 110 newborn lambs into two groups (55/group): control (untreated) and treated with two doses of 0.33 mL/kg of a mineral complex (zinc, copper, selenium, and manganese) on day of life (DOL) 1 and 30. Treated animals were heavier ($P < 0.05$) than untreated lambs on DOL 15 and 45, but not on DOL 30 due to a coccidiosis outbreak. Catalase activity did not differ between groups, while superoxide dismutase and xanthine oxidase activities were higher ($P < 0.05$) in treated lambs compared with control animals. Serum levels of total protein and globulins were higher ($P < 0.05$) in treated animals (DOL 15, 30 and 45). A significant increased on the number of lymphocytes (DOL 45), as well as on seric levels of immunoglobulins (IgM and IgG) was observed in treated animals (DOL 15 and 30). Serum Ig levels remained constant throughout the experiment in the treated group, but fluctuated in the control group. Serum glucose levels were greater in treated animals (DOL 15 and 30). It is possible to conclude that subcutaneous administration of minerals has beneficial effects on lambs by increasing antioxidant and immunological defenses, reflected by greater weight gain, which could mitigate the impact of coccidiosis.

Keywords: lambs; nutraceutical effect; immunological response; animal health; antioxidant action; coccidiosis.

1. Introduction

Mineral supplementation of sheep is an important practice from a practical and economic point of view, especially regarding animal productivity. In general, this type of supplementation is an indispensable component of several production systems throughout

Brazil (Silva-Sobrinho, 2000). Optimal sheep mineral supplementation is essential for several vital functions, including digestion, respiration, circulation, and enzymatic reactions (Ortunho, 2013). Mineral deficits may cause many problems, including decrease in productivity, poor growth and weight gain, in addition to higher susceptibility to diseases and infertility. Approximately 5% of animal body weight is mineral, which can vary with age, species, breed and individual characteristics (Martin, 1993; Ortunho, 2013). Some minerals are required in major quantities and are called macro-minerals (calcium, phosphorus, sodium, chlorine, magnesium, manganese, potassium and sulfur); those required in smaller quantities (zinc, iron, copper, selenium, cobalt, manganese and fluoride) by the animals body are known as micro-minerals (Martin, 1993). All these minerals are essential in order to maintain all vital physiological functions and deficiencies may limit animal survival and performance.

Delays on body development and high mortality rates during the first few weeks of live may cause huge economic losses to farmers. These problems might be associated with improper animal handling, lack of adequate food, or poor facilities to raise these animals from birth to weaning (Martin, 1993). When exposed to this environment, animals are susceptible to various pathogens. Since 1985, several studies have demonstrated the importance of mineral supplementation for optimization of tissue structure, ionic homeostasis, acid-base equilibrium, and enzymatic systems (NRC, 1985); however, at this early stage of life, the consumption of solid diets is low, and therefore, lambs dependent on the minerals present in the colostrum and the milk of their mothers. In the literature, high deficiency of selenium was found in lactating ewes compared to non-lactating females of other species (Khan et al., 2010), suggesting that lambs may suffer of mineral deficiencies in the first days of life when they consume only milk.

Therefore, the development of strategies to deal with these problems is essential in order to raise healthy and productive herds and the oral supplementation or injectable application of minerals may mitigate several risks surrounding newborn lambs. Minerals are essential to the immunological and inflammatory responses and protect the animals against performance impairments (Garg et al., 2008; Ortunho 2013). Recent studies have shown that the administration of mineral complexes to calves activates the immunological and antioxidant responses, as well as reduces health problems, including diarrhea (Glombowsky et al., 2018; Tomasi et al., 2018). However, we found no records on the metaphylactic or nutraceutical effect of mineral supplementation for lambs. Thus, the aim of this study was to evaluate the metaphylactic effect of minerals (zinc, copper, selenium, and manganese) on the immunological and antioxidant responses, as well as its impact on weight gain and prevention of coccidiosis of newborn lambs.

2. Materials and Methods

2.1. Products

A commercial product (Adaptador MIN®, Biogen) was used to evaluate the effect of mineral supplementation in newborn lambs. This product (100 mL) is composed of copper edetate (1 g), zinc edetate (4 g), manganese edetate (1 g), and sodium selenite (0.5 g).

2.2 Animals

We used a total of 110 newborn lambs, Lacaune breed, weighing approximately 3 kg at birth. They were housed in pens (5 animals each) and divided into two groups (n = 55 each): a control group and a treated group. Animals from both groups received colostrum in the first few hours of life. Thereafter, newborns were fed milk from their mothers until day of life

(DOL) 7. After this period, the animals received also artificial milk (Table 1). A dose of 0.33 mL/kg mineral compound was administered subcutaneously on DOL 1 (until 2 hours post-birth) and DOL 30. This commercial product has no indication for sheep, and we used the same dose indicated for calves as recommended by the manufacturer in order to satisfy their physiological needs and according to the literature (Glombowsky et al., 2018; Tomasi et al., 2018). Animals were weighed on DOL 1, 15, 30, and 45. Table 1 also shows the measures of minerals (copper, zinc, selenium and manganese) determined in the concentrate by the Near Infrared Spectroscopy (NIRS) method in a commercial laboratory (Shankar, 2015), and feeding of lambs.

2.3 Sample collection and blood analyses

Total blood from 10 animals per group was collected by the jugular vein in tubes containing EDTA for complete blood counts, and also in tubes containing sodium citrate that were used to measure antioxidant enzymes. Blood collected without anticoagulant was used to obtain serum (3500 RPM for 10 min) for biochemical analyses and IgM and IgG quantification. All samples were stored at – 20 ºC until analysis.

2.4 Hematological analyses

The number of erythrocytes, total leukocytes, and hemoglobin concentration were performed using a semi-automated blood cell counter (CELM CC530), and for hematocrit a micro centrifugation method (Feldman et al., 2000) was used. Blood smears were prepared and stained according to Romanowski´s method for microscopic examination to perform cell morphology and leukocyte differentiation (Feldman et al., 2000).

2.5 Serum biochemistry

Serum levels of total protein, glucose, albumin, and urea were measured using commercial kits (Analisa®) and a semi-automated analyzer (BioPlus-2000®). Serum levels of globulins were obtained by the formula: total protein – albumin.

2.6 Antioxidant enzymes: catalase, superoxide dismutase and xanthine oxidase

Catalase (CAT) activity in total blood was measured according to the method described by Nelson and Kiesow (1972) and the results were expressed in U CAT/ mg of protein. Superoxide dismutase (SOD) activity was measured and quantified according to the technique described by McCord and Fridovich (1976) and the results were expressed in U SOD/ mg of protein.

Serum xanthine oxidase (XO) activity was determined using the method described by Westerfeld and Richert (1949). The reaction mixture contained 1 mM of xanthine as substrate and 50 mM of phosphate buffer (pH 7.4). This mixture was incubated with approximately 0.5 mg of protein at 37 ºC for 60 min in a final volume of 0.5 mL. The rate of urate formation from xanthine degradation was determined by measuring absorbance at 290 nm. Activity was expressed as U/L.

2.7 Immunoglobulins

Immunoglobulins (IgG and IgM) were quantified using ELISA commercial kits (eBIOSCIENCE, San Diego, USA), according to the manufacturer´s.

2.8 Determination of seric concentrations of minerals

The seric concentrations of selenium, cooper, manganese and zinc were determined (DOL 1, 15 and 45) as described by Flores et al. (2001) using the Hydride Generation Atomic Absorption Spectrometry (HG–AAS) technique and chemicals of analytical grade (Merck, Darmstadt). Thus, 250 µL of HNO_3 and 62.5 µL of H_2O_2 were added to 125 µL of serum. Milli-Q water was added to achieve the final volume and the solution was analyzed by Inductively Coupled Plasma–optical Emission Spectrometry (ICP-OES).

2.9 Sampling and fecal analyses

Fecal samples (DOL 15, 30 and 45) from all animals were collected from the rectal ampulla to count for the number of oocysts per gram of feces (OOPG) using the McMaster technique (Gordon and Whitlock, 1939) with sucrose solution.

2.10 Statistical analysis

Data was analyzed using the descriptive statistics for contingency of information and for further assumptions, which were presented as descriptive (mean and standard deviation) for blood cell parameters: hematocrit, erythrocyte count, hemoglobin, leukocytes, lymphocytes, monocytes, and eosinophils. The second group of parameters measured were immunoglobulins (G and M) and XO. The third set of data was for CAT and SOD activities, followed by biochemical components: total protein, urea, glucose, albumin, and globulin. Finally, measurements associated with animal weight: body weight and weight gain. For each group and day of observation (DOL 1, 15, 30, and 45), all parameters were tested for normality using the Shapiro-Wilk test (Shapiro-Wilk, 1965). Skewness, kurtosis and homogeneity were evaluated by the Levene test, or log transformation when needed. A t-test was used to analyse all parameters, i.e. between groups (controlling data dependency due to dependence in time), and over time for weight (DOL 1, 15, 30, and 45). Significant difference was set at $P < 0.05$. Statistical manipulations were performed using R-language, v.3.1 (R Development Core Team 2012).

3. Results

3.1 Weight gain

Treated animals showed higher body weight and weight gain ($P < 0.05$) on days 15 and 45 of age compared with untreated lambs (Figure 1-A-B). Over time, body weight increased in both groups ($P < 0.001$).

3.2 Parasitological examination

On DOL 15, all animals were negative for oocysts of *Eimeria* spp. On DOL 30, the number of *Eimeria* spp. oocysts was higher ($P < 0.05$) in the control group (2800 ± 1435 OOPG) compared with the treated group (750 ± 574 OOPG). Most animals showed signs of diarrhea, and thus, all animals were treated with toltrazuril at DOL 31 (Baycox Ruminantes®) at a dose of 5 mg/kg. At DOL 45 all animals were negative for any type of oocysts.

3.3 Blood counts

No difference was observed between groups regarding red blood cells, hematocrit, and hemoglobin concentration ($P > 0.05$ – Table 2); however, over time, these variables differed in both groups ($P < 0.001$). In summary, there was an increase in these variables on days 15 and 45 of life compared to day 1 in both groups. In general, there was a reduction in some erythrogram variables on day 30 of age (Table 2), a period that coincided with natural coccidial infection.

Total leukocytes on DOL 45 were significantly higher in treated animals compared with untreated lambs ($P < 0.05$), due to an increased number of lymphocytes (Table 2). The number of neutrophils was lower in treated lambs (DOL 45). The other white cells (eosinophils and monocytes) did not differ between groups ($P > 0.05$, Table 2). In summary, over time there were changes in the leukogram of lambs, except for the number of eosinophils (Table 2). We would like to draw attention to lymphocyte counts that increased over time in treated lambs ($P < 0.05$; Table 2).

3.4 Serum biochemistry

Serum levels of total proteins and globulins were higher ($P < 0.05$) in treated animals (DOL 15, 30, and 45) compared with control (Figure 2 A, C), while serum albumin levels were lower in the treated group (DOL 15) (Figure 2-B). Treated animals showed constant serum levels of urea throughout the experiment, but it was lower on DOL 30 and higher on DOL 45 in the control group (Figure 2-D). Serum levels of glucose were higher in treated animals on DOL 15 and 30 compared with the control group ($P < 0.05$, Figure 2-E). Over time, all biochemical variables increased ($P < 0.05$) in both groups comparing to DOL 1 to DOL 15 of age. However, no differences were found on total protein and globulins on day 1 to day 30 and 45 only in the control group, but there was a reduction from day 15 to 30 and 45 in both groups ($P < 0.05$).

3.5 Antioxidant enzymes and xanthine oxidase activity

The activity of antioxidant enzymes was shown in Figure 3. CAT activity did not differ between groups (P > 0.05, Figure 3-A). Superoxide dismutase and xanthine oxidase activities were higher (P < 0.05) on DOL 15, 30, and 45 in treated compared with untreated lambs (Figure 3 B, C). In general, CAT, SOD and XO activities increased in both groups (P < 0.05). However, at the first moment, the XO activity was lower on DOL 1 to 15.

3.6 Immunoglobulins

Elevated levels of IgM and IgG on DOL 15 were observed in mineral treated compared to untreated lambs (Figure 4). However, IgM levels decreased (P < 0.05) on DOL 30 in treated animals compared with control lambs, while IgG levels increased (Figure 4-B). Levels of IgM increased over time (DOL 1 to DOL 15 and 30), and subsequently it reduced on DOL 15 and 30 to DOL 45). Over time, IgG levels increased in both groups (P < 0.05).

3.7 Mineral in serum

Selenium (DOL 45) and zinc (DOL 15 and 45) levels in serum were higher in treated compared to untreated lambs (P < 0.05; Table 3). Cooper and manganese levels in serum did not differ between groups (P > 0.05). Numerically (P > 0.05), all four minerals showed higher levels in treated animals (DOL 15 and 45). In summary, the levels of all minerals increased over time (P < 0.05), with the exception of manganese that did not differ over time untreated lambs (P > 0.05).

4. Discussion

The metaphylactic effects of minerals in newborn lambs remain poorly understood. However, Arthington et al. (2014) demonstrated that the administration of a mineral complex in beef cattle based on zinc, manganese, copper, and selenium stimulated humoral responses and increased mineral deposition in the liver. In the present study, a mineral complex based on zinc, manganese, copper, and selenium stimulated the immune and antioxidant responses. These results are similar to those observed by Soldá et al. (2017) while studying cows injected with mineral complex during the transitional period. The use of minerals (subcutaneously or intramuscularly) might be beneficial to prevent some negative interactions that may occur during digestion and absorption of minerals and to increase mineral levels at times of greater demand (i.e., growth, lactation and reproduction). After parenteral injection, these elements circulate in the animal´s body and may be incorporated into the cells as needed, excedent amounts are bounded to liver proteins for futher use or filtered and excreted by this organ (Suttle, 2010). In this way, minerals can be better utilized by the animal.

Mineral application in lambs did not alter blood counts, but increased the number of total leukocytes on DOL 45 as a result of increased lymphocytes, and this increase can be attributed to selenium mitogenic property, i.e., selenium induces the proliferation of peripheral blood lymphocytes by changes on the mitotic index (Hawkes et al., 2011). Also, a study conducted by Prasad (2008) reported that zinc is capable to induce T-lymphocytes proliferation by the inhibition of interleukin-2, since this interleukin is an inhibitor of the cellular cycle of the immune cells, such as lymphocytes. There were also increased serum levels of IgM and IgG probably a consequence of increased lymphocyte counts, since immunoglobulins are molecules synthesized by lymphocytes (Fernández-Cruz et al., 2009). Moreover, increased seric IgM and IgG levels can be attributed to copper, since a study conducted by Makhlouf et al. (1998) demonstrated its ability to stimulate the synthesis of antibodies and the formation of immune complexes, which consequently, rises the immunoglobulin levels. Immunoglobulin G is found in large quantities in blood and extracellular fluids, serving essential functions in antibody-mediated defense, as well as in the neutralization of toxins, immobilization of bacteria, sensitization of natural killer cells, and activation of the complement system (Tizard, 2013). Lambs supplemented with minerals showed elevated serum levels of total proteins and globulins, suggesting an activation of the immune response. On the other hand, we found that untreated animals were more affected by coccidiosis due to poor immune responses. Mineral supplementation for lambs was able to enhance their immune responses, meliorating their overall health, explaining their greater weight gain.

Treated animals showed better metabolic stability throughout the experiment compared to untreated lambs. Interestingly, untreated animals showed relatively higher serum levels of albumin on DOL 15, which may be interpreted as a compensatory mechanism in response to loss of other proteins, since albumin is involved in several vital functions, including blood homeostasis and coagulation (Bern et al., 2015). Lambs in the control group showed fluctuations in serum urea levels, suggesting a higher susceptibility to metabolic disorders and other risks to the animal’s health (Peixoto and Osório, 2007). In the current study, the control group showed only minor weight gain and serum levels of glucose were greater in treated animals, suggesting better carbohydrate availability. These findings may suggest that the mineral application improved the digestion and absorption of nutrients, mainly carbohydrates. In addition, increased serum glucose levels may be considered beneficial overall for several metabolic functions, including ATP synthesis (Alberts et al., 2002).

SOD and XO activities increased in treated animals compared with control. This may be attributable to the role of copper and zinc in the SOD molecule. This enzyme participates in the control of free radicals, molecules that mediate oxidative stress (Andrade and Marreiro, 2011). Although the treatment did not alter CAT activity, a study conducted by Soldá et al. (2017) showed that mineral complex supplementation augments the antioxidant system of cows during the transitional period. This effect may be due to improved CAT activity. This enzyme is also an important antioxidant associated with the decomposition of hydrogen peroxide (van der Vliet and Janssen-Heininger, 2014). XO activity is associated with catalysis of purine proteins (adenine, guanine and hypoxanthine) to uric acid, the final product of purine metabolism (Stangassinger et al., 1995). Augmentation of serum XO activity contributes to increased levels of uric acid, a molecule that also shows antioxidant properties (Ames et al., 1981). All these effects benefit animal´s health by preventing or dimishing oxidative stress (Birten et al., 2012). Therefore, mineral application may help lamb´s health indirectly by enhancing some anti-oxidant activities.

Eimeriosis is a common disease in newborn lambs (Urquhart et al., 1996), principally in intensive systems (Lima, 2004). In lambs, this disease is usually associated with severe diarrhea, fever, anorexia, weight loss, decreased wool quality, and death (Mc Dougald, 1979). Farms that do not employ coccidiostatics in animal feed, commonly treat affected animals with toltrazuril due to f its high efficacy, as observed in this study. Both groups showed diarrhea, but treated animals shed lower amounts of *Eimeria* spp oocysts. This can be explained by an enhancement of the immune system by zinc, copper and selenium, since they are important minerals to stimulate the immunological response, leading to greater protection against aggressive infectious agents such as *Eimeria* spp. In addition, Cu is involved in antibody synthesis and secretion (mainly IgG), cellular immunity, and inflammatory responses (Saker, 2006); as well as zinc and copper by playing an important role in the redox metabolism, suggesting an antioxidant effect, mainly due to the activation of some enzymes, such as superoxide dismutase (Overback et al., 2008). Similarly, selenium helps to maintain many defense mechanisms, including antibody production, cell proliferation, cytokine production, prostaglandin metabolism, in addition to proper function of immune cells in the innate response (Smith et al., 1986; Hoffmann, 2007), as well as to enhance protection of cell membranes from oxidative damage (McDowell, 1996). Therefore, the mechanism involved in minimizing the negative effects of coccidiosis in lambs was indirect, i.e., the application of mineral caused a nutraceutical effect seen by a greater inflammatory and antioxidant responses, thus, protecting them from an exacerbated infection by *Eimeria* spp.

Based on these evidences, we conclude that the subcutaneous use of mineral complex based on zinc, manganese, copper and selenium in newborn lambs increases their concentration in the blood, and indirectly these minerals mediate the activation of the antioxidant system and enhance their immune responses. As a consequence, there was a better response of the animals against *Eimeria* spp, and the excretion of oocysts and clinical signs were minimized in treated animals. Moreover, mineral complex applied subcutaneously to lambs may favor the metabolism of proteins and carbohydrates, leading to increased body weight gain.

Ethics Committee

This study was approved by the Ethics Committee of Use of Animals (CEUA) of Universidade do Estado de Santa Catarina (CETEA/UDESC), under protocol number 7398301116.

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Table 1 - Ingredients used to feed lambs a different phases of life.

	Age (days)		
Ingredients	1 to 7	8 to 20	21 to 45
Natural milk[1] (mL)	500	200	-
Replacer milk[2] (mL)	-	300	500
Concentrate[3] (g)	-	100	300

[1] Milk offered to lambs soon after ewe's milking. [2] Replacer milk prepared by mixing 1.0 kg of powder to 4 L of water (according to manufacturer´s instructions), followed by heating at 80 ºC and offered to lambs at 37 ºC. [3] Nutritional composition of concentrate: 20 % of crude protein; 3 % of ethereal extract; 10 % of fibrous material, 12 g of calcium; 6 g of phosphorus, and 72 % of total digestible nutrients. [3] Levels of selenium, zinc, copper and manganese in the concentrate was 0.61; 80.2; 30.0; and 81.0 mg/kg, respectively.

Table 2 - Mean and standard deviation of blood components: total erythrocytes, hematocrit, hemoglobin concentration, total leukocytes, lymphocytes, neutrophils, monocytes and eosinophils of treated (n=10) and untreated (control; n=10) lambs on days 1, 15, 30 and 45 of age.

Variable	**Days**	**Mean ± standard deviation**		
		Treated group	Control group	***p-value**
Erythrocytes (x10^6 µL)	1	5.13 (0.25)^b	5.23 (0.40)^c	**0.19**
	15	8.51 (1.89)^a	9.10 (1.67)^{ab}	**0.46**
	30	8.40 (1.22)^a	8.30 (0.75)^b	**0.84**
	45	9.78 (1.09)^a	9.63 (0.41)^a	**0.69**
p-value$^{\&}$		**0.001**	**0.001**	
Hematocrit (%)	1	33.33 (3.06)^{ab}	30.00 (1.00)^b	**0.96**
	15	39.60 (5.21)^a	43.10 (7.20)^a	**0.23**
	30	26.15 (1.96)^c	25.17 (2.56)^c	**0.35**
	45	30.54 (3.19)^{bc}	29.59 (1.57)^b	**0.41**
p-value$^{\&}$		**0.001**	**0.001**	
Hemoglobin (g/dL)	1	8.83 (0.70)^c	8.87 (0.90)^b	**0.76**
	15	10.75 (1.28)^{ab}	11.10 (1.45)^a	**0.62**
	30	9.68 (1.07)^{bc}	9.04 (0.75)^b	**0.14**
	45	11.13 (1.18)^a	10.89 (0.53)^a	**0.56**
p-value$^{\&}$		**0.001**	**0.001**	
Leukocytes (x10^3 µL)	1	8.33 (1.21)	8.73 (1.03)^b	**0.68**
	15	11.44 (3.78)	12.20 (3.66)^a	**0.65**
	30	8.94 (1.50)	8.59 (3.61)^{ab}	**0.78**
	45	9.59 (0.83)	8.83 (0.74)^b	**0.04***
p-value$^{\&}$		**0.08**	**0.01**	
Lymphocytes (x10^3 µL)	1	3.93 (0.51)^b	4.10 (0.85)	**0.78**
	15	4.65 (1.82)^{ab}	4.93 (1.82)	**0.73**
	30	5.29 (1.70)^{ab}	5.42 (1.48)	**0.50**
	45	6.17 (0.50)^a	4.71 (0.33)	**0.001***
p-value$^{\&}$		**0.001**	**0.27**	
Neutrophil (x10^3 µL)	1	4.00 (1.18)^{ab}	3.97 (0.85)^b	**0.97**
	15	5.88 (2.51)^a	6.37 (2.23)^a	**0.64**
	30	2.52 (1.43)^{bc}	2.40 (1.44)^c	**0.85**
	45	2.36 (0.51)^c	2.89 (0.65)^{bc}	**0.04***
p-value$^{\&}$		**0.001**	**0.001**	
Monocytes (x10^3 µL)	1	0.30 (0.20)^c	0.33 (0.25)^c	**0.86**
	15	0.54 (0.42)^{bc}	0.59 (0.46)^{bc}	**0.80**
	30	1.10 (0.57)^a	1.44 (0.21)^a	**0.06**
	45	0.85 (0.23)^{ab}	1.00 (0.35)^{ab}	**0.29**
p-value$^{\&}$		**0.001**	**0.001**	
Eosinophils (x10^3 µL)	1	0.17 (0.12)	0.10 (0.10)	**0.49**
	15	0.35 (0.28)	0.32 (0.27)	**0.81**
	30	0.28 (0.10)	0.26 (0.13)	**0.63**
	45	0.21 (0.07)	0.24 (0.10)	**0.41**
p-value$^{\&}$		**0.45**	**0.24**	

*P < 0.05 represents significant differences between groups. & Similarly, P<0.05 in the same column shows difference between moments after repeated analysis over time (illustrated with different subscript letters). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

Table 3 - Mean and standard deviation of minerals (selenium, cooper, manganese and zinc) used for treated (n=10) and untreated control; (n=10) lambs on days 1, 15 and 45 of age.

Mineral	**Days**	**Mean ± standard deviation**		
		Treated	**Control**	***p-value**
Selenium (µg/L)	1	39.7 ± 5.6^{c}	42.0 ± 8.4^{b}	**0.65**
	15	89.0 ± 6.2^{b}	81.0 ± 12.1^{a}	**0.59**
	45	103.5 ± 9.5^{a}	85.5 ± 13.9^{a}	**0.01***
p-value&		**0.001**	**0.001**	
Cooper (µmol/L)	1	6.2 ± 0.9^{b}	5.6 ± 1.2^{b}	**0.72**
	15	14.7 ± 4.3^{a}	11.1 ± 5.1^{a}	**0.41**
	45	13.4 ± 3.6^{a}	10.0 ± 1.9^{a}	**0.19**
p-value&		**0.001**	**0.001**	
Manganese (µg/L)	1	6.1 ± 0.5^{b}	6.2±0.8	**0.84**
	15	7.8 ± 0.7^{a}	6.9±1.3	**0.31**
	45	7.9 ± 1.4^{ab}	6.7±1.0	**0.18**
p-value&		**0.03**	**0.52**	
Zinc (µmol/L)	1	9.80 ± 4.0^{b}	12.1 ± 5.2^{b}	**0.68**
	15	28.9 ± 3.3^{a}	21.0 ± 6.1^{a}	**0.01***
	45	39.0 ± 11.1^{a}	24.2 ± 10.5^{a}	**0.01***
p-value&		**0.001**	**0.001**	

*P < 0.05 represents significant differences between groups. & Similarly, P<0.05 in the same column shows significant differences between moments after repeated analysis over time (illustrated with different subscript letters). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

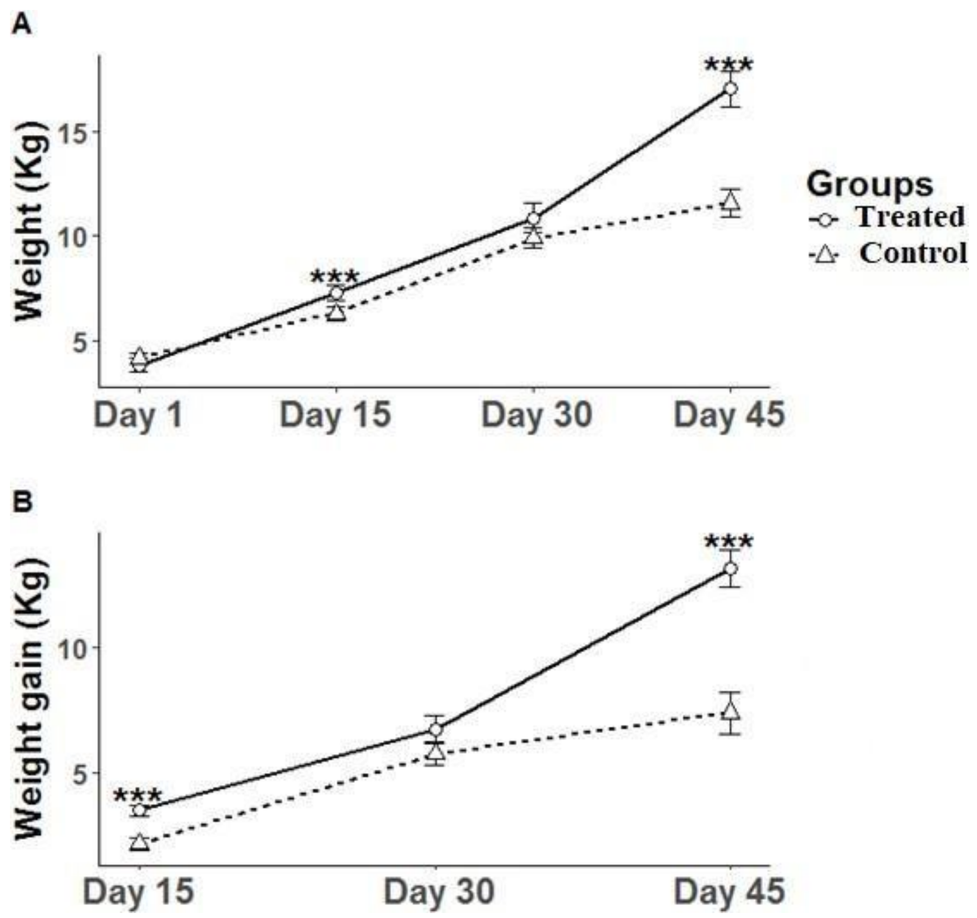

Figure 1: Body weight (A) and weight gain (B) of treated (n=55) and untreated lambs (control; n=55) on days 1, 15, 30 and 45 of life. Asterisks indicate significant differences between groups (***$P<0.05$). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

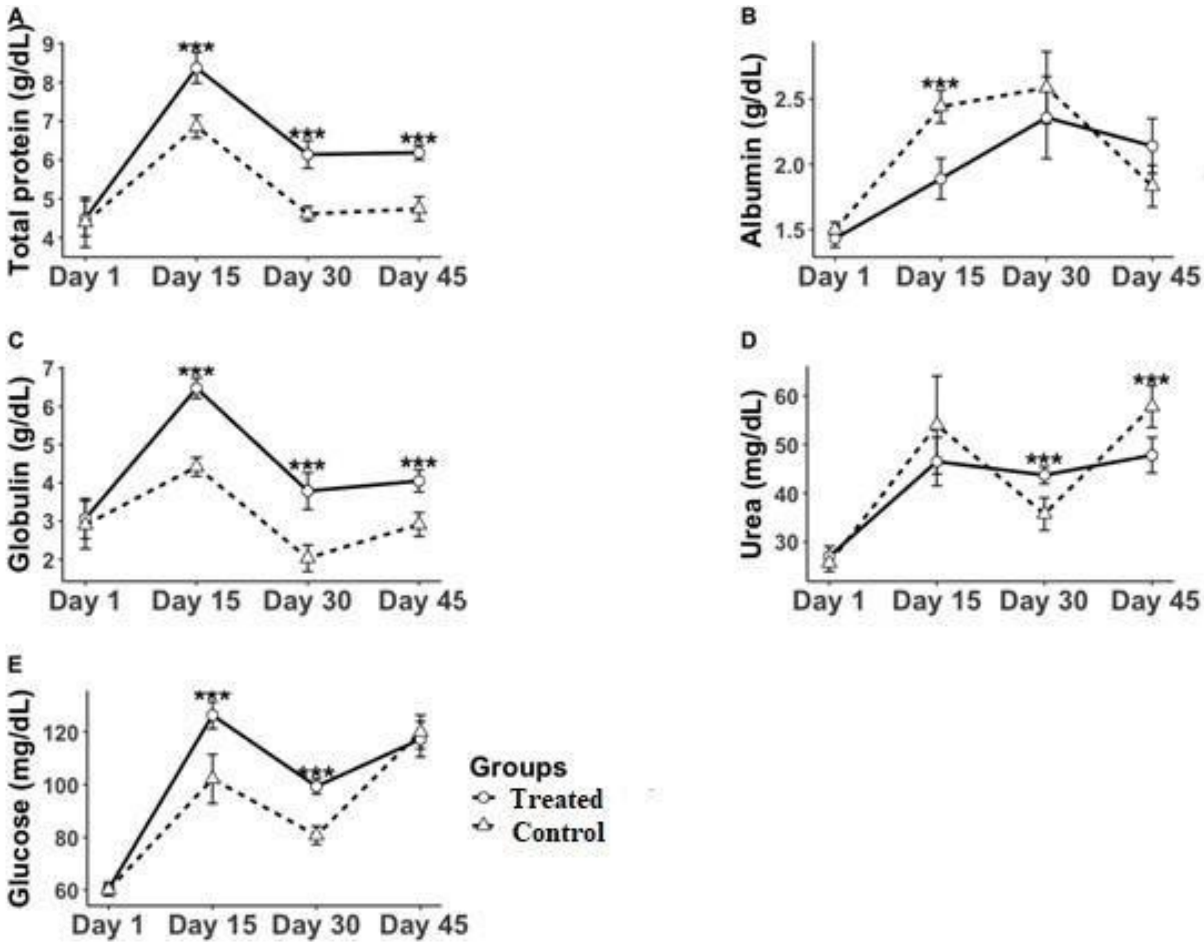

Figure 2: Seric levels of total protein (A), albumin (B), globulin (C), urea (D) and glucose (E) of treated (n=10) and untreated (control; n=10) lambs on days 1, 15, 30 and 45 of age. Asterisks indicate significant differences between groups (***P<0.05). Note: treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

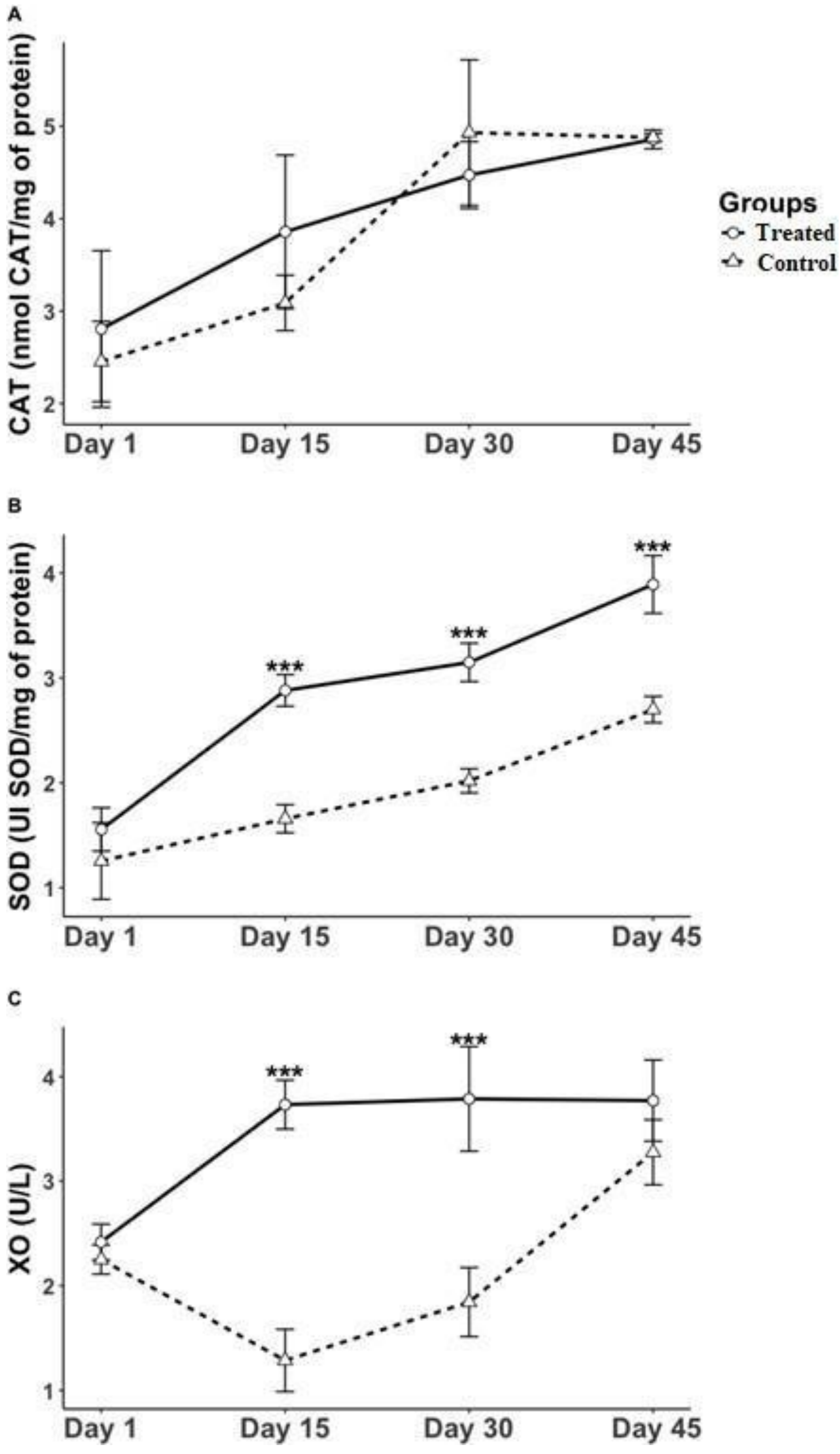

Figure 3: Superoxide dismutase (SOD) [A], catalase (CAT) [B] and xanthine oxidase (XO) [C] activities in treated (n=10) and untreated (control; n=10) lambs on days 1,15,30 and 45 of age. Asterisks indicate significant differences between groups (***P<0.05). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

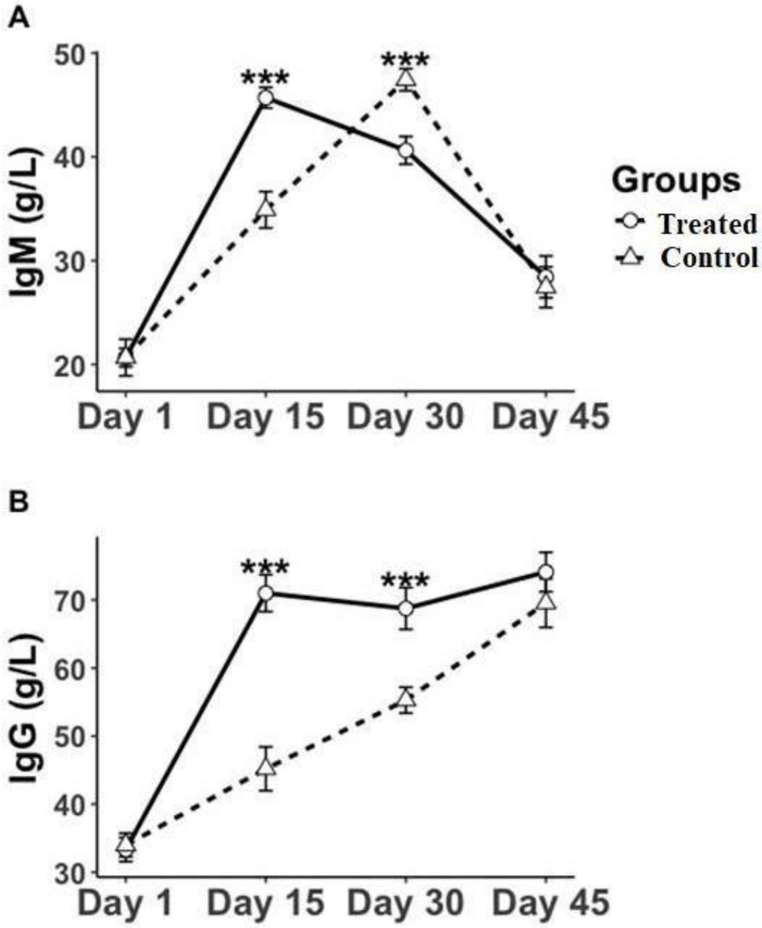

Figure 4: Seric levels of immunoglobulins: IgM (A) and IgG (B) in treated (n=10) and untreated (n=10) lambs on days 1,15, 30 and 45 of age. Asterisks indicate significant differences between groups (***P<0.05). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

MANUSCRITO II

Nutraceutical effect of trace elements as injectable doses reduces mortality, modulates oxidant and antioxidant status, and improves the quality of lamb meat

Chrystian J. Cazarotto1, Jhonatan P. Boito1, Patrícia Glombowsky1, Rafael A. Baggio1, Gabriela M. Galli1, Gustavo Machado2, Nathiele B. Bottari3, Marta L.R. Leal4, Julcemar D. Kessler1, Matheus D. Baldissera5, Aleksandro S. da Silva1,3*

1 Graduate Program in Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, Santa Catarina (SC), Brazil.

2 Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, USA.

3 Graduate Program in Biochemical Toxicologicology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil.

4 Department of Large Animal, UFSM, Santa Maria, RS, Brazil.

5 Department of Microbiology and Parasitology, UFSM, Santa Maria, RS, Brazil.

*Author for correspondence: Department of Animal Science, University of Santa Catarina State. 680 D, Beloni Trombeta Zanin Street, Chapecó/SC, Brazil Zip: 89815-630, Phone: 55 49 3322-4202. Fax: 55 49 3311-9316. (E-mail: aleksandro_ss@yahoo.com.br).

ABSTRACT

Our study aimed to evaluate whether zinc, copper, selenium and manganese subcutaneous mineral supplementation reduced mortality, improved performance, and modulated oxidant and antioxidant balance in lamb meat, thereby improving its quality. We divided the 110 newborn Lacaune lambs into two groups: non-supplemented (control), and supplemented (treated) with three doses of 0.33 ml/kg mineral complex on days of life 1, 30, and 60. The animals were fed the same diet, and consumption did not differ between groups ($P > 0.05$). All animals were weighed on day of life 1, 30, 60, 90, and 150. The mortality of treated animals was 7.27% lower than that of control. At 150 days of life, 12 animals were slaughtered for physical and chemical analysis of meat, oxidant and antioxidant status, and for allometric analysis. Mineral-supplemented animals had greater body weight ($P < 0.05$) on days of life 60 and 90. There was an increase in fat thickness ($P < 0.004$); pH levels ($P < 0.002$) were lower in mineral-supplemented animal meat than in that of the control group. Meat was paler (according to Lightness (L color)) in the control group ($P < 0.04$). Weight loss from cooking was greater in control animals ($P < 0.004$). Shear strength values were lower in the meat of supplemented lambs ($P < 0.008$) suggesting that mineral supplementation was associated with increased meat tenderness. In addition, catalase and superoxide dismutase activities were higher ($P < 0.01$) in mineral-supplemented animals, associated with a reduction in reactive oxygen species levels ($P < 0.01$), and lipid peroxidation products ($P = 0.02$). These data suggest that mineral supplementation modulated oxidant and antioxidant status, reflecting better meat quality.

Keywords: performance; lipid oxidation; reactive oxygen species; antioxidant system.

TOC Abstract'

In sheep breeding, the first days of life are critical. Low immunity and high exposure to pathogenic agent's favor disease and mortality. The metaphylactic effects of the minerals copper, zinc, selenium and manganese strengthen immune and antioxidant responses. In addition, mineral supplementation contributes to improved meat quality. Healthy animals grow rapidly and increase the profitability of this product.

INTRODUCTION

With the increasing demand for sheep meat, sheep breeding has become an increasingly attractive pursuit in the agricultural economy (Morris 2009). Because they are considered fast-cycling animals with accelerated metabolism, sheep are susceptible to various diseases that compromise the entire production cycle, depreciating value of the final product, *i.e.*, lamb meat (Martin 1993). Mineral deficiencies cause substantial morbidity in these animals, manifesting as decreased productivity, growth impairment, poor weight gain, susceptibility to disease, and decreased fertility (Martin 1993). Minerals such as manganese, zinc, selenium, and copper are required in various quantities by these animals, and all are essential for maintaining proper physiological function. Deficiencies of one or more limits animal performance (Martin 1993).

The micronutrient content of animal meat varies, depending on muscle type, age, species, nutritional status, breed, genetics, sexual maturity and presence of a suitable production system (Pethick *et al.* 2011). Quality sheep meat (especially lamb) is characterized by a combination of attributes including flavor, juiciness, texture, softness, and appearance (Silva Sobrinho 2001; Thompson *et al.* 2005). It is essential to implement new alternatives, aiming for greater productivity and quality, to satisfy the increasingly demanding consumer (Garcia *et al.* 2005). Retailers view meat color as a primary consideration for consumers who prefer bright red color (oxymyoglobin) in fresh meat and avoid brown color (metmyoglobin) (Truscott *et al.* 1984). The pH of sheep meat ranges from 5.5 to 5.8; however, higher values (≥ 6.0) may be found in animals with depleted muscle glycogen prior to slaughter. This may influence the quality of the final product (Garcia *et al.* 2005).

Oxidative stress caused by heat stress and incorrect management reduced meat quality, principally texture and protein contents (Wang *et al.* 2009). For this reason, several interventions have been implemented to reduce animal deaths and aid production of good quality product for consumers, including the use of mineral products in the form of injectable micronutrient solutions. The latter are increasingly employed in conjunction with initial immunization in young animals in order to prevent and treat micronutrient deficiencies. These products may prevent or minimize oxidative stress during the weaning period (Silva *et al.* 2016), consequently improving meat quality (Khan *et al.* 2018). Khan *et al.* (2018) demonstrated that supplementation with sodium selenite, a compound used in the present study, was capable of improving meat quality via prevention of meat oxidation and augmentation of antioxidant status in breast muscle of broiler chickens. In addition, Holen *et al.* (2018) reported that zinc, another compound used in the present study, improved carcass composition and meat quality in pigs housed under crowded conditions. Recently, Soldá *et al.* (2017) demonstrated that injectable mineral supplementation based on copper and sodium selenite reduced lipid oxidation and improved antioxidant capacity in dairy cows during the transition period, improving animal health. Paz Matias (2014) suggested that zinc supplementation improved SOD activity, thereby boosting the antioxidant system. This occurs because zinc is a cofactor of the enzyme, and is necessary for its proper function. Thus, our hypothesis was that mineral supplementation based on zinc, copper, selenium and manganese would improve meat quality via reduction of oxidative process and increase of antioxidant levels. The objective of this study was to evaluate whether supplementation with zinc, copper, selenium and manganese in lambs reduced mortality, improved performance, and modulated oxidant and antioxidant status, with consequent improvements in meat quality.

MATERIALS AND METHODS

Product

The commercial product used in this study was Adaptador® (Biogen). The product included copper edetate (1.0 g), zinc edetate (4.0 g), manganese edetate (1.0 g), and sodium selenite (0.5 g) per 100 mL.

Animals

We studied 110 male newborn Lacaune lambs weighing approximately 3 kg. The animals were randomized into two groups, a non-supplemented group (control, 55 animals), and a group given mineral supplementation (treated, 55 animals); in addition, the lambs were sub-grouped by chronological order of birth. Both groups were raised simultaneously. Adaptador was injected at 0.33 ml/kg subcutaneously on day of life (DOL) 1 (hours after birth) and the injection was repeated on DOLs 30 and 60. Animals from both groups received colostrum in the first hours of life. Subsequently, the lambs were allocated at five per stall and fed sheep's milk until DOL 7, followed by artificial and sheep's milk on DOL 8-20, artificial milk on DOL 21-45 (weaning) and commercial concentrate with 20% crude protein (CP) on DOL 8-45 (Table 1).

After weaning, twenty lambs were allocated in two stalls (control and treatment groups: 10 animals each) in order to evaluate feed intake. The remaining animals were kept in a collective bay, receiving the same diet as the others. The feeding regimen was determined according to life stage of the animal (Table 1), and diet was formulated according to their physiological needs up to 150 days (NRC 2007). The animals received daily concentrate, hay and silage (Table 1). The measures of dry matter, crude protein and minerals (copper, zinc, selenium and manganese) were determined in silage, hay and concentrate by a near infrared spectroscopy method in a commercial laboratory (Shenk and Westerhaus, 1994; Shankar, 2015) (Table 2).

Blood collection

Whole blood from 10 animals per group was collected from the jugular vein. Tubes without anticoagulant were used to obtain serum after centrifugation (3500 RPM x 10 min) for measurement of minerals, biochemical and immunological variables. All samples were stored at – 20ºC until analysis.

Mineral analysis in serum

Concentrations of selenium, cooper, manganese and zinc were measured at DOL 1, 30 and 60. We followed a protocol according to Flores et al. (2001), using the hydride generation atomic absorption spectrometry (HG–AAS) technique and analytical grade chemicals from Merck (Darmstadt). We added 250 µL of HNO_3 and 62.5 µL H_2O_2 to 125 µL of serum. Milli-Q water was added to achieve the final volume and the solution was analyzed by inductively coupled plasma–optical emission spectrometry.

Total antioxidant and cytokine levels

Total antioxidant levels in serum were measured through the ferric reducing ability of plasma (FRAP) in serum of according to the technique described by Benzie and Strain (1996) and the results were expressed in µmol/L. Serum concentrations of interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) were determined using the commercial immunoassay Quantikine®, according to manufacturer’s recommendations (R & D Systems, Minneapolis, MN, USA). The presence and concentration of cytokine were determined by colour intensity measured spectrophotometrically in a micro ELISA reader. Results were expressed in pg/mL.

Performance

Measures of animal performance, including weight, feed intake, and mortality were evaluated. Animals were weighed on DOL 0, 30, 60, 90, and 150, and food consumption was evaluated at three time intervals of 5 days: DOL 60-64, 90-94 and 146-150. Animals were offered feed at 2% live weight/animal twice a day (8:00 AM and 6:00 PM). Leftovers were quantified.

Allometric analysis, slaughter and muscle collection

At DOL 150, 12 animals were slaughtered. Prior to slaughter, the animals were weighed and measured for posterior height (cm), conformation (scale 1-5), thoracic circumference (cm), anterior height (cm), body length (cm), and scrotal circumference (cm). Subsequently, the viscera and non-carcass components were weighed separately in order to evaluate the influence of treatment on the supplemented animals. After slaughter, the carcasses were weighed, and the cold carcass weight (CCW) was obtained. In addition, we calculated cooling weight loss (CWL), and cold or commercial (CC) carcass yield. Subsequently, the carcasses were divided longitudinally. Left halves were sectioned into anatomical regions and were weighed individually. The longissimus dorsi muscle at the 13th thoracic vertebra was collected for chemical and physical meat analysis, as well as for oxidant and antioxidant status described below.

Chemical and physical meat analysis

The longissimus dorsi muscle was refrigerated at 6°C for 24 h, and was used for color determination, water retention capacity (WRC), cooking weight loss (CL) and shear strength (SS) determinations. Loin eye area (LEA) was calculated according to Yamamoto *et al.* (2013). Color was calculated as follows: L* (luminosity), a* (intensity of red) and b* (intensity of yellow), using a colorimeter (Minolta CR-200). Water retention capacity was determined according to Hamm's methodology, adapted by Yamamoto *et al.* (2013). To determine weight losses during cooking, samples were weighed before and after cooking on a portable grill (Grill Mondial Due Grill Smart Pretoforno), preheated to 170°C until achieving 75°C internal temperature. Subsequently, we measured areas of portions of cooked samples and measured shear strength using a texture analyzer coupled to a Warner-Bratzler device (Yamamoto *et al.* 2013).

Determination of oxidant/antioxidant status in meat

A muscle fragment of approximately 20 g was removed and was frozen for oxidant and antioxidant analysis. The sample was homogenized in buffer solution containing 10 mM Tris-HCl, pH 7.4 on ice and was centrifuged at 9000 g for 15 min at 4ºC. Aliquots were stored at −20ºC until utilization. Protein levels in the pancreas were standardized between 1.4–1.8 for biochemical tests.

Lipid peroxidation in meat was evaluated by measuring levels of malondialdehyde (MDA), using the measurement of thiobarbituric acid reactive substances (TBARS) levels (Ohkawa *et al.* 1978). Results were expressed in nanomoles MDA per g of tissue (nmol MDA/g). Oxidation of 2′−7′-dichlorofluorescein determined the index of peroxide produced by cellular components, according to the modified method of Colpo *et al.* (2008) for determining ROS levels in meat (Halliwell and Gutteridge 2007; Murphy *et al*. 2011). Briefly, 50 µL of supernatants (S1) from muscle were incubated with 12 µL DCF and 1 mM CF at 37°C for 1 h in the dark. Fluorescence was determined using 488 nm for excitation and 520 nm for emission. Fluorescence measurements were normalized to time 0 values; rates of increased fluorescence reflected ROS levels. Results were expressed as U DCFA mg^{-1} protein.

SOD activity in meat was determined spectrophotometrically by measuring inhibition of the autocatalytic rate of adrenochrome (McCord and Fridovich 1969). Results were expressed as U SOD/mg protein. CAT activity in meat was evaluated using the methodology of Nelson and Kiesow (1972), that determines the decomposition rate of H_2O_2. CAT results were expressed as U CAT/mg protein.

Statistical analysis

Data from each group were first analysed by descriptive statistics for contingency of information and for further assumptions that are presented as descriptive for mean and standard deviation. For weight, biochemical and immunologic analyses, related variables for each group and day of observation were tested for normality using the Shapiro-Wilk test. Skewness, kurtosis and homogeneity were evaluated by the Levene test, or log transformation when needed. A t-test was used to analyse all parameters, i.e. between groups (controlling for data dependency due to dependence in time), and over time for weight (DOL 1, 30, 60, 90 and 150), FRAP and cytokines (DOL 1, 30 and 60). Significant difference was assumed if $P < 0.05$. The statistical analyses were performed using R-language, v.3.1 (R Development Core Team 2012).

RESULTS

Mineral levels in serum

Serum selenium, copper, zinc and manganese results showed in Figure 1. In the treated group, there was an increase in levels of selenium and copper (DOL 60) and zinc (DOL 30 and 60) compared to control. Generally, and numerically, the four minerals were higher in treated animals than in control animals (DOL 30 and 60). In the control animals, a reduction of the four minerals occurred after weaning (at 45 days), an effect that was not observed in the group supplemented for three minerals, since they maintained their levels of copper, selenium and zinc (DOL 60).

Total antioxidants and cytokines

On DOL 30 and 60, FRAP levels were higher ($P < 0.05$) in animals from the treated group than in the control group (Table 3). On DOL 60, TNFα- and IL-1 levels were also higher ($P < 0.05$) in the treated group than in the control group (Table 3). Over time, the FRAP, TNF and IL-1 levels increased in both groups (Table 3).

Performance

There was a significant difference between the groups at DOL 60 and 90, where the treated group gained more weight (Figure 2). Weight gain was significantly greater in the treatment group on DOL 60 and 90. Despite the fact that there was no significant difference at 150 days ($P > 0.05$), the animals in the treated group had 8.7% higher weight compared to the control group. Food intake did not differ between groups ($P > 0.05$), and the daily leftovers were 10% lower during the three intervals to both groups. Mortality was 12.75% (7/55) and 5.45% (3/55) in the control and treatment groups, respectively. The dead animals first exhibit hyperthermia and diarrhea, followed by hyperpyrexia, dehydration, weight loss and weakness.

Hypothermia (≥ 40ºC) was achieved with antibiotic therapy (sulfamethoxazole + trimethoprim), but without therapeutic success. The period from the appearance of clinical signs to death ranged from 2 to 5 days.

Allometric evaluations

Allometric measurements are displayed in Table 4. No differences between groups were observed for any allometric evaluation ($P > 0.05$).

Chemical and physical meat analysis

Chemical and physical analyses are presented in Table 5. Fat thickness was higher in the treated group than in the control group ($P < 0.004$). pH levels ($P < 0.002$) were lower in the treated group than in the control group. Regarding colour, the only difference between groups was observed in L, returning higher values in control animals than in supplemented animals ($P < 0.04$). Cooking weight loss was higher in control animals than in supplemented animals ($P < 0.004$). The shear strength was lower in the supplemented group than in the control group ($P < 0.008$).

Oxidant and antioxidant status

Antioxidant enzyme activity in meat is shown in Figure 3. The treated group had lower values of ROS ($P < 0.001$) and TBARS ($P = 0.02$) compared with the control group (Figure 2- B). CAT and SOD activities differed between groups ($P < 0.01$), with higher levels in the treated group (Figure 2 C-D).

DISCUSSION

According to the literature, trace elements are essential for multiple organic processes, including skeletal development, immune responses and productive and reproductive performance in animals (Underwood and Suttle, 1999). Unfortunately, cattle diets do not always contain adequate amounts of trace elements to meet metabolic demands. In addition, although cattle may receive mineral supplementation ad libitum, it is not always enough to block the mineral antagonists that may be present in the diet. The use of parenteral minerals (subcutaneous or intramuscular) may be beneficial for preventing negative interactions that may occur during the digestion and mineral absorption processes or to augment the mineral status of an animal before periods of greatest need (i.e., growth, lactation and reproduction). After parenteral injection, the elements circulate and are incorporated into the cells as needed, the rest being filtered by the liver, where minerals are required to maintain proteins for long-term use, or where they are excreted from the body (Suttle, 2010). In this way, minerals can be better utilized by the animal. In the present study, parenteral mineral supplementation improved performance in the growth phase and reduced mortality in the lambs' suckling phase. In addition, supplementation improved the quality of the meat, reflected in the reduction of oxidation levels. This result was similar to that observed by Carmo *et al.* (2017) in cattle supplemented with minerals. According to Carmo *et al.* (2017), mineral supplementation may be considered a novel approach to improving animal performance and beef quality. In the present study, we observed a reduction in mortality in lambs supplemented with minerals, similar to results reported by Zakaria et al. (2017) and by Kralik and Kralik (2017) in chickens supplemented with minerals. Salim *et al.* (2011), reported that organic mineral supplementation based on Zn stimulated the development of the immune system, possibly reducing mortality rates. In addition, the presence of selenium may contribute to the reduction of mortality by improving the humoral and innate immune response in sheep (Hall *et al.,* 2013). Based on these results, we hypothesized that the reduction of mortality in lambs supplemented with organic minerals was mediated by the activation and modulation of the immune system, as observed in this study, with increase of pro-inflammatory cytokines (TNF-α and IL-1) and total antioxidant (FRAP).

Mineral supplementation in the lambs did not alter the allometric analysis, however, it promoted significant differences in some chemical and physical characteristics of the meat. The fat content was greater, and pH was lower in mineral-supplemented animals. According to Siqueira *et al.* (2001), subcutaneous fat directly contributes to meat softness by acting as an insulator, preventing sudden cooling of the carcass, thereby shortening sarcomeres and making the meat harder. The high fat content in mineral-supplemented animals may be responsible for the tenderness of their meat. Garcia *et al.* (2005) reported that the final pH in sheep meat varied from 5.5 to 5.8. However, higher values (≥ 6.0) could be found in cases of depletion of muscle glycogen deposits. This may negatively influence the quality of the final product. Supplemented animals had lower pH than that reported in the literature (5.4). However, since the 1990s, investigators have argued that meat quality at moderately low final pH (5.4) characterized normal, usually soft meat (Bray *et al.* 1989; Devine *et al.* 1993).

The intensity of meat colour was determined by the total concentration and structure of myoglobin, which was itself affected by ante-mortem factors such as species, gender and age of the animal, and by post-mortem factors such as anatomical region, temperature and pH (Seideman *et al.* 1984). L* indicated the luminosity of the meat (Seideman *et al.* 1984). Luminosity values were lower in the treated group than in the control group, suggesting that the meat became paler in control animals, associated with improvement of antioxidant/oxidant status (Khan *et al.* 2018). However, there were no changes in colour values a* and b*. Xu *et* *al.* (2017) and Zakaria *et al.* (2017) reported that animal diets containing Zn did not change factors associated with L* or a* and b* colour values. These differences may be related to the dosing and the timing of supplementation, as well as to the difference of species studied (chickens as opposed to lambs). The low pH in the treatment group associated with lower L* is desirable, as is the red colour, an important factor for retail customers (Simões and Ricardo 2000). Xu *et al.* (2017) showed that pigs supplemented with zinc showed improvement in pH-associated meat quality, similar to the effect observed in the present study. However, Zakaria *et al.* (2017) reported that zinc did not alter the pH of chicken breast 24 h post-mortem. Taken together, these results suggest that supplementation with zinc and other microminerals may improve meat quality in lambs. Cooking weight loss and shear strength were lower in the treatment group. According to the softness classification, described by Boleman *et al.*(1997) values from 2.3 to 3.6 correspond to very soft meat, 4.1 to 5.4 moderately soft, and 5.9 to 7.2 to slightly soft. By this measure, lamb meat in the treatment group can be classified as moderately soft, with mean values between 2.79 and 3.53. This suggests that greater forces were required to disrupt meat samples from the control group. Zakaria *et al.* (2017) demonstrated that a diet containing zinc produced more succulent breast meat. The mechanisms involved in this process remain unknown, but we speculate that they may involve the diminution of oxidative reactions in post-mortem meat; this is because the metaphylactic effects of the minerals used in this study have potent antioxidant effects, thus protecting from free radicals in cells during the transition period from muscle to meat, as well as during meat storage.

The treated group had lower ROS and TBARS levels than did the control group. According to Schneider and Oliveira (2004), excessive production of ROS can lead to oxidative stress with subsequent harmful effects on the animal. Increases in ROS and TBARS levels may lead to oxidation of proteins, lipids and nucleic acids, with consequent loss of function and tissue damage (Halliwell and Whiteman 2004). Excessive levels of ROS and other free radicals cause lipid peroxidation, as observed in the present study with the increase in MDA levels. MDA accumulation indicates oxidative stress and tissue damage, possibly contributing to changes in weight (Kassahn *et al.* 2009). In addition, high levels of ROS and increased lipid peroxidation may impair the activity of antioxidant enzymes such as CAT and SOD, as observed by Baldissera *et al.* (2017). Damage to CAT activity may lead to increased levels of hydrogen peroxide, a molecule with pro-oxidative and pro-inflammatory effects (Asad 2004). SOD inhibition may increase levels of hydroxyl radicals, in turn leading to impaired performance and risk of mortality (Barreiros *et al.* 2006). In the present study, supplementation with organic minerals produced lower values of ROS and TBARS, suggesting reduced radical formation and lipid peroxidation, similar to results reported by Soldá *et al.* (2017) in mineral-supplemented cows. For example, a study conducted by Yeo and Kang (2007) demonstrated that sodium selenite reduced ROS content through elevated glutathione peroxidase (GPx) activity and stimulation of selenoprotein P via redox regulation, decreasing free-radical-mediated lipid peroxidation. It is important to highlight that sodium selenite was not immediately used for synthesis of selenoprotein, i.e., it was converted to hydrogen selenite that acted as a central pool of selenium for specific integration of selenium in selenoproteins, contributing to protective effects against oxidation (Brodin *et al.* 2015). Using organic compounds, studies have suggested that zinc edetate inhibited lipid peroxidation, as observed in the present study, through improvement of GPx activity and consequent reduction of ROS content (Girotti *et al.* 1985; Farinati *et al.* 2003). In summary, organic and/or inorganic compounds may prevent lipid peroxidation via enhancement of the antioxidant system, as evaluated in the present study by stimulation of SOD and CAT activities. The finding of higher SOD and CAT activity in supplemented lambs may be accounted for by the presence of copper, which is a component of the SOD molecule, and zinc, which is a cofactor of the CAT enzyme. Recall that these enzymes participate in regulation of free radicals and non-radical species that are associated with oxidative reactions (Andrade and Marreiro 2011). In addition, is important emphasize that improvement of antioxidant/oxidant status was directly associated to improvement of meat quality, as observed by Khan *et al.* (2018). Xu *et al.* (2017) demonstrated that Zn supplementation in swine improved SOD anti-oxidant activity.

CONCLUSIONS

In lambs, among the metaphylactic effects of zinc, manganese, copper and selenium, we highlighted the stimulation of important pro-inflammatory cytokines to the benefit of animal defences and modulated oxidative reactions via activation of the antioxidant system. Consequently, there was a reduction in mortality and greater weight gain in the initial phase of life, as well as improved physical characteristics of the meat. These changes are desirable as they contribute to animal health and offer the final consumer a better product. Therefore, in general, we conclude that the subcutaneous application of minerals was beneficial in lamb production.

ETHICS COMMITTEE

This study was approved by the Ethics Committee of Use of Animals (CEUA) of Universidade do Estado de Santa Catarina (CETEA/UDESC), protocol number 7398301116.

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Table 1: Ingredients and diet used to feed lambs from birth to slaughter. Animals from both groups received the same diet throughout the experimental period.

Ingredients	Age of animals (days)				
	1 to 7	8 to 20	21 to 45	46 to 90	91 to 150
Ewe milk (mL/animal/day)	500	200	-	-	-
Artificial milk (mL/animal/day)	-	300	500	-	-
Commercial concentrate (g/animal/day)*	-	140	300	-	-
Concentrate (g/animal/day) #	-	-	-	400	600
Corn silage (kg/animal/day)	-	-	-	1.00	1.50
Hay (kg/animal/day)	-	-	0.12	0.25	0.30
& ***Ingredients of the concentrate***					
Ground corn (%)	-	-	-	61.0	70.0
Soybean meal (%)	-	-	-	25.0	25.0
Powdered milk (%)	-	-	-	10.0	-
$Nucleus (mineral/vitamin) (%)	-	-	-	4.0	3.0
Ammonium chloride (%)	-	-	-	-	1.0
Calcitic limestone (%)	-	-	-	-	1.0
Calculated mineral composition of total diet+					
Calcium (g/kg)	2.39	2.22	3.60	5.48	8.38
Phosphorus (g/kg)	1.72	1.49	2.23	3.18	4.58
Copper (mg/kg)	1.79	4.80	9.45	0.00	0.00
Iron (mg/kg)	30.2	23.02	37.55	22.75	25.26
Manganese (mg/kg)	6.44	13.15	25.61	24.8	27.9
Selenium (mg/kg)	0.50	0.23	0.31	0.35	0.40
Zinc (mg/kg)	25.0	19.78	30.25	48.0	54.0

* Specific commercial concentrate (pelleted) for lambs: crude protein (min 210 g/kg), crude fat (min 30 g/kg), crude fiber (max 100 g/kg), acid detergent fiber - ADF (max 120 g/kg), ash (max 100 g/kg), calcium (min 8 g/kg, and max 12 g/kg), phosphorus (min 6 g/kg), *Saccharomyces cerevisiae* (min $1.5x10^7$ CFU/kg), vitamin A (min 15,000 IU/kg), vitamin D3 (min 2250 IU/kg), vitamin E (min 50 IU/kg), copper (min 30 mg/kg), iron (min 100 mg/kg), iodine (min 1.8 mg/kg), manganese (min 80 mg/kg), selenium (min 0.6 mg/kg), cobalt (min 1 mg/kg), zinc (min 80 mg/kg), growth promoter additive (Lasalocid) (45 mg/kg).

Concentrate (mash) produced on the farm with ingredients described in this table (&), using Y-type mixer.

$ Nucleus composition: phosphorus (min 55g/kg), calcium (min. 215 g/kg, max 225 g/kg), sulphur (min 12 g/kg), sodium (min 80 g/kg), cobalt (min 60 mg/kg), chromium (min 12 mg/kg), iron (min 1420 mg/kg); iodine (min 100 mg/kg), magnesium (min 14 mg/kg), manganese (min 1550 mg/kg), selenium (min 22 mg/kg), vitamin A (min 20000 IU/kg), vitamin D (min 40000 IU/kg), vitamin E (min 550 IU/kg), and fluorine (max 550 mg/kg).

+ The levels of minerals in the diet were calculated according with requirements of the NRC (2001).

Table 2: Chemical composition of ingredients used in lamb diets.

Chemistry composition	Silage	Hay	*Concentrate (8-45 days)	#Concentrate (46-150 days)
Dry matter (%)	29.8	86.2	88.0	87.5
Crude Protein (%)	7.78	14.5	20.8	16.4
Copper (mg/kg)	nd	nd	30.0	Nd
Zinc (mg/kg)	0.15	nd	80.2	89.8
Selenium (mg/kg)	0.03	nd	0.61	0.66
Manganese (mg/kg)	24.1	nd	81.0	46.5

Note: not-detected (nd); * Commercial concentrate; #Concentrate produced with ingredients described in Table 1.

Table 3: Mean and standard deviation of ferric reducing ability of plasma (FRAP), tumor necrosis factor (TNF-α) and interleukin 1 (IL-1) in serum of supplemented (n = 10) and control (n = 10) animals on days of life 1, 30 and 60.

Variable	**Days**	**Mean ± standard deviation**		
		Control	Treated	**p-value**
FRAP (µmol/L)	1	63.5 (8.6)[b]	60.9 (10.3) [b]	0.796
	30	95.6 (18.9)[a]	128.7(16.4) [a]	0.027
	60	88.4 (12.2)[a]	118.3 (17.5) [a]	0.001
	p-value	0.001	0.001	
TNF-α (pg/mL)	1	50.4 (11.6) [c]	47.6 (9.4) [b]	0.821
	30	152.20 (26.7) [a]	143.10 (37.2) [a]	0.569
	60	96.15 (21.9) [b]	125.17 (12.5) [a]	0.034
	p-value	0.001	0.001	
IL-1 (pg/mL)	1	28.8 (4.70) [c]	21.9 (10.90) [b]	0.260
	30	80.7 (13.2) [a]	91.1 (14.8) [a]	0.620
	60	60.7 (11.0) [b]	79.0 (8.7) [a]	0.001
	p-value	0.001	0.001	

Note: P <0.05 on the same line shows difference between groups. Similarly, P<0.05 in the same column shows difference between moments, a repeated analysis over time (illustrated with different subscript letters).

Table 4: Distribution of the animal measurements for supplemented and control groups to allometric evaluations.

Variable	Groups (mean and standard deviation)		p-value
	Treated	Control	
Weight of day of slaughter (kg)	37.9 (4.27)	36 (5.02)	0.92
Pelvic limb height (cm)	59.6 (2.50)	58. (3.10)	0.42
Weight in slaughterhouse (kg)	14 (1.62)	13.8 (2.03)	0.81
Perimeter (cm)	74.5 (2.78)	73.0 (11.74)	0.51
Thoracic limb height (cm)	65.0 (2.45)	61.5 (4.09)	0.11
Body length - in vivo (cm)	56.5 (4.59)	56.6 (3.33)	0.68
Length of lamb carcass (cm)	50.3 (2.14)	53.1 (2.86)	0.10
"Green viscera" (digestive system) (kg)	10.6 (1.20)	10.9 (1.26)	0.87
Heart (kg)	0.1 (0.02)	0.1 (0.02)	0.74
Spleen (kg)	0.05 (0.01)	0.06 (0.02)	0.46
Internal fat (kg)	0.5 (0.14)	0.7 (0.25)	0.13
pH	6.6 (0.32)	6.5 (0.13)	0.37
Testicle weight (kg)	0.1 (0.06)	0.19 (0.08)	0.99
Perimeter of the testicle (kg)	20.5 (3.70)	21.3 (3.67)	0.87
Distal extremity of the limbs (Legs) (kg)	0.7 (0.07)	0.77 (0.12)	0.93
Penis (kg)	0.07 (0.02)	0.06 (0.02)	0.68
Skin of lamb (skin and wool) (kg)	2.6 (0.29)	2.7 (0.61)	0.81
Lung (kg)	0.6 (0.09)	0.60 (0.12)	0.74
Kidneys (kg)	0.08 (0.01)	0.09 (0.01)	0.93
Head (kg)	1.7 (0.18)	1.6 (0.15)	0.69
Urinary bladder (kg)	0.02 (0,02)	0.03 (0.02)	0.46
Liver (kg)	0.6 (0.11)	0.7 (0.10)	0.42
Depth of thorax (cm)	23.7 (0.71)	23.6 (0.75)	0.74
Length of pelvic limb (cm)	35.3 (1.19)	34.6 (1.46)	0.42
Length of thoracic limb (cm)	12.8 (1.79)	12.3 (0.82)	0.87
Pelvic limb width (cm)	8.9 (0.60)	9.8 (1.14)	0.17

Note: there were no statistical differences in these analyses between groups. As central tendency measurement we show the mean and dispersion by standard deviation

Table 5: Distribution of the animal measurements for treated and control groups for meat quality analyses of lambs.

Variables	Groups (mean and standard deviation)		p-value
	Treated	Control	
Colour: a	17.9 (1.26)	17.4 (0.95)	0.48
Colour: b	7.8 (1.37)	6.4 (0.81)	0.13
Colour: L	40.4 (1.98)	43.2 (2.32)	0.04*
Fat thickness (mm)	1.9 (0.58)	1.3 (0.21)	0.004*
Ribeye - depth (cm^2)	58.0 (4.21)	55.7 (4.63)	0.24
Ribeye - width (cm^2)	28.1 (2.08)	28.2 (1.75)	0.98
pH	5.4 (0.04)	5.5 (0.03)	0.002*
Loss of water by cooking (%)	0.06 (0.01)	0.09 (0.02)	0.004*
Water holding capacity (g water/g dry matter)	4.0 (0.22)	3.9 (0.11)	0.58
Shear force (kgf/cm^2)	4.4 (0.34)	5.2 (0.46)	0.008*
Carcass weight(kg)	14.7 (1.49)	13.8 (1.62)	0.18

Note: * significate difference between groups. As central tendency measurement we show the mean and dispersion by standard deviation

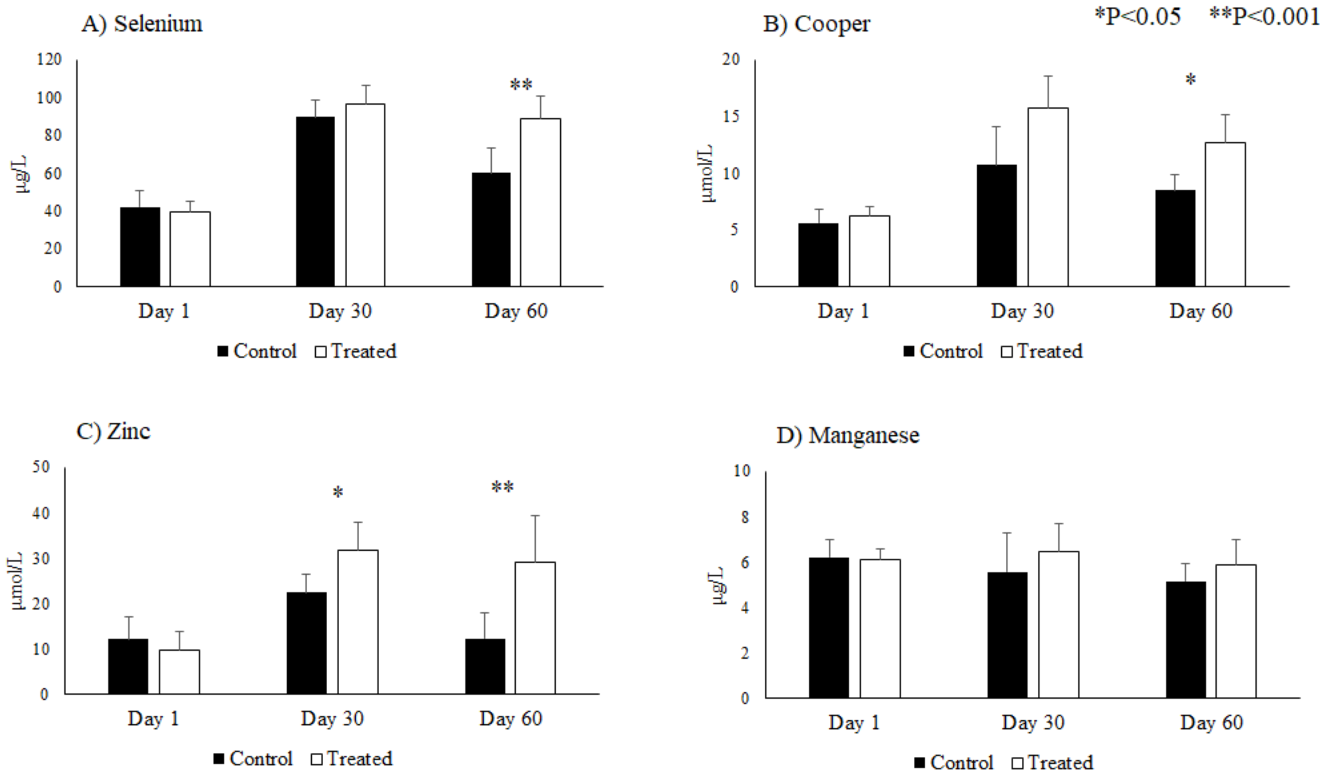

Figure 1: Serum levels of minerals (A) selenium; B) copper; C) zinc; and D) manganese) in lambs of the control and treated groups.

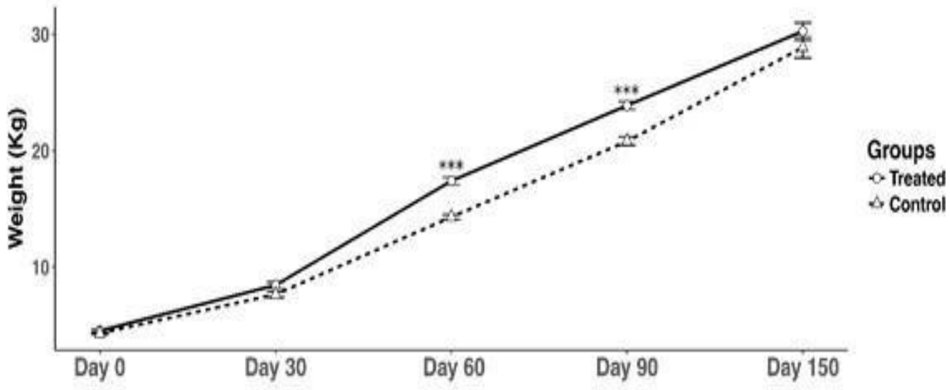

Figure 2: Distribution by group of weight in lambs treated with minerals. Each day of sampling the mean and standard deviation of each variable are represented. *** represents significant differences between two groups in DOL 60 and 90.

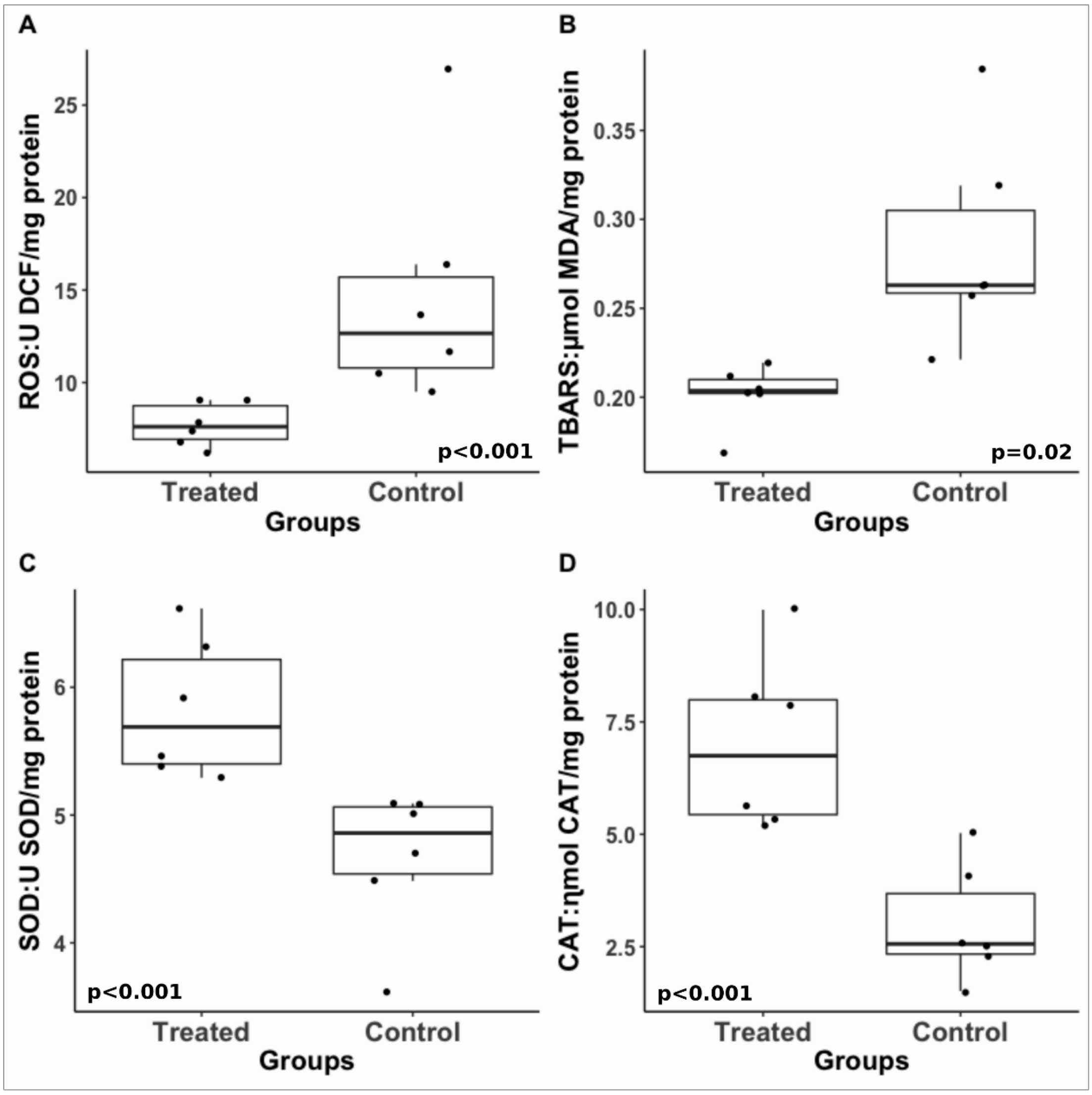

Figure 3: Distribution of biochemical variables for each group in meat. (A) A significant difference between groups ($P < 0.001$) was observed, i.e. higher reactive oxygen species [ROS] in the control group; (B) and followed the same tendency with the control group having more thiobarbituric acid reactive substances [TBARS] ($P = 0.02$); (C and D) the treated group show higher values ($P < 0.001$) for both enzymes (catalase [CAT] and superoxide dismutase [SOD]). Note: The dots represent the individual observations, this is a regular box-and-whisker bars are depicting groups of numerical data through their quartiles. Box plots may also have lines extending vertically from the boxes (whiskers) indicating variability outside the upper and lower quartiles, the centre line is always the median value.

CONSIDERAÇÕES FINAIS

Os desafios enfrentados pela criação de cordeiros e produção de carne no Brasil e de maneira especial, em Santa Catarina ainda são grandes. Pois além da busca por atender o mercado e pela apresentação de produtos que sejam atrativos aos consumidores, é necessário investir conhecimento nos sistemas de manejos e alternativas que viabilizem a atividade.

Os cordeiros merecem maior atenção e os estudos devem ser intensificados visando buscar maior produção com maior qualidade. A modulação dietética ainda é a ferramenta mais eficaz para estimular essas características nos animais e o uso de suplementos vem ao encontro a esse pensamento. Minerais, especialmente o selênio, cobre, zinco e manganês são exemplos de como é possível realizar alterações satisfatórias, através da modulação do metabolismo do animal.

Os efeitos metafílicos do zinco, manganês, cobre e selênio, foram destacados pela estimulação de importantes citocinas pró-inflamatórias em benefício das defesas animais e reações oxidativas moduladas via ativação do sistema antioxidante. Consequentemente, observamos a redução da mortalidade e maior ganho de peso na fase inicial da vida, bem como melhora das características físicas da carne. Além disso, houve uma melhor resposta dos animais à coccidiose, e a excreção de oocistos e sinais clínicos foi em menor proporção. A utilização do complexo mineral pode favorecer condições para o metabolismo de proteínas e carboidratos, contribuindo para o aumento do ganho de peso. Essas mudanças são desejáveis, pois contribuem para a saúde animal e oferecem ao consumidor final um produto melhor.

Ainda há muito a ser descoberto sobre as propriedades dos minerais, muitas informações podem ser acrescentadas á futuros trabalhos. Descobertas satisfatórias foram feitas ao utilizarmos esses minerais na forma injetável em cordeiros recém-nascidos. Esse estudo possibilitou concluir que a aplicação de minerais pela via subcutânea é capaz de modular o sistema antioxidante dos animais, assim como estimular a imunidade. Observamos que indiretamente esses minerais contribuem para saúde dos animais sendo assim benéfica na produção de cordeiros.

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ANEXOS

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 "Benefícios da aplicação de complexo mineral (zinco, cobre selênio e magnésio) em cordeiros recém nascidos: avaliação da resposta imune, níveis de antioxidantes e parâmetros bioquímicos", protocolada sob o CEUA nº 7398301116 (ID 000309), 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 26/05/2017.

We certify that the proposal "Benefits of the application of mineral complexes (zinc, copper selenium and magnesium) in newborn lambs: evaluation of the immune response, antioxidant levels and biochemical parameters", utilizing 120 Ovines (males and females), protocol number CEUA 7398301116 (ID 000309), 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 05/26/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 12/2016 a 12/2017 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Machos e Fêmeas	idade:	1 a 6 meses	N:	120
Linhagem:	Lacaune			Peso:	4 a 50 kg		

Local do experimento: Cabanha chapecó, em Chapecó. Propriedade que tem convênio com UDESC Oeste.

Lages, 17 de maio de 2018

Marcia Regina Pfuetzenreiter
Coordenadora da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Prof. Dr. Ubirajara Maciel da Costa
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina