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USO DE INGREDIENTES E ADITIVOS ALTERNATIVOS NA DIETA DE OVINOS: IMPACTOS SOBRE SAÚDE, DESEMPENHO ZOOTECNICO E QUALIDADE DE CARNE

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

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Elaborada por
Karoline Wagner Leal

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

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RESUMO

Dissertação de Mestrado
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Chapecó, 11 de fevereiro de 2020

A ovinocultura no Brasil possui grande potencial para exploração e apresenta melhoras nos índices produtivos, graças a adoção de novas tecnologias. Através da utilização racional do potencial genético dos rebanhos tem se aumentado a oferta de produtos lácteos e cárneos. Para isso o planejamento nutricional é muito importante, tendo em vista que a alimentação é o fator que mais onera o custo de produção. Desta forma, está pesquisa apresenta algumas informações para aprimorar o crescimento e o desenvolvimento de ovinos de diferentes categorias e raças; tendo como objetivo avaliar se a utilização de resíduos de pré-limpeza de soja, como ingrediente alternativo, e a biocolina vegetal, como aditivo, influenciam na saúde, no desempenho zootécnico e na qualidade da carne de ovinos confinados. Para isso três experimentos distintos foram realizados. No experimento I, foram utilizados 40 cordeiros da raça Lacaune em um delineamento inteiramente casualizado, distribuídos em quatro grupos com cinco repetições e dois animais por repetição. A biocolina vegetal (BV) foi adicionada como aditivo ao concentrado, em diferentes níveis durante 60 dias (30 dias pré-desaleitamento e 30 dias pós-desaleitamento). Os tratamentos foram formados da seguinte forma: Grupo controle, T0 sem adição de biocolina vegetal; T2 adição de 2g de BV por animal/dia; T4 adição de 4g de BV por animal/dia; T6 adição de 6g de BV por animal/dia. A administração de biocolina vegetal melhorou o desempenho dos cordeiros do T2 em comparação com o grupo controle; observamos um efeito quadrático que indica a dose de 3,63 gBV/animal/dia é adequada para suplementação desta categoria. De modo geral, a adição de biocolina vegetal na dieta dos cordeiros foi benéfica para a saúde, tendo em vista que houve redução dos radicais livres e estimulação da produção de globulinas durante o período de transição alimentar. No experimento II, foram utilizadas 48 borregas da raça Lacaune em um delineamento inteiramente casualizado, distribuídas em três grupos com 16 repetições. Os tratamentos foram: grupo controle (T0: sem adição de BV), T4 com adição de 4g de BV por animal/dia e T8 com adição de 8g de BV por animal/dia; a biocolina foi homegeneizada no concentrado e ofertada aos animais uma vez ao dia, durante 75 dias de experimento. Como principais resultados, observamos maior ganho de peso no T8, nos primeiros 30 dias de experimento, no entanto nas avaliações subsequentes não foi evidenciado esse resultado. Em relação aos parâmetros ruminais observamos que houve redução da atividade microbiana ruminal e verificamos menor porcentagem de ácidos graxos de cadeia curta no líquido ruminal dos animais suplementados com BV. A suplementação de BV para borregas na recria apresentou benefícios para a saúde, pois verificamos efeito hepatoprotetor, além de estimulação da ação antioxidante e resposta imunológica. Mesmo

que a suplementação de BV tenha interferido na microbiota ruminal, essas mudanças não refletiram no ganho de peso. No experimento III, foram utilizados 32 cordeiros machos, oriundos do cruzamento fixo entre as raças Texel e Ile de France, distribuídos em um delineamento inteiramente casualizado com quatro grupos e oitro repetições. Os tratamentos consistiam em diferentes níveis de substituição da silagem de sorgo pelo resíduo de pré-limpeza de soja (RPLS) (0%, 33,5%, 66,5% e 100%). Os principais resultados indicam que a inclusão do resíduo de pré-limpeza de soja para terminação de cordeiros em confinamento não influencia negativamente os parâmetros instrumentais da carne; no entanto verificamos alterações relevantes no perfil lipídico da carne, dependente do nível de inclusão do RPLS, que podem influenciar negativamente a qualidade da carne. Altos niveis de substituição aumentam a quantidade de acidos graxos saturados na carne, o que não é benéfico a saúde do consumidor. Em resumo, concluímos que a suplementação de biocolina vegetal para cordeiros na fase de cria apontou resultados satisfatórios que indicam melhora no desempenho dos animais; já a suplementação de BV para borregas na recria não influenciou o ganho de peso dos animais, no entanto foi observado melhora no metabolismo; por fim quando testamos o RPLS evidenciamos que existem limitações de utilização, dependentes do nível de utilização desse resíduo.

Palavras-chave: alimentação, ovinos, produtividade, suplementação.

ABSTRACT
Master's Dissertation
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USE OF INGREDIENTS AND ALTERNATIVE ADDITIVES IN SHEEP DIET: IMPACTS ON HEALTH, ZOOTECHNIC PERFORMANCE AND MEAT QUALITY

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Sheep farming in Brazil has great potential for exploration and shows improvements in production rates, thanks to the adoption of new technologies. Through the rational use of the genetic potential of herds, the offer of dairy and meat products has increased. For this, nutritional planning is very important, considering that food is the factor that most costs the production cost. In this way, this research presents some information to improve the growth and development of sheep of different categories and breeds; aiming to evaluate whether the use of soy pre-cleaning residues, as an alternative ingredient, and vegetable biocolline, as an additive, influence health, zootechnical performance and meat quality of confined sheep. For this, three different experiments were carried out. In experiment I, 40 Lacaune lambs were used in a completely randomized design, distributed in four groups with five repetitions and two animals per repetition. Vegetable biocholine (BV) was added as an additive to the concentrate, at different levels for 60 days (30 days before weaning and 30 days after weaning). The treatments were formed as follows: Control group, T0 without adding vegetable biocolline; T2 adding 2g of BV per animal / day; T4 addition of 4g of BV per animal / day; T6 addition of 6g of BV per animal / day. The administration of vegetable biocolina improved the performance of lambs of T2 compared to the control group; we observed a quadratic effect that indicates a dose of 3.63 gBV / animal / day is adequate for supplementation in this category. In general, the addition of vegetable biocolline to the lambs' diet was beneficial to health, considering that there was a reduction in free radicals and stimulation of globulin production during the food transition period. In experiment II, 48 Lacaune lambs were used in a completely randomized design, distributed in three groups with 16 repetitions. The treatments were: control group (T0: without addition of BV), T4 with addition of 4g of BV per animal / day and T8 with addition of 8g of BV per animal / day; the biocholine was homogenized in the concentrate and offered to the animals once a day, during 75 days of the experiment. As main results, we observed greater weight gain at T8, in the first 30 days of the experiment, however in subsequent evaluations this result was not evidenced. Regarding ruminal parameters, we observed that there was a reduction in rumen microbial activity and we verified a lower percentage of short chain fatty acids in the rumen liquid of animals supplemented with BV. BV supplementation for rearing lambs presented health benefits, as we verified hepatoprotective effect, in addition to stimulation of antioxidant action and immune response. Even though BV supplementation interfered with the rumen microbiota, these changes did not reflect on weight gain. In experiment III, 32 male lambs were used, coming from the fixed cross between the Texel and Ile de France breeds, distributed in a completely randomized design with four groups and eight repetitions.

The treatments consisted of different levels of substitution of the sorghum silage by the soy pre-cleaning residue (RPLS) (0%, 33.5%, 66.5% and 100%). The main results indicate that the inclusion of soy pre-cleaning residue for finishing lambs in confinement does not negatively influence the instrumental parameters of the meat; however, we verified relevant changes in the meat's lipid profile, depending on the level of inclusion of the RPLS, which can negatively influence the quality of the meat. High levels of substitution increase the amount of saturated fatty acids in the meat, which is not beneficial to the consumer's health. In summary, we conclude that the supplementation of vegetable biocholine for lambs in the breeding phase showed satisfactory results that indicate improvement in the animals' performance; BV supplementation for rearing lambs did not influence the animals' weight gain, however, an improvement in metabolism was observed; finally, when we tested the RPLS, we found that there are usage limitations, depending on the level of use of this waste.

Keywords: (food, sheep productivity, supplementation).

SUMÁRIO

CAPÍTULO I

1. REVISÃO DE LITERATURA

1.1 PANORAMA DA OVINOCULTURA NO BRASIL E NO MUNDO

O desenvolvimento da cadeia produtiva da ovinocultura é uma importante estratégia para o desenvolvimento rural em algumas regiões. De acordo com dados apresentados pelo Instituto Brasileiro de Geografia e Estatística (IBGE) em 2017 o efetivo do rebanho ovino brasileiro apresenta um total de 18.948.934 cabeças. A nível mundial a produção de ovinos está amplamente distribuída, totalizando 1.202.430.935 animais. A Organização das Nações Unidas (FAO) em 2016 observou uma taxa de crescimento do rebanho ovino de 1,5% ao ano, sendo o maior produtor mundial de ovinos a China, seguida pela Austrália e Índia.

A ovinocultura possui variados sistemas de criação, a adequação do rebanho além do clima depende da determinação do propósito de produção; este relaciona-se com a infraestrutura da propriedade, a genética do rebanho e as opções de mercado (Guimarães e Souza, 2014). Tendo o Brasil vasta extensão territorial a criação de ovinos possui diferentes sistemas de produção de acordo com a região.

Na região Sul do Brasil a ovinocultura apresenta efetivo de 4.010.916 cabeças (IBGE, 2017). No Rio Grande do Sul a produção de lã foi o principal objetivo de exploração econômica no século 20; após crises no setor com a entrada da fibra sintética a ovinocultura gaúcha passou por um processo de reestruturação (Silva et al., 2013). Assim, os produtores inseriram raças especializadas para a produção de carne, que aumentaram a competitividade do setor, devido a grande demanda de proteína animal. O estado de Santa Catarina figura como um dos pioneiros na produção de ovinos leiteiros, tem captado recursos e incentivos governamentais, já que a atividade é vista no estado com alternativa de renda para pequenos produtores (Pires et al., 2014). Além disso, os criadores também investem na produção de carne e lã. No Paraná a produção de cordeiros excluvisos para o abate é o principal objetivo de exploração, apresenta sistema de criação intensivo, com uso de tecnologia e sistema de organização em cooperativas (Debortoli, 2017).

A criação de ovinos na região Sudeste do Brasil está voltada a produção de carne, os estados de São Paulo e Minas Gerais contribuem significativamente para as estatísticas da região. Em relação ao efetivo nacional há baixa representatividade, segundo censo

agropecuário totaliza 610.784 cabeças, atualmente apresenta o menor efetivo brasileiro de ovinos (IBGE, 2017). No entanto, os pecuaristas têm se destacado pela qualidade da carne e, além disso, houve crescimento de mercado. Assim, pequenas propriedades buscam uma atividade econômica viável, utilizam sistemas intensivos de produção e animais especializados para carne (Costa e Barbosa, 2014). Já no centro-oeste do país a maior concentração de criadores de ovinos encontra-se no Mato Grosso e no Mato Grosso do Sul. O propósito de exploração também é voltado a produção de carne, mesmo com instruções do Projeto Aprisco realizado pelo SEBRAE-MS há baixa tecnificação e produtividade (Vargas Jr e Sorio, 2014). Entretanto, o rebanho está em expansão na região, segundo IBGE (2017) possui o terceiro maior efetivo brasileiro, totaliza 1.027.452 cabeças.

A região nordeste do Brasil é caracterizada por possuir o maior rebanho ovino brasileiro, 12.634.412 cabeças (IBGE, 2017). O mercado consumidor é intenso, porém há baixa oferta regular de produtos com qualidade. O rebanho está distribuído entre muitos estados o que dificulta a organização do setor. Feiras onde são comercializados os animais consistem na principal fonte de abastecimento dos abatedouros formais e informais (Guimarães et al., 2014). Este importante e tradicional mercado consumidor de carne ovina oportuniza aos criadores chances de empreender e agregar valor aos produtos regionais, a média de consumo regional ultrapassa a média de consumo nacional. No entanto, há dificuldades no setor de vendas, pois os produtos são dissociados e dispersos. Além disso, a produção é basicamente formada por pequenos produtores que não têm poder de negociação. Em resposta às oportunidades de mercado existentes a Empresa Brasileira de Pesquisa Agropecuária (Embrapa) oferece orientações de mercado para os produtores.

A região norte do país possui 665.370 cabeças segundo o censo agropecuário (IBGE, 2017). A espécie ovina compõe o sistema de produção das propriedades familiares na região amazônica, seja para complementar a renda ou autoconsumo. Houve crescimento do rebanho na região Norte por iniciativa dos produtores juntamente com programas e incentivos governamentais. Ademais, a migração de criatórios tradicionais do Nordeste e a utilização de tecnologia e investimento em raças especializadas para o abate visa intensificar a produção e com isso abrir caminhos para a exportação com os países vizinhos da Amerécia do Sul. No entanto, os rebanhos da região estão distribuídos em pequenas propriedades, a comercialização é informal e realizada por meio de atravessadoresque realizam aquições de

animais excedentes e negociam com marchantes e açougues sem nenhum tipo de fiscalização (Monteiro et al., 2014).

Os sistemas de criação têm se expandido para além das fronteiras das regiões nordeste e sul do Brasil, tradicionalmente produtoras, o aumento do efetivo ovino nas demais regiões do país e a evolução da capacidade produtiva das espécies ovinas, através do melhoramento genético, nutricional e sanitário, aumentam a participação da ovinocultura no agronegócio brasileiro (Rogério et al., 2016). De modo geral, a ovinocultura brasileira necessita de atenção quanto a estruturação da cadeia produtiva; ações governamentais e parcerias institucionais podem levar conhecimento e tecnificação para os produtores, tendo em vista a falta de utilização das informações sobre parâmetros nutricionais e reprodutivos (Guimarães e Souza, 2014). Independente da região a expansão da atividade trará benefícios tanto para pequenos quanto para grandes produtores; a implantação de órgãos fiscalizadores pode auxiliar no crescimento da atividade no país.

A produção de carne está em pleno avanço e requer implementação tecnológica para atender o mercado consumidor exigente (Leite e Medeiros, 2014); e a produção de leite ovino ainda incipiente no país, fortemente ligada à industrialização de produtos lácteos, possui um mercado promissor para produção de queijos (Corrêa et al., 2014). Desta forma, a ovinocultura brasileira apresenta crescimento pouco acelerado, porém constante, desde 2002; a produção de proteína animal aliada a geração de renda promove desenvolvimento do meio rural, sendo esta atividade de grande importância para o país (Guimarães e Souza, 2014).

1.2 OVINOCULTURA DE CARNE

A produção de carne ovina no Brasil está em pleno avanço, porém não é suficiente para abastecer o mercado interno. Desta forma, Osório et al. (2014) explicam que é importante observar a cadeia produtiva, pois o animal desejado é aquele que produz em maior quantidade, em máxima qualidade, em menor tempo e área e com o menor custo. Inicialmente, o foco da produção cárnea ovina estava no produtor e no animal, com os avanços nas pesquisas a carcaça tomou este lugar. No entanto, atualmente a carne adquire importância, os estudos relacionados a qualidade de carne estão cada vez mais elucidativos. Assim, através de sofisticados cortes, modo de preparo e apresentação nos pratos dos

restaurantes se alcança os consumidores, sendo esses, hoje, o principal foco da cadeia produtiva (Osório et al., 2014).

Nesse seguimento, uma pesquisa de mercado realizada no sudeste do país por Frias et al. (2018) mostra que o mercado da carne ovina está consolidado. Seus resultados expõem que 64% dos entrevistados consomem carne ovina e que 31% afirmam consumir pelo menos uma vez por semana. Desta maneira, com a grande diversidade de condições ambientais das áreas de criação, espera-se o surgimento de novos sistemas de produção. Conforme citado por Siqueira (1996), que já expunha a tendência de intensificação da criação e utilização de terminação de cordeiros em regime de confinamento. Assim, a produção de carne de cordeiro viabiliza, economicamente, o setor ovino e a busca de alternativas de produção tornam-se uma necessidade atual (Gois et al., 2018).

Azeredo et al. (2005) explicam que os ovinos produzem carne de maneira mais econômica em seu estágio de crescimento e conversão alimentar máxima. Portanto, uma opção para diminuir os custos de produção é buscar alimentos alternativos para cordeiros em terminação. Devido a ampla diversidade de áreas de produção procura-se disponibilizar alimentos que atinjam o maior número de produtores. A criação de ovinos em confinamento se justifica em áreas de terras valorizadas, nesse sistema busca-se elevados ganhos de peso, em torno de 0,300 g/animal/dia, o que possobilita a produção de cordeiros precoces com menor quantidade de gordura na carcaça para atender as exigências do mercado consumidor (Silva Sobrinho, 2014). Os sistemas de criação intensivos, como o confinamento, podem ser uma alternativa para auxiliar na oferta regular de carne ovina (SÁ e OTTO de SÁ, 2013).

1.3 OVINOCULTURA DE LEITE

A produção de ovinos destinados à produção leiteira está em fase de desenvolvimento, há pouca disponibilidade de índices zootécnicos quando comparados à produção caprina ou bovina, por exemplo. No ano de 2017, foram produzidas 11.567.441 T de leite ovino fresco no mundo, contra 18.894.731 T de leite caprino e 706.393.439 T de leite bovino (FAOSTAT, 2019). Entretanto, existe grandes possibilidades de crescimento e inclusão de pequenos produtores neste nicho de mercado. Além de meio de subsistência de famílias com menores condições, proporciona nutrientes em quantidade semelhante ou até superior (em alguns aspectos) ao leite bovino.

A raça Lacaune, muito utilizada para a produção de ovinos de leite, teve origem na Década de 50, pela união de diversas raças francesas, com o início de seu melhoramento genético a partir de 1960, por duas empresas de Inseminação Artificial (IA), Confédération Générale de Roquefort (Millau, França) e Ovitest (Onet-le-Château, França) (Baloche et al., 2014). Sequencialmente, houve a necessidade de implantação de ordenha mecanizada, que causou a diminuição de produtores dispostos a investir, em contrapartida um aumento significativo na quantidade de leite produzido por animal. O destaque da raça para a produção de leite atraiu outros países que começaram a importar animais, o Sul do Brasil, por exemplo, em 1992 iniciou a formação de um plantel nacional da raça Lacaune. O estado do Rio Grande do Sul iniciou o primeiro laticínio especializado do país, e a raça Lacaune foi difundida para Santa Catarina, Paraná, Minas Gerais, Rio de Janeiro, São Paulo e Distrito Federal (Figueira et al., 2018).

As fêmeas da raça Lacaune podem variar de 60 a 80 kg em média, enquanto os machos variam de 80 a 100kg. Sua produção de leite pode durar até 180 dias (Figueira et al., 2018). Esta raça possui uma produção inicial 30% maior quando comparado à East Friesian, entretanto, a queda de sua produção tende a ser mais acentuada, chegando a 8g/dia, comparado a 2g/dia da East Friesian (Ticiani et al., 2013). Este percentual de produção superior da raça Lacaune no início de produção causa um aumento na produção final significativo. A produção total dessa raça chega a 153,64 kg de leite, com média de produção diária de 1,67kg, enquanto a raça East Friesian produz 124,20 kg durante toda a lactação, com produção diária de 1,35 kg (Ticiani et al., 2013).

O planejamento do sistema de criação em fazendas leiteiras é muito importante, visto que o leite produzido pelas matrizes é destinado a comercialização. Diferentes sistemas de produção de ovinos podem ser adotados para evitar perdas de produção durante o aleitamento de cordeiros. De acordo com Flamant e Casu (1978), citado por Bianchi (2018), existem 6 formas de produção: Os cordeiros ficam com as mães até o final do aleitamento (3 a 4 meses) e após esse período as mães são secas; os cordeiros permanecem com as mães por volta de 3 meses pós parto, onde são desmamados e é feita a ordenha das ovelhas por mais 1 mês; os cordeiros são desmamados por volta de 4 a 6 semanas pós parto e as ovelhas continuam sendo ordenhadas por 5 meses aproximadamente; os cordeiros são separados das mães durante o dia enquanto estas pastejam, as ovelhas são ordenhadas uma vez durante o dia e a

noite os cordeiros são soltos para permanecerem com elas; os cordeiros são soltos junto com as mães após uma ordenha no dia por aproximadamente 8 semanas, onde ocorre o desmame, após este período as ovelhas são ordenhadas duas vezes ao dia; os cordeiros são separados das mães com 24 horas de vida, sendo alimentados artificialmente e as ovelhas permanecem sendo ordenhadas por até 10 meses. Nesta perspectiva, é importante adaptar o sistema de cria e proporcionar uma preparação do cordeio aos alimentos que irá receber no confinamento, quando estes são separados das mães logo após a ingestão do colostro (Silva Sobrinho, 2014).

1.4 NUTRIÇÃO DE OVINOS

O manejo nutricional dos rebanhos tem papel essencial nos sistemas de produção. A utilização da vegetação disponível é um desafio para os criadores, tendo em vista a capacidade de suporte, a qualidade e a quantidade de material e o tipo de exploração do ambiente. A escolha por sistemas de produção sustentáveis e competitivos permite modificações no manejo nutricional que apresentam impactos positivos e refletem nos índices produtivos, reprodutivos e sanitários (Pereira et al., 2007).

De acordo com Resende et al. (2008) quando há aprimoramento dos índices produtivos (taxa de ganho de peso, conversão alimentar, rendimento de carcaça, produção leiteira) eleva-se o requerimento nutricional. No Brasil as recomendações de exigências nutricionais seguem padrões internacionais. Por esta razão o desempenho observado em outros países não condiz com a realidade encontrada nas mais distintas regiões brasileiras, já que as exigências nutricionais são influenciadas por fatores ambientais, nutricionais e genéticos. Os sistemas de alimentação comumente utilizados são NRC, INRA e AFRC; diferenças metodológicas são observadas quanto aos fatores de correção e eficiência de utilização; assim há reflexos nos valores preconizados para as exigências nutricionais. Por isso é de suma importância avaliar o sistema de alimentação e suas peculiaridades a fim de escolher aquele que se adequa as condições em que os animais serão submetidos.

Os sistemas de alimentação utilizam métodos fatoriais para determinar as exigências nutricionais de acordo com a fase que o animal se encontra (mantença, crescimento, gestação, lactação ou produção de fibra ou lã) (Resende et al., 2008). A adequação da dieta requer conhecimento não somente das exigências nutricionais dos animais; o valor nutriocional dos alimentos, que por meio de métodos específicos são combinados em

proporções adequadas, tem a finalidade de atender os requerimentos dos animais, reduzir transtornos digestivos, custos e perdas de nutrientes (Cabral et al., 2008). Destacam-se estimativas acuradas de consumo e requisitos em energia, proteína, minerais e vitaminas.

A alimentação de pequenos ruminantes segue critérios básicos sobre o comportamento ingestivo dos animais, o plano alimentar disponível, o custo mínimo da dieta, as práticas de conservação de volumosos, o manejo do pasto nativo ou cultivado e a possibilidade de substituir alimentos tradicionais por alimentos alternativos (Rogério et al., 2016). A suplementação estratégica em períodos pré-determinados é uma alternativa interessante, quando há escassez de forragens para categorias mais exigentes. As variações na disponibilidade de forragens ao longo do ano refletem significativamento no desempenho dos rebanhos, há diminuição da matéria seca das pastagens por consequência do processo de lognificação da parede celular das plantas (Pereira et al., 2007). Por isso o planejamento alimentar nas propriedades garante oferta regular de alimento todo ano, sendo indispensável para o correto funcionamento do manejo nutricional.

Dado que a alimentação é o fator que mais aumenta o custo de produção, a possibilidade de utilizar ingredientes alternativos, disponíveis na região a custos acessíveis, pode melhorar a qualidade de alimentos volumosos e refletir na diminuição dos custos (Pereira et al. 2007). Ao considerar que na maioria das situações as forragens disponíveis carecem de alguns nutrientes essenciais, suplementos são necessários para se obter níveis aceitáveis de desempenho, ainda que não seja possível predizer o impacto que esse fornecimento terá sobre o rebanho (Silva Sobrinho, 2014). Os sistemas de criação de ovinos, sejam extensivos ou intensivos, necessitam manter o aporte de nutrientes aos animais indepentende da categoria ou ciclo de produção; assim alimentos concentrados, nutracêuticos e funcionais, tendo em vista a estacionalidade forrageira, contribuem para manter a produtividade dos rebanhos e estimulam o crescimento da ovinocultura.

1.4.1 RESÍDUO DE PRÉ-LIMPEZA DE SOJA - RPLS

Segundo Oliveira et al. (2006) os resíduos originados na produção agrícola e na agroindústria necessitam de estudos para serem aproveitados na alimentação dos animais domésticos. Neste contexto, o resíduo de pré-limpeza de soja acumula-se em abundância nos pátios das usinas de beneficiamento e dos secadores das fazendas. Pesquisas direcionadas ao aproveitamento de subprodutos agroindustriais tem um papel ímpar no processo de gerar

tecnologias para esses produtos e benefícios na pecuária, dando um destino mais ecológico e social para esses resíduos (Nunes et al., 2007).

Entretanto, a caracterização dos alimentos de acordo com sua composição química e constituição de suas diferentes frações degradáveis ou não no rúmen é o grande objetivo dos nutricionistas para alcançar com êxito o balanceamento de rações que proporcionem nutrientes para o crescimento e desenvolvimento dos microrganismos do rúmen e para o animal (Oliveira et al., 2006). A soja foi inserida em todo Brasil porque assumiu uma importância muito grande para a economia e alimentação, conquistando todo o território, uma vez que o clima, a terra e a fertilidade promovem a sua ascensão nos estados que possui a agricultura como fator econômico (Hirakuri & Lazzaroto, 2014). A oleaginosa teve uma ótima adaptação no território brasileiro, sendo hoje um dos principais produtos de exportação (Conab, 2017). A disponibilidade de resíduos de soja chega a ser de aproximadamente 2% do total de grãos colhidos (Mello et al., 2004).

São avaliados por amostragem, na matéria-prima recebida, os seguintes parâmetros: teor de umidade, quantidade de material estranho e incidência de grãos quebrados, avariados e ardidos. Muitas impurezas, frequentemente, se misturam aos grãos. A eliminação da sujidade mais grossa antes do armazenamento na indústria é denominada pré-limpeza, máquinas especiais realizam essa seleção através de peneiras vibratórias e outros dispositivos, que separam os grãos dos resíduos (EMBRAPA SOJA, 2015).

O resíduo utilizado no experimento de Mello et al. (2004) consistia em grãos quebrados, grãos miúdos, grãos chochos, bandas, casca, tegumento e sementes de plantas daninhas, obtido no momento que se realiza a pré-limpeza dos grãos para o armazenamento. Da mesma forma como utilizamos nesse estudo, porém Mello et al. (2004) verificou a utilização desse subproduto como fonte proteica na alimentação de bovinos confinados, a análise bromatológica demonstrou níveis proteicos de 29,49%, esses níveis apresentam-se superiores ao encontrado no presente estudo, proteína bruta de 9,6%.

O problema com o uso desses resíduos é a grande variabilidade na sua composição bromatológica, o que dificulta o balanceamento nutricional das dietas (Thiago et al., 2003). Goes et al. (2008) avaliou a composição bromatológica e a degradabilidade ruminal de resíduos de pré-limpeza de soja na alimentação de ovinos, os autores explicam que existem diferentes subprodutos, pois são utilizadas diferentes peneiras para a separação dos grãos,

além disso expõem que esses subprodutos demonstraram baixa degradabilidade ruminal para matéria seca (MS), proteína bruta (PB) e fibra em detergente neutro (FDN), e concluem que a composição física dos resíduos influência na composição bromatológica, Sendo assim, é válido realçar que para serem utilizados na alimentação animal, análises preliminares devem ser realizadas.

Babilônia et al. (2000) utilizaram o resíduo úmido de pré-limpeza de soja tratado com ureia, ainda assim alertam que a composição dos resíduos é muito variável. Os autores apontam níveis de proteína bruta de até 33,50%, foram avaliados o consumo médio diário, a composição bromatológica, o ganho de peso médio diário e a relação custo/benefício. Como pode-se observar os trabalhos supracitados não verificaram questões relacionadas à saúde e o metabolismo, aos parâmetros sobre qualidade de carne e ao perfil lipídico perante a saúde dos consumidores.

Contudo, nosso trabalho propõe uma abordagem diferente acerca da utilização de resíduos de pré-limpeza soja na alimentação de cordeiros em terminação. Questões relacionadas a saúde e bem-estar dos animais serão abordadas, porém a avaliação da qualidade e do perfil lipídico da carne são os principais objetos de estudo. Visto que a produção de produtos derivados de animais está cada vez mais inclinada a saúde dos consumidores.

1.4.2 COLINA VEGETAL COMO ADITIVO NA NUTRIÇÃO ANIMAL

A colina é considerada um nutriente essencial, biologicamente importante, essa biomolécula é responsável por desempenhar funções no organismo como: síntese de fosfolipídios, membranas celulares e lipoproteínas, que atuam na integridade do organismo, no transporte e na quebra de gorduras, sendo a fosfotidilcolina o principal fosfolipídio das membranas biológicas. A colina também é fonte de doares de metil (via betaína), necessários para a metilação do DNA, RNA e proteínas. Além disso, realiza a síntese de neurotransmissores como a acetilcolina, fundamental para o funcionamento do sistema nervoso (Neill et al., 1978; Zeisel et al., 1991; Zeisel et al., 2003; Saeed et al., 2017).

A colina está presente em pequenas quantidades nos alimentos e pode ser sintetizada no próprio organismo (Zeisel et al., 2003). Estudos indicam que animais alimentados com uma dieta deficiente de colina podem desenvolver retardo no crescimento, disfunção renal, perda de hepatócitos, hemorragias ou anormalidades ósseas; pesquisas em humanos e

animais relatam que a colina pode prevenir fígado gorduroso (Saeed et al., 2017). Os benefícios da suplementação de colina ainda são pesquisados, para aves e suínos valores de referência já foram relatados e os resultados são satisfatórios com a utilização de colereto de colina, forma sintética (Rostagno et. al., 2005; Santos e Pereira, 2010; Farina, 2014). No entanto, o requerimento nutricional de colina para ruminantes não foi definido pelo NRC (2007), mesmo que sejam utilizadas fontes ricas de colina ou cloreto de colina nas dietas de ruminantes a biodisponibilidade da molécula para o animal é moderada, já que ocorre extensiva degradação ruminal de colina (Baldi e Pinotti 2006).

Por consequência disso, a colina ruminalmente protegica (RPC) foi desenvolvida e tem sido utilizada para ruminantes como fonte de suplementação. Bryant et al. (1999) realizaram experimentos com adição de RPC em novilhos e cordeiros, os pesquisadores apresentaram resultados instigantes quanto a melhora do desempenho dos novilhos, já os cordeiros não apresentaram efeito da suplementação quanto o desempenho, por isso os pesquisadores explicam que os níveis de utilização não foram consistentes. Estudos em bovinos verificaram que ao suplementar 30 g por dia de RPC há aumento na produção de leite e melhora nos parâmetros metabólitos do sangue (Xu et al., 2006). Segundo Pinotti et al (2009) a suplementação de 5 g por dia de RPC para bovinos de corte em terminação aumentou o peso corporal e o ganho médio diário, os autores afirmam que a suplementação de colina ruminalmente protegida pode melhorar o desempenho de crescimento dos bovinos de corte.

A suplementação de RPC em vacas leiteiras no período de transição auxilia na mobilização lipídica, melhora o balanço energético, aumenta o consumo voluntário e melhora o metabolismo hepático fortemente desafiado nesse período (Sun et al., 2016). Segundo Wu et al. (2014) mesmo com poucos estudos em ruminantes, em outras espécies há indícios que a suplementação com colina melhora a resistência a doenças, uma vez que há melhora da função imune e da capacidade antioxidante, pela diminuição dos fatores de necrose tumoral (Sun et al., 2016). A utilização de colina ruminalmente protegida trouxe uma nova alternativa de suplementação com resultados positivos, no entanto os resultados foram variados, dependentes da quantidade e da qualidade de colina biodisponivel para o ruminante, por isso novas fontes de colina estão sendo desenvolvidas.

Com o intuito de melhorar o desempenho zootécnico dos animais, aditivos são comumente utilizados na formulação das dietas. Compostos secundários de plantas estão sendo manipulados e testados, pesquisas apontam que esses apresentam benefícios à saúde dos animais, previnem ou tratam distúrbios, ou seja, reduzem o risco de doenças (Silva Sobrinho, 2014; Campos e Itaya, 2016). A biocolina vegetal (BV) possui colina na forma conjugada e pode ser utilizada para substituir por exemplo a colina ruminalmente protegida; é extraída de quatro plantas *Trachyspermum amni, Citrullus colocynthis, Achyranthus aspera e Azadirachta* (Godinez-Cruz et al., 2015; Rohr, 2018). A principal forma biodisponível para o ruminante na biomolécula é a fosfotidilcolina, que possui resistência natural a degradação ruminal. Godinez-Cruz et al. (2015) suplementaram biocolina vegetal para cordeiros em terminação e verificaram aumento no ganho de peso dos animais com apenas 4g de BV por animal por dia. Tanto em aves de corte quanto aves de postura a biocolina vegetal foi adicionada as dietas e pode substituir o cloreto de colina na dieta (Calderano et al., 2015; Mena-Bustamante, 2018). Estudos complementares em ruminantes devem ser realizados para ratificar esses resultados, a fim de assegurar que a biocolina vegetal é resistente a degradação ruminal e possui potencial de utilização auxiliando na saúde e no bem-estar dos animais.

1.5 OBJETIVOS

1.5.1 OBJETIVO GERAL

A presente dissertação teve como objetivo avaliar variáveis relacionadas ao desempenho zootécnico, saúde animal, metabolismo ruminal e qualidade da carne de ovinos expostos a dietas contendo ingredientes e aditivos alternativos em diferentes períodos: período de transição alimentar pré e pós desaleitamento, período de recria de borregas e período de terminação de cordeiros.

1.5.2 OBJETIVOS ESPECÍFICOS

Três objetivos específicos foram planejados distintamente:

1. Avaliar os efeitos da suplementação com biocolina vegetal sobre a bioquimica sérica, o status oxidante e antioxidante, a resposta imune e o desempenho zootécnico de cordeiros Lacaune no período de transição alimentar.
2. Verificar se a suplementação de biocolina vegetal para borregas Lacaune na recria influencia as análises sobre bioquimica sérica, status oxidante e antioxidante, resposta imune, produção de ácidos graxos voláteis no rumen e desempenho zootécnico.
3. Analisar os efeitos da substituição da silagem de sorgo por resíduo de pré-limpeza de soja sobre a qualidade da carne, o perfil lipido da carne e a bioquimica sérica do sangue de cordeiros Texel terminados em confinamento.

CAPÍTULO II

MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de três manuscritos, com sua formatação de acordo com as orientações das revistas ao quais foram submetidos:

2.1 MANUSCRITO I

Vegetable biocholine supplementation in lambs during the feed transition period improves health and enhances weight gain

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SUBMETIDO

Vegetable biocholine supplementation in lambs during the feed transition period improves health and enhances weight gain

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ABSTRACT

Lambs from dairy ewes are removed from their mothers within hours of ingesting colostrum; this influences their growth and development, especially when artificial milk has poor quality and/or is of insufficient volume. Alternatives have been proposed to assist lambs during this phase, including providing concentrate with additives, including choline, which is involved in various physiological processes. The objective of this study was to determine the effects of vegetal biocholine (VB) supplementation on growth, biochemistry and antioxidant responses in dairy lambs during the feed transition period. We used 40 Lacaune lambs in a randomized block design, distributed in four groups with five replications (two animals by replication). VB was added in the concentrate and was consumed between 30 days pre-weaning to 30 days post-weaning (total 60 days). The treatments were as follows: T0, without added vegetable biocholine, control group; T2, addition of 2 g VB per animal/day; T4, addition of 4 g VB per animal/day; T6, addition of 6 g VB per animal/day. Supplementation with VB increased the weight gain of T2 lambs compared to control; regression analysis suggested that optimal supplementation dose is 3.63 g VB/animal/day. After weaning, there were lower serum concentrations of creatine kinase enzyme in lambs supplemented with VB than in controls. No differences were observed with respect to levels of total protein, albumin, or globulins. Alanine aminotransferase enzyme activity was greater at T2 and T4 than at T0 and T6. Serum concentrations of glucose were greater in T2 and T4 lambs than in T0 and T6 lambs. In general, VB supplementation decreased serum levels of reactive oxygen species from day 15 of supplementation; these animals had lower levels of lipid peroxidation after weaning. The activities of glutathione S-transferase did not differ. These data suggest that VB supplementation increases weight gain and improves health, primarily by reducing free radical levels during the dietary transition period.

Keywords: Additives. Choline. Growth. Sheep.

1. Introduction

The Lacaune breed has a dual purpose: breeders are directed to milk production, while their (male) offspring are intended for meat production (Barillet et al., 2001; Figueira et al., 2018). The rearing phase of lambs is a critical period for production, because newborns

are separated from their mothers a few hours after birth (Monteiro et al., 2014). Lambs that do not receive nutritional alternatives to fill the breast milk deficit suffer high mortality rates in the first weeks of life (Silva et al., 2010). In general, greater weight gain is correlated with lower mortality rates; therefore, it is necessary to offer the best environment and nutrition conditions to avoid losses during this period, despite the fact that lambs are capable of compensatory weight gain (Bôas et al., 2003; Rosa et al., 2007; Godoy et al., 2017).

A farm's productive efficiency depends heavily on the production system; therefore, feed systems that provide improved nutrition should be considered to enhance growth performance (Ribeiro et al., 2006). Dairy farms adopt weaning systems after colostrum ingestion, and they offer artificial feeding until 45 to 60 days. The evidence suggests that bodily condition at weaning is more important than age; therefore, it is important to pay attention to newborn care, that is, the adequacy of nutritional management with complementary feeds (Monteiro et al., 2014). The initiation of solid feeds in lambs' diets accelerates rumen development, despite the fact that infant lambs cannot obtain energy from fiber fermentation; concentrates in this category are important (Silva Sobrinho, 2014). Concentrated additives can improve lamb growth and development, as feedlot decreases the possibility of feed selection. Powdered additives may enhance animal growth, particularly vegetable biocholine (VB), a choline source that differs from conventional feed additives.

Choline participates in several bodily functions; it ensures the integrity of cells and tissues (Zeisel et al., 1991; Saeed et al., 2017). Choline participates in the synthesis of phospholipids, neurotransmitters, triglyceride, and lipoprotein transport, as well as stimulating the immune system and antioxidant responses (Zeisel et al., 2009; Wu et al., 2014; Repetto et al., 2010). Research on choline supplementation in humans and animals has been carried out precisely because it possesses a range of physiological actions that impact health (Zeisel et al., 1991; Molano et al., 2017; Saeed et al., 2017). Some authors have quantified the concentration of choline in feeds and found that the available quantities are small (Neill et al., 1978; Zeisel et al., 2003; Baldi and Pinotti 2006). Choline is degraded in the rumen (Sharma and Erdman et al., 1989; Bindel et al., 2000) and ruminally protected choline has positive effects on milk production; the molecule minimized the negative energy balance suffered by lactating animals in the transition period (Pinotti et al., 2005; Aires et al., 2016). In dairy cows, rumen-protected choline supplementation improved metabolic profiles,

decreased triglyceride and cholesterol levels, increased immunity with emphasis on stimulation of interleukin production, and decreased free radical levels that cause oxidative stress (Hartwell et al., 2000; Pinotti et al., 2003; Ardalan et al., 2010; Sun et al., 2016).

A new source of choline extracted from plants, known as vegetable biocholine (VB), is rich in phosphatidylcholine. Some researchers suggest that this molecule has natural resistance to ruminal degradation, i.e., bioavailability would increase for dairy cows and lambs (Rodríguez-Guerrero et al., 2018; Valencia Narváez, 2019). Although the NRC (2001) did not determine the nutritional requirement for choline for ruminants, studies suggest that growing and developing animals have greater requirements for the molecule (Al-Ali et al., 1985; Zeisel et al., 2006; Pinotti et al., 2009). We are unaware of studies evaluating the effects of VB supplementation on growth, biochemistry and antioxidant response of dairy lambs during the feed transition. Our hypothesis is that, for dairy lambs, strategic supplementation with VB before and after weaning would stimulate immune responses, with antioxidant and hepatoprotective effects, consequently favoring growth. For these reasons, the objective of this study was to determine the effects of VB supplementation on growth, biochemistry, and antioxidant responses of dairy lambs during the feed transition period.

2. Materials and Methods

2.1. Vegetable biocholine

Phosphatidylcholine was extracted from the plants *Azadirachta indica*, *Citrullus colocynthis*, *Trachyspermum ammi*, and *Achyranthes aspera*. The commercial product is known as Biocholine Powder® (VB). Guaranteed levels of total phosphatidylcholine (natural choline conjugates) are 16 g/kg VB. A sample of this additive was sent to a specialized laboratory for actual quantification of phosphatidylcholine in VB using high-performance thin-layer chromatography (Kupke and Zeugner 1978); we found that the levels of total phosphatidylcholine were 16.8 g/kg.

The chemical composition of the commercial product (VB) was evaluated: 92.6% dry matter, 9.77% crude protein, 5.72% ether extract, 36.4% acid detergent fiber, and 45.1% neutral detergent fiber.

2.2. Animals and experimental design

The experiment was carried out on a sheep farm in Chapecó (southern Brazil). The project was approved by the Animal Experimentation Ethics Committee (CEUA/UDESC), protocol number: 8560130319.

After birth, the animals were adapted to the premises and received sheep's milk for 10 days (500 mL/animal/day). Between days 11 to 14, there was a period of adaptation of milk (natural to artificial), the same being provided in the first 2 days in the proportion of 50:50, and in the last two days in the proportion 30% natural and 70% replacement milk (Desmamelac®). All this food and care management in the first 15 days of the experiment was done by the farmer.

To start the experiment, we selected 40 male Lacaune lambs at 15-days of age. All animals were clinically evaluated at day 15, when they were also vaccinated against clostridiosis and preventively medicated for coccidiosis. All were deemed clinically healthy prior to the start of the experiment. The animals were organized in a completely randomized design with four treatments and five replications, with two animals per replication. During the 60 days of experiment (30 days pre-weaning and 30 days post-weaning) the animals were housed in covered stalls (two lambs per pen), equipped with drinking water and collective feeders.

The lambs were nursed between days 15 to 45 of age, corresponding to the first 30 days of the experiment. Replacement milk (Desmamelac®) was offered twice daily to all experimental animals (0800h and 1600h), divided in a volume of 500 mL/animal/day. 200 g of concentrate was made available daily to the lambs for the first 30 days of the experiment. The available feed intake was 100% in this production phase.

Between the 45th and 52nd day of age, the lambs passed through an adaptation period of feeding (i.e., weaning, when the volume of replacement milk was reduced to 250 mL/animal/morning period). During this adaptation period, 400 g of concentrate was made available daily to the lambs (twice a day), as well as 400 g of silage/animal/day (once per day in the afternoon). During this adaptation period, food intake was 100%.

Between days 53 and 75 of age, the amount of feed offered was based on the weekly body weight of the animals to calculate the amount of concentrate and silage offered. The animals were offered a quantity of feed (dry matter) to standardize to 4.5% of body weight in a ratio of 60:40 (silage: concentrate). The total amount of feed supplied changed weekly.

Food consumption was evaluated only in the three final days of the experiment (days 73, 74 and 75). All groups consumed 100% of the available concentrate. The silage consumption was also similar between the groups, that is, 92.6% (T0), 94.0% (T2), 90.7% (T4) and 93.1% (T6).

VB was added to the concentrate at various levels and was consumed by the lambs for 60 days (30 days before and 30 post-weaning). The treatments were as follows: T0, without added vegetable biocholine, control group; T2, addition of 2 g VB per animal/day; T4, addition of 4 g VB per animal/day; T6, addition of 6 g VB per animal/day. During the experimental period, the amount of concentrate differed with the production phase of the lambs and the body weight of the animal; nevertheless, it is important to note that the level of VB in the concentrate was adjusted according to daily consumption of concentrate, ensuring that the animals ingested 0, 2, 4, and 6 g/lamb/day.

2.3. Analysis of the chemical composition of feed

The feed (concentrate and silage) was collected, frozen and subsequently lyophilized and analyzed for percentage of dry matter (DM), ash, ether extract (EE), crude protein (CP) and neutral detergent fiber (NDF) (AOAC 2000; 2005). The results are displayed in Table 1.

2.4. Growth performance

Animal performance was determined by measurements of the body weight on days 0, 30 and 60 of the experiment. Using an analytical digital scale, all animals were weighed individually. Weight gain and daily average weight gain were calculated.

2.5. Blood sample collection

Blood was collected from the jugular veins into vacuum tubes without anticoagulant on days 0, 15, 30, 45 and 60 of the experiment. It is important to make clear that day 0 corresponds to 15 days of lamb age; day 30 corresponds to the weaning date; and day 60 corresponds to day 75 of life. The collected samples were placed in styrofoam boxes at 10 °C. Upon arrival at the laboratory, the tubes were immediately centrifuged at 3,500 *g* for 10 minutes. Samples were properly identified and stored in a freezer (–21 ºC) until the time of analysis.

2.6. Serum biochemistries

To evaluate metabolic profiles, we performed biochemical analyses. When thawing serum at room temperature, we used a semi-automatic Bio-2000 (BioPlus®) and commercial kits (Analisa®) to determine serum levels of total protein (TP), albumin, globulin, glucose, urea, alanine aminotransferase (ALT), and creatinine kinase (CK). Globulin levels were calculated by difference between total protein and albumin levels.

2.7. Lipid peroxidation (TBARS)

Lipid peroxidation was measured in serum samples by measuring malondialdehyde (MDA) formation as described by Jentzsch et al. (1996). When heated, MDA reacts with thiobarbituric acid (TBA) to form a pink complex. We incubated 200 µl of the sample in a 90 °C water bath for 45 min in medium containing distilled water (550 µl), 0.2 M orthophosphoric acid (1 mL) and 0.1 M TBA (250 µl). Absorbances were measured at 532 nm.

2.8. Reactive species (RS)

Determination of 2´-7´-dichlorofluorescein (DCFH) levels were determined as an index of the peroxide production by the cellular components, i.e. levels of reactive species. This experimental method of analysis is based on the deacetylation of the probe DCFH-DA, and its subsequent oxidation by reactive species to DCFH, a highly fluorescent compound (Halliwell and Gutteridge 2007). Serum (10 µL) was added to a medium containing Tris–HCl buffer (10 mM; pH 7.4) and DCFH-DA (1 mM). After DCFH-DA addition, the medium was incubated in the dark for 1 h until the start of fluorescence measurement procedure (excitation at 488 nm and emission at 525 nm, and both slit widths = 1.5 nm). The results were expressed as U DCF/mg protein.

2.9 Glutathione S-transferase (GST)

GST activity was assayed spectrophotometrically at 340 nm using the method of Habig et al. (1974). The mixture contained serum to be tested, 0.1 M potassium phosphate

buffer (pH 7.4), 100 mM GSH and 100 mM CDNB used as substrate. The enzymatic activity was expressed as μmol CDNB/min/mg protein.

2.10. Statistical analysis

All dependent variables were tested for normality using the Univariate procedure in SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all variables were normally distributed. All data were then analyzed using MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Weight gain and average daily gain were tested for fixed effect of treatment using animal (treatment) and animal (pen) as random effects. All other variables were analyzed as repeated measures and tested for fixed effects of treatment, day, and treatment × day, using animal (treatment) and animal (pen) as random variables and animal (treatment) as subject. The results of the day 0 for each variable were included as covariates in each respective analysis; but if they have $P > 0.10$ for removed from the covariates we removed the day 0 from the model. The compound symmetric covariance structure was selected for concentration of ALT and albumin and the first order autoregressive covariance structure was selected for all other variables. The covariance structures were selected according to the lowest Akaike information criterion. Weight gain data were also subjected to regression analysis to determine the ideal VB concentration for lamb diets. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined as $P \leq 0.05$, and tendency was defined as $P > 0.05$ and ≤ 0.10.

3. Results

3.1. Growth performance

The results of growth performance are presented in Table 2. Effects of treatment × day ($P = 0.05$) were detected for body weight. T2 and T4 lambs had greater body weight than T0 lambs. Effects of treatment were detected for weight gain and average daily gain. T2, T4, and T6 lambs, had greater gain from d 0 to 60, compared to T0 lambs. By simple regression from treatment and weight gain (d 0 to 60), the linear ($P = 0.03$; $R^2 = 0.09$), quadratic ($P = 0.005$; $R^2 = 0.210$) and root ($P = 0.002$; $R^2 = 0.250$) effects were evaluated. Regression

analysis revealed that the optimal concentration of biocholine was 3.63 g/kg of feed (Figure 1).

3.2. Serum clinical biochemistry: liver function and metabolism

The biochemical profiles are presented in Table 3. Effects of treatment × day showed a tendency (P = 0.10) while significant effects of treatment were detected (P = 0.02) for serum activity of ALT, and T2 and T4 lambs, with greater concentration on d 30 than in T0 and T6 lambs. Effects of treatment × day were detected for serum activity of CK ($P \leq 0.05$). The T2, T4, and T6 lambs had lower activity of CK on days 45 and 60 than did T0 lambs; T4 lambs had the lowest concentration on d 45, compared to the others. Effects of treatment × day (P = 0.01) were detected for serum concentrations of glucose. T2 and T4 lambs showed higher glucose concentrations on d 15 than did T0 and T6 lambs. However, no effects of treatment × day and treatment were detected for serum concentrations of total protein, albumin, globulin or urea.

3.4. Serum oxidant and antioxidant status

The results of serum oxidant and antioxidant status are presented in Figure 2. Effects of treatment × day (P = 0.01) were detected for serum concentration of TBARS. The T6 animals had lower concentrations on d 45 than did T0 animals, and T4 and T6 animals had lower concentrations on d 60 than did T0 animals. Effects of treatment × day (P = 0.01) were detected for serum concentration of ROS. The T2 and T4 lambs had lower concentrations on days 15 and 30, than did T0 and T6 lambs. The T4 and T6 lambs had lower concentrations on d 45, than did T0 animals. The T2, T4 and T6 lambs had lower concentrations on d 60 than did T0 lambs. However, no effects of treatment × day or treatment were detected for serum concentration of GST.

4. Discussion

The VB supplementation increased weight gain during the experimental period (days 0 to 60) in the group of animals that consumed 2 g VB/animal/day. By contrast, Al-Ali et al. (1985) found that addition of choline chloride to the feed of growing lambs did not give rise to significant differences in animal weight gain. These authors stated that the first two weeks

of animal life are critical; nevertheless, after this period, endogenous choline synthesis appears to be adequate to meet bodily requirements. This discrepancy may be explained by the source of choline used, because we used different molecules; our VB has low ruminal degradability. Similarly, Bryant et al. (1999) found better performance of ruminally-protected choline supplemented steers and lambs related to changes in lipid metabolism and metabolic hormones responsible for fat metabolism.

Lower activity of CK were observed in our study in lambs that consumed VB after weaning. CK acts as a low-threshold sensor for ADP and it has some control over glycolytic flow (Wallimann et al., 1992). Some researchers suggest that choline supplementation improves energy metabolism by making more glucose available for cells (Xu et al., 2005; Rodríguez Guerrero et al., 2018). Taken together, the findings suggest that there was little need to activate anaerobic cell metabolism for ATP production, consequently decreasing the need for CK enzyme action because of greater availability of cellular glucose for ATP production.

In general, the groups of lambs supplemented with VB presented lower levels of free radicals and TBARS; this is desirable as it indicates lower lipid peroxidation. Choline and phosphatidylcholine possess substantial antioxidant potential (Baldissera et al., 2019), possibly explaining the reduction of oxidant profiles in sera of VB-supplemented animals. Studies in rats suggested that choline deficiency led to increased lipid peroxidation and oxidative stress (Ossani et al., 2007; Bagnyukova et al., 2008; Saeed et al., 2017). Therefore, by supplementing choline, it is possible to establish balance in the mechanism of cellular homeostasis; as seen our results by decreasing RS starting at the beginning of the experiment. Studies using VB as additive in fish feed gave results similar to ours, with emphasis on antioxidant stimulation and consequent reduction of oxidation (Zhao et al., 2016; Baldissera et al., 2019; Souza et al., 2020). In ruminants, there are few studies with VB supplementation. There are no studies regarding oxidant and antioxidant status information; therefore, to the best of our knowledge, this is the first study to document these effects.

We found that ALT levels were greater in T2 and T4 than in T0 and T6. According to Saeed et al. (2017) ALT activity is associated with liver health; choline is a lipotropic nutrient that may prevent liver disorders. Salman et al. (2017) used ruminally protected choline for cows during the transition period; they observed differences in ALT levels

between the treated groups and emphasized the need for complementary studies to clarify the effect of choline in ruminants with known metabolic profiles. It is noteworthy that these are animals of different species and categories; nevertheless, our results were similar to those obtained by Salman et al. (2017); these researchers also observed increased ALT activity in the 60 g ruminally protected choline group. However, the increase of this enzyme was associated with choline deficiency (Zeisel et al., 1991). According to the literature, long-term choline deficiencies may result in liver dysfunction. This finding may indicate that there had been an increase in choline requirements by animals. Nevertheless, choline requirements for ruminants have not yet been defined (NRC, 2001).

5. Conclusion

Vegetable biocholine supplementation improved lamb growth. Regression analysis revealed that the ideal dose was 3.63 g VB/animal/day. There was a decrease in free radicals and lipid peroxidation in lambs that consumed VB; this may be related to antioxidant stimulation already described in several studies with VB. Taken together, the data suggest that VB was beneficial for lambs during the feed transition period, improving antioxidant responses and weight gain.

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Table 1: Chemical composition of feed used in the diet of lambs supplemented with vegetable biocholine.

Chemical composition (%)	**Silage**	**Concentrates[1]**			
		T0	**T2**	**T4**	**T6**
Dry matter	32.92	87.84	87.16	88.25	88.38
Ash	4.21	7.25	7.6	7.13	6.98
Crude protein	8.01	19.7	18.32	18.04	18.6
Ether extract	4.34	3.52	3.54	3.58	3.61
NDF	33.03	12.32	12.56	12.57	11.91
ADF	17.8	3.94	3.81	3.94	3.67

Note: 1 Total of 100 kg concentrate base was formulated with corn (70%), soybean meal (25%) and premix (5%). The mineral and vitamin premix used in this study contained phosphorus (min 55 g/kg), calcium (215 g/kg, max 225 g/kg), sulfur (min 12 g/kg), sodium (min 80 g/kg), cobalt (min 60 mg/kg) chromium (min 12 mg/kg), iron (1420 mg/kg), iodine (min 14 mg/kg), magnesium (min 14 mg/kg), manganese (min 1550 mg/kg), selenium (min 22 mg/kg), vitamin A (2000 IU/kg), vitamin D (min 40000 IU/kg), vitamin E (min 550 IU/kg) and fluorine (max 550 mg/kg). The milk substitute contained: moisture (max 70 g/kg), crude protein (min 200 g/kg), lactose (min 260 g /kg), ethereal extract (min 100 g/kg), fibrous matter (max 15 g/kg) kg), mineral matter (max 82 g/kg), ADF (max 4,000 mg/kg), phosphorus (min 7,000 mg/kg), calcium (min 10 g/kg, max 11 g/kg), cobalt (min 1.20 mg/kg), copper (min 8.50 mg/kg), sulfur (min 4,500 mg/kg), iodine (min 2.5 mg/kg), iron (min 150 mg/kg), magnesium (min 650 mg/kg), manganese (min 32 mg/kg), selenium (min 2.50 mg/kg), zinc (min 125 mg/kg), nicotinic acid (min 30 mg/kg), pantothenic acid (min 14.50 mg/kg), vitamin A (min 70,000.00 IU/kg), vitamin B1 (min 5 mg/kg), vitamin B2 (min 12 mg/kg), vitamin B6 (min 7 mg/kg), vitamin B12 (min 80 mg/kg), vitamin D (min 14,000.00 IU/kg), vitamin E (min 150 IU/kg), vitamin K3 (min 6 mg/kg), choline (min 600 mg/kg), BHT (min 600 mg/kg), beta-glucans (min 3,400.00 mg/kg), glucomannans (min 4,771.00 mg/kg), mannan oligosaccharide (min 1,363.00 mg/kg), *Bacillus licheniformis* (min 3.63 x 10^x CFU/kg) and *Bacillus subtilis* (min 3.63 x 10^x CFU/kg).

Table 2: Growth performance of lambs supplemented with biocholine concentrate during the feed transition period [30 days pre-weaning (d 0) until 30 days after weaning (d 60)].

Variables	Treatments[1]				SEM	*P*-value	
	T0	T2	T4	T6		Treat	Treat × day
Body weight, kg						0.70	0.05
d 0	6.71	6.81	6.86	6.85	0.62		
d 30	15.56	15.59	15.52	16.25	0.62		
d 60	20.83^{b}	22.88^{a}	22.44^{a}	22.24^{ab}	0.62		
Weight gain, kg							
d 0 to 30	8.86	8.78	8.66	9.40	0.70	0.88	
d 30 to 60	5.27	7.30	6.92	5.99	0.79	0.29	
d 0 to 60	14.13^{b}	16.07^{a}	15.58^{a}	15.39^{a}	0.38	0.01	
Average daily gain, kg							
d 0 to 30	0.295	0.294	0.289	0.313	0.02	0.88	
d 30 to 60	0.176	0.243	0.231	0.200	0.03	0.29	
d 0 to 60	0.235^{b}	0.268^{a}	0.260^{a}	0.256^{a}	0.01	0.01	

[1]The treatments T0, T2, T4 and T6 represents 0, 2, 4 and 6 g of biocholine per animal/day, respectively. $^{a\text{-}b}$Differs ($P \leq 0.05$) between treatments.

Table 3: Biochemical profile of lambs supplemented with biocholine concentrate during the feed transition period [30 days pre-weaning (d 0) until 30 days after weaning (d 60)].

Variables[1]	Treatments[2]				SEM	*P*-value	
	T0	T2	T4	T6		Treat	Treat × day
ALT (U/L)						0.02	0.10
d 0	74.04	75.32	79.23	73.12	10.69		
d 15	100.80	103.49	104.90	89.32	10.69		
d 30	85.44^{b}	152.95^{a}	145.73^{a}	102.22^{b}	11.69		
d 45	73.32	80.78	91.07	103.59	10.67		
d 60	93.84	111.49	113.40	112.52	11.69		
CK (U/L)						0.05	0.01
d 0	86.33	93.55	102.17	101.00	25.13		
d 15	80.60	88.06	106.30	124.43	25.13		
d 30	146.63	123.02	193.65	160.46	25.13		
d 45	316.83^{a}	219.26^{b}	153.28^{bc}	164.40^{c}	28.09		
d 60	285.68^{a}	181.19^{b}	186.30^{b}	159.45^{b}	28.10		
Glucose (mg/dL)						0.51	0.01
d 0	84.84	90.56	92.01	93.21	5.84		
d 15	93.94^{b}	117.22^{a}	124.67^{a}	96.54^{b}	6.49		
d 30	44.44	47.39	48.84	44.21	5.84		
d 45	64.64	53.06	55.01	51.54	5.84		
d 60	61.39	53.06	47.34	48.21	6.49		
Total protein (g/dL)	7.17	6.84	6.97	7.65	0.33	0.26	0.66
Albumin (g/dL)	2.73	2.87	2.75	2.76	0.10	0.76	0.58
Globulin (g/dL)	4.48	3.98	4.30	4.84	0.26	0.12	0.42
Urea (mg/dL)	36.43	37.91	39.48	39.79	2.94	0.82	0.70

[1] ALT, alanine aminotransferase and CK, creatinine kinase.

[2]The treatments T0, T2, T4 and T6 represents 0, 2, 4 and 6 g of biocholine per animal/day, respectively. [a-b]Differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.

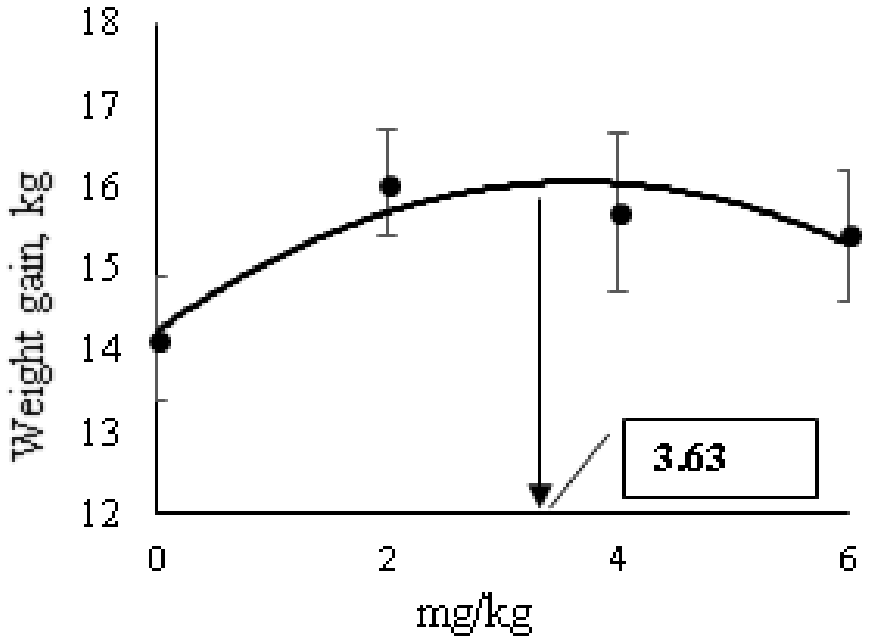

Figure 1: Simple regression analysis to verify the optimal point of vegetable biocholine supplementation have a quadratic effect to weight gain (WG) during experimental period (day 1 to 60) [$WG = 14.135 + 2.38048X - 0.771116X^2$ (R^2=0.369)].

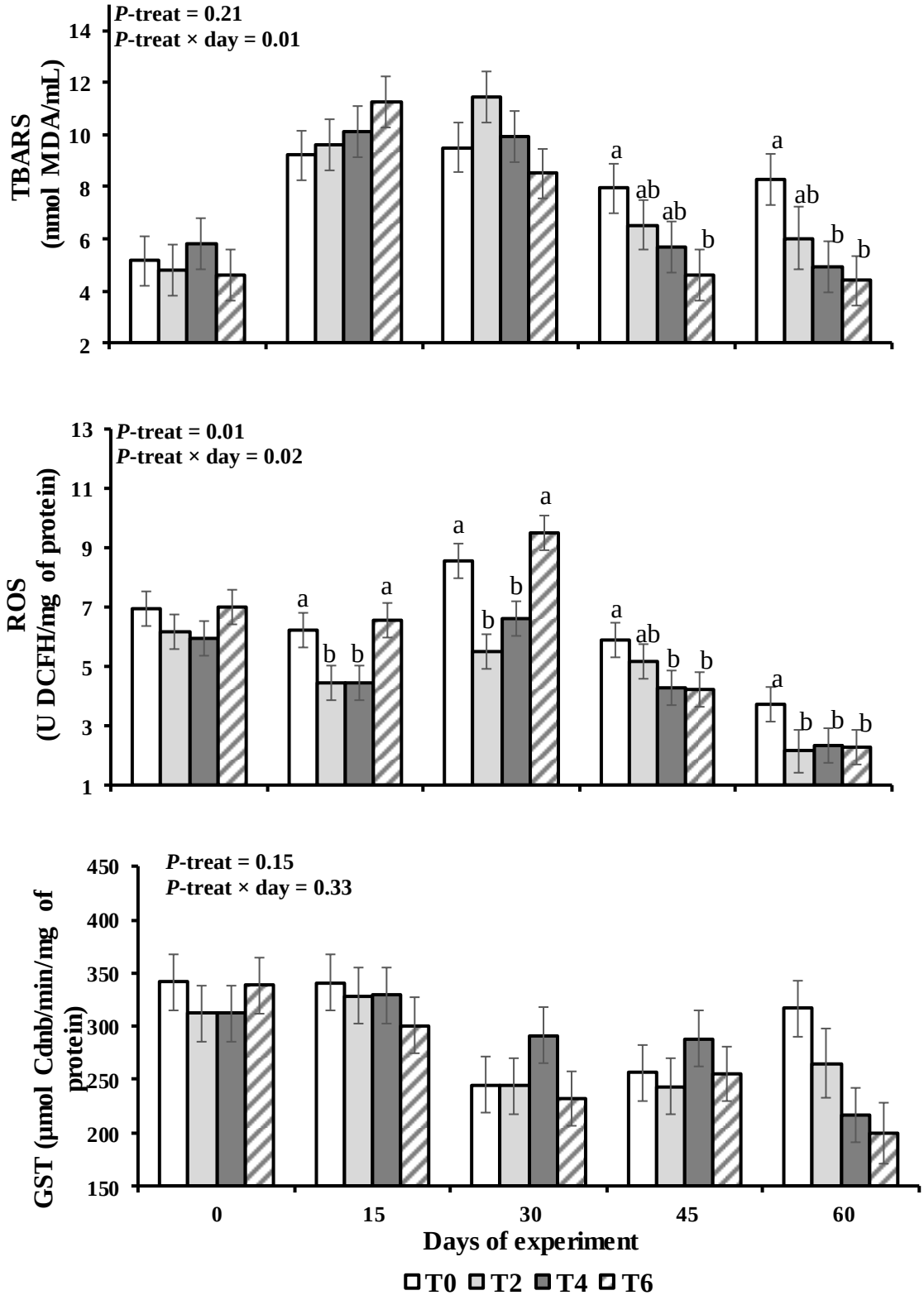
P-treat = 0.21
P-treat × day = 0.01
TBARS (nmol MDA/mL)
14
12
10
8
6
4
2
a
ab
ab
b
a
ab
b
b
P-treat = 0.01
P-treat × day = 0.02
ROS (U DCFH/mg of protein)
13
11
9
7
5
3
1
a
b
b
a
a
b
b
a
a
ab
b
b
a
b
b
b
P-treat = 0.15
P-treat × day = 0.33
GST (µmol Cdnb/min/mg of protein)
450
400
350
300
250
200
150
0
15
30
45
60
Days of experiment
T0
T2
T4
T6

Figure 2: Lipid peroxidation (TBARS), reactive oxygen species (ROS) and glutathione S-transferase (GST) activity in serum of lambs supplemented with biocholine concentrate during the feed transition period [30 days pre-weaning (d 0) until 30 days after weaning (d 60)]. The treatments T0, T2, T4 and T6 represents 0, 2, 4 and 6 g of biocholine per animal/day, respectively. $^{a\text{-}b}$Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM.

2.2 MANUSCRITO II

The effects of biocholine supplementation in young female sheep: performance, volatile fatty acid concentration in ruminal fluid, antioxidant status and metabolism

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The effects of biocholine supplementation in young female sheep: performance, volatile fatty acid concentration in ruminal fluid, antioxidant status and metabolism

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ABSTRACT

The aim of this study was to determine whether supplementation with vegetable biocholine (VB) in the feed of young sheep would improve zootechnical performance, ruminal volatile fatty acid profiles, antioxidant status, and liver health, as well as carbohydrate and protein metabolism. We randomly allocated 48 4-month-old female Lacaune lambs into three groups with 16 replications; each animal was considered an experimental unit: control group (T0: no added VB), T4 with 4 g VB per animal/day; T8 with 8 g VB per animal/day. VB was homogenized in concentrate and offered to animals once a day for 75 days. T8 animals showed greater weight gain in the first 30 days of the experiment (days 1 to 30); a positive result that was not observed in subsequent evaluations. Ruminal microbial activity was reduced in lambs consuming VB. In general, we found lower percentages of short chain fatty acids (acetate, propionate and butyrate) in the ruminal fluid of animals supplemented with VB. With biocholine supplementation, there were greater serum activities of superoxide dismutase (days 15, 60 and 75) and glutathione S-transferase (day 75) than in the control group. Intake of VB by young animals reduced levels of aspartate aminotransferase (days 60 and 75) and gamma glutamyltransferase (day 75). Similar behavior was observed for serum glucose levels (days 15 and 75). Overall protein levels (days 45, 60 and 75) and globulin levels (days 15, 30, 45, 60 and 75) were higher in lambs consuming VB than in controls. These data suggest that supplementation with VB in growing lambs improved health, manifested as hepatoprotective, antioxidant and immunological effects. By contrast, biocholine supplementation interfered with the ruminal microbiota and reduced the total percentage of short chain fatty acids. These changes did not affect weight gain in animals that consumed VB over the long-term

Keywords: Sheep. Nutrition. Supplements. Vegetable Choline.

1. Introduction

Lamb rearing systems have direct impacts on parameters related to reproductive efficiency. On milk production farms, intensive feeding systems of young animals ensures their growth and development, as these animals require greater care. Monteiro et al. (2014) found that animals undergoing early weaning required intensive feeding, including

interventions that could be carried out in sheds or in pasture. Simplicio and Maia (2014) reported that body development and the age of puberty in lambs depend on the environment, genetics and gender; nevertheless, these events are strongly influenced by the production system, nutrition and feed.

Females who enter puberty before achieving minimum body weight may have diminished body development, resulting in smaller offspring. Simplício and Maia (2014) pointed out that this happens when management, feeding, nutrition and health care are deficient. Because weaning occurs after ingestion of colostrum, a common practice in milk sheep production, newborns begin to receive artificial breastfeeding when weaning occurs, up to 60 days of age (Monteiro et al., 2014). Therefore, it is necessary, in addition to providing appropriate feeding systems, to use strategic supplementation to enhance animal performance. According to Silva Sobrinho (2014), on most farms, there are deficiencies of essential nutrients attributable to the lack of commercial products formulated specifically for sheep. Therefore, is important to provide supplements so as to minimize these negative effects. Researchers have suggested, for example, supplementation with minerals, vitamins or herbal extracts (Mendoza-Martínez et al., 2018).

Silva Sobrinho (2014) suggested that dietary supplements may alter the digestibility and/or the consumption of roughage. An important ingredient in animal diet is choline, generally available to animals as choline chloride (Xu et al., 2005). Choline is essential for maintenance of various physiological functions, including metabolism, maintenance and cell integrity (Zeisel et al., 1991). Deficiencies contribute to heart disease, growth and bone development abnormalities, as well as compromised liver and kidney functions (Saeed et al., 2017). For ruminants, choline is degraded in the rumen; therefore, it is necessary to provide protected choline (Atkins et al., 1988; Savoini et al., 2010).

The bioavailability of choline is influenced by dose, mode of administration, stage of animal development, and dietary composition (Baldi and Pinotti, 2006). In concentrate, choline is present in the form of lecithin; in compound and commercial feed, choline chloride is added as an alternative but is easily degraded, resulting in low bioavailability for intestinal absorption (Baldi and Pinotti, 2006). Recent studies have shown good results with vegetable biocholine (VB) (Demattê Filho et al., 2015; Koujalagi et al., 2017; Rohr et al., 2018; Souza et al., 2020).

Vegetable biocholine is extracted from plants; it has natural resistance to ruminal degradation in the form of phosphatidylcholine (Valencia Narváez, 2019). Nevertheless, information about its ruminal fermentation is scarce. In the 1970s, researchers observed that choline chloride was rapidly metabolized, leaving doubts as to its true influence on bacterial growth mechanisms (Neill et al., 1978). Vegetable biocholine needs to be further explored, especially with respect to interactions at the ruminal level and to influences on fermentation products as well as consequent short and/or long-term effects on animal performance.

Therefore, our objective was to determine whether VB supplementation in young ovine diets would improve performance, influence ruminal volatile fatty acid profiles, and alter antioxidant status and liver health, as well as carbohydrate and protein metabolism in these animals.

2. Materials and Methods

2.1. Vegetable biocholine (VB)

Biocholine Powder® is the trade name of a phosphatidylcholine product, a molecule extracted from the plants *Azadirachta indica*, *Citrullus colocynthis*, *Trachyspermum ammi* and *Achyranthes aspera*. Importantly, the total phosphatidylcholine level is 16 g/kg of VB.

The chemical composition of the commercial product (VB) was chemically evaluated: 92.6% dry matter, 9.77% crude protein, 5.72% ether extract, 36.4% acid detergent fiber, and 45.1% neutral detergent fiber.

2.2. Animals and experimental design

The experiment was carried out in a dairy farm located in Chapecó, SC, southern Brazil. After approval by the Animal Experimentation Ethics Committee (CEUA/UDESC), protocol 8560130319, we selected the animals as experimental unit.

Forty-eight female Lacaune lambs up to 120 days of age were used. All animals were clinically evaluated and found to be apparently healthy. The study animals were housed in a covered sheepfold, distributed in three stalls, with total area of 90 m², outfitted with drinkers and collective feeders. The experimental period was 75 days and the animals were distributed in a completely randomized design with three treatments and 16 replications per treatment, i.e., each animal was considered an experimental unit. The diet was composed of ad libitum

corn silage and concentrate (Table 1) that was fed 400 g/animal/day, twice a day (0700h and 1700h).

In the concentrate increasing levels of VB were used: Control group had no VB in the concentrate (T0); group T4 received concentrate with 10 g/kg (corresponding to consumption of 4 g VB/animal/day); group T8, received concentrate with 20 g/kg (corresponding to consumption of 8 g VB/animal/day).

2.3. Analysis of the chemical composition of feed

Silage and concentrated feed were sampled, frozen and analyzed for centesimal composition (Table 1). The methodology described by Silva and Queiroz (2002) was followed to determine percentages of dry matter (DM), mineral matter (MM), ether extract (EE), crude protein (CP) and neutral detergent fiber (NDF).

2.4. Body weight and weight gain

Animal performance was evaluated by measuring body weight on days 1, 15, 30, 45, 60, and 75 of the experiment. All animals were weighed individually on an analytical digital scale. Weight gain (kg) and daily weight gain (g) were calculated based on the body weight of each animal.

2.5. Sample collection

Blood samples were collected from jugular veins of eight animals per treatment, using vacuum tubes without anticoagulant to obtain serum on days: 1, 15, 30, 45, 60 and 75 of the experiment. Immediately after collection, the tubes were refrigerated at 10 °C, then centrifuged at 5,100 RPM for 10 minutes. The serum was collected, identified and stored frozen (–20 °C) until analysis.

Ruminal fluid was collected two hours after feeding in the morning on the 15th, 45th and 75th day of the experiment, using a 1.5-m, 11-mm diameter oro-rumen tube. The first 50 mL were discarded for possible salivary contamination, as previously described (Borges et al., 2002; Gonçalves et al., 2003; Vieira et al. 2007; Rodrigues et al., 2013; Furtado et al., 2014). A total of 200 mL of ruminal fluid were collected per animal, stored in two 200 mL glass Becker® containers. We measured pH using a portable digital pH meter, model AK103.

One part of the sample was used for the methylene blue reduction test to identify microbial activity (Vieira et al., 2007).

The remaining ruminal fluid was filtered through gauze and stored in two 50 mL Falcon® tubes per sample in a thermal box, previously heated with water at 39 °C. The tubes were then frozen at –20 °C for further analysis of short chain fatty acids.

2.7. Analysis of ruminal short chain fatty acids

The short fatty acids (SFA) acetic acid, propionic acid and butyric acid were determined in ruminal fluid. After thawing in a water bath (final temperature 10 °C), samples were centrifuged at 1050 × *g* for 5 min. We transferred 1 mL of supernatants to 2 mL polypropylene tubes and added 100 μL of citric acid (1.0 mol L^{-1}). Subsequently, the contents were homogenized for 30 s in a vortex shaker, followed by a centrifugation for 5 min at 17000 × *g*. Then, 100 μL of supernatants were diluted in 900 μL of methanol and added to 100 μL of an ethyl hexanoate internal standard (8.7 mg mL^{-1} in methanol).

Samples were analyzed in a gas chromatograph equipped with a flame ionization detector (GC-FID; Varian Star 3400CX, Chrompack, Middelburg) and an auto sampler system. A total of 1 μL of extract were injected in a split/splitless injector operated in splitless mode for 1 min (the splitter-valve was open 20:1). Hydrogen was used as a carrier gas, at a constant pressure of 25 psi. Analyte separation was carried out on a capillary column CP-WAX 52 CB (60 m × 0.25 mm × 0.25 μm). The initial temperature was adjusted to 50 °C for 1 min, and increase up to 185 °C at 15 °C min^{-1}, and increasing at 5 °C min^{-1} until 195 °C. Injector and detector temperatures were constant at 240 °C.

Method validation was carried out according to the Eurachem Guide (Magnusson and Örnemark, 2014) with the following parameters: selectivity, linearity, linear range, repeatability, accuracy and limited of detection (LOD) and limit of quantification (LOQ). Linearity was evaluated by calculating a regression equation using the least-squares method. The analytical calibration curves are displayed Supplementary Material 1. The LOD and LOQ values were obtained by sequential dilution until obtaining signal-to-noise ratios of 3:1 and 10:1, respectively. Precision was assessed by evaluating the repeatability of six replicate analyses. Accuracy was determined by recovering known amounts of standard substances

added to the samples. The results were expressed as mmol of each SFA per 100 mL^{-1} rumen fluid.

2.8 Serum clinical biochemistry

We performed blood tests to monitor metabolic adaptation and to diagnose possible metabolic-nutritional imbalances. Serum levels of total protein (TP), albumin, glucose and urea were quantified using a Bio-2000 semi-automatic analyzer (BioPlus®) and commercial kits (Analisa®). Globulin values were calculated as the difference between TP and albumin. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) were measured using commercial kits for the Bio-2000.

2.9 Oxidant status and antioxidant enzymes

Glutathione S-transferase (GST) activity was assayed spectrophotometrically at 340 nm using the method of Habig et al. (1974). The mixture contained serum as test, 0.1 M potassium phosphate buffer (pH 7.4), 100 mM GSH and 100 mM CDNB, which was used as the substrate. The enzymatic activity was expressed as μmol CDNB/min/mg protein.

The antioxidant enzyme superoxide dismutase (SOD) activity were assayed in serum by measuring the inhibition of 1 mM adrenaline auto-oxidation by absorbance at 480 nm using a glycine buffer (50 mM, pH 10.2) as described by Bannister and Calabrese (1987).

2.10. Statistical analysis

The data were tabulated and first subjected to descriptive analysis. Then, they were subjected to the normality test (Shapiro–Wilk). Data that did not present normal distribution were transformed to logarithms for the purpose of normalization (glucose, ALT, AST, GGT and urea). For the data that were normally distributed, a two-way analysis of variance (ANOVA) was applied for comparison between groups (T0, T4, T8) and repetitive data over time (Days 1, 15, 30, 45, 60 and 75). The Tukey test to verify the accuracy of the data to comparison between groups. Significance was considered when $P < 0.05$. Results were presented as mean and standard deviation.

3. Results

3.1. Body weight and weight gain

The body weight of lambs supplemented with biocholine showed no significant differences throughout the experimental period. The weight range was 38 kg to 46 kg, a large individual variation. However, the initial weight of the treatments was similar: 40.8 kg (T0), 41.0 kg (T4) and 40.7 kg (T8). A significant difference was found for body weight gain over time in the first 30 days of the experiment (day 1 to 30) in animals from T8 compared to T0. This greater weight gain was not observed at other times (days 1 to 60; days 1 to 75). Regarding average daily gain (ADG) throughout the experimental period, no significant differences were observed (Table 2).

3.2. Ruminal fluid: pH, microbiological activity and short chain fatty acid concentration

Results of the ruminal fluid response parameters are displayed in Table 3. The ruminal pH was lower on day 45 of the experimental period for T8 compared to the other treatments. Also microbiological activity was lower in T8 than in T0 (days 15, 45 and 75). The acetic acid concentration was lower in the groups supplemented with VB than in T0 (days 45 and 75). Propionic acid concentrations were lower in T4 than in T0 and T8 (days 45 and 75). Butyric acid concentration was generally lower in the supplemented groups than in the control group (days 45 and 75). Total short chain fatty acid (SCFA) levels were lower in T4 than in other treatments (day 45). At the end of the experimental period, there were lower concentrations of SCFA in supplemented groups (T4 and T8) compared to T0 (day 75).

Results of repeated analyzes over time are shown in Table 3. The pH differed over time at T8 (days 15 to 45). The concentration of acetic acid and propionic acid decreased over time only at T4 (days 15 to 45; and days 15 to 75). Butyric acid also decreased in animals that consumed BV (T4 and T8) between days 15 to 45; and days 15 to 75. Total short chain fatty acid was lower only at T4 over time (days 15 to 45; days 15 to 75). The acetate/propionic ratio increased over time in groups T0 and T4; and reduction in T8, as detailed in Table 3. The other variables in rumen fluid did not differ over time.

3.3. Serum clinical biochemistry: liver function and carbohydrate and protein metabolism

There were lower AST levels in T8 lamb serum on days 60 and 75 of the experiment than in T0; similarly, GGT levels were lower in serum at 75 days of experiment in animals consuming VB (T8) than in T0 (Table 4). ALT levels did not differ between treatments. Serum glucose levels were lower in lambs supplemented with VB (days 15 and 75) than in T0. Significant differences were found regarding the total protein concentrations in lamb serum (days 45, 60 and 75); in general, the supplemented groups had higher levels than those of T0. Similarly, globulin levels were greater in supplemented groups (days 15, 30, 45, 60 and 75) than in T0. No changes in albumin and urea values were observed (Table 4).

Results of repeated analyzes over time of clinical biochemistry are shown in Table 4. AST activity decreased in T8 animals (days 1 to 60; days 1 to 75). ALT activity increase in T0 from day 1 to 30; and reduced its activity in T8 animals (days 30 to 45; days 30 to 60). GST activity decreased in ovine that consumed BV (T4 and T8) over time, as detailed in Table 4. Also, over time, glucose and albumin levels differed in all groups (T0, T4 and T8) as observed in Table 4. There was a reduction in globulin levels in the control animals (T0) over the days (days 1 to 45; 1 to 60; and 1 to 75). Oscillations in the levels of urea, sometimes increase and sometimes decrease, in groups T0 and T4, as detailed in table 4. Total protein did not differ over time.

3.4. Serum oxidant and antioxidant status

Serum SOD activity was higher in T8 than in T0 (days 15 and 60), as well as at 75 days of experiment (Table 5). Serum GST activity was higher in T4 and T8 on day 75 (Table 5). SOD activity decreased over time in animals from groups T0 (days 1 to 60; 1 to 75) and T8 (days 1 to 45; 1 to 60; 1 to 75) (Table 5). GST activity increased in sheep from groups T4 (days 60 to 75) and T8 (days 45 to 75; days 60 to 75) (Table 5).

4. Discussion

The objective of this study was to augment the development of lambs in the rearing phase by offering VB via concentrate, because these animals have high growth capacity, and they require nutrition of adequate quality to support their development. Pinotti et al. (2009) evaluated growth performance of beef cattle and found that supplementation with ruminally-protected choline increased body weight and daily weight gain at day 89 of experiment.

Importantly, in the present study, we did not observe significant differences in body weight and average daily gain. Nevertheless, significant differences were observed for weight gain at the first 30 days of supplementation for the dose of 8 g/animal/day. This effect did not remain until the end of the experimental period, similar to findings reported by Pinotti et al. (2009) in cattle. The authors believed that, in the growth phase, 5 g of choline per animal/day was sufficient to influence weight gain; however, with increasing body weight by the end of the study, the lack of response to choline was attributed to the fact that the animals were receiving 30% less choline per kg body weight. This accords with our results, because the dose used in our experiment did not change as the animals' body weights increased. Because the experiment was carried out on a commercial farm, we respected the farmer's management; they did not permit increases in the amount of concentrate during the experiment.

Studies have evaluated the effect of choline chloride supplementation on ruminal fermentation; these did not find significant changes in ruminal pH (Erdman et al., 1984; Atkins et al., 1998). By contrast, in our study, the pH of the group supplemented with VB was in the range of 6.0, lower than that of the control group. Although presenting significant differences, this pH range does not cause ruminal disturbances. Atkins et al. (1988) evaluated the concentration of volatile fatty acids (VFA) and found a higher concentration of acetate in animals supplemented with a chemical form of choline, whereas the concentration of propionate and butyrate showed no changes with choline chloride supplementation. The authors explain that any effect attributed to choline supplementation depends on interactions of the molecule with ruminal fermentation; did not find any apparent effects, although they identified a higher rate of ruminal degradation in the supplemented animals. It is important to emphasize that in our study we used vegetable choline, a different molecule. Researchers suggest that phosphatidylcholine has greater bioavailability for ruminants, with natural resistance to ruminal degradation, suggesting that choline is able to pass through the rumen (Valencia Narváez, 2019; Rodríguez-Guerrero et al., 2018; Mendoza et al., 2019). These properties that may account for the differences between VFA levels in our study and others that used choline chloride. Importantly, phosphatidylcholine is present in only 10% of bacterial membranes, playing a key role in symbiotic and pathogenic interactions (Aktas et al., 2010). Our results suggest that plant biocholine modifies the concentration of volatile

fatty acids. In general, the supplemented showed lower concentrations of VFA. Our results suggest that there is a reduction in the bacterial population, because we believe that plant biocholine has some antimicrobial potential as shown by the methylene blue reduction test showing longer color retention times in supplemented groups. Because this is a preliminary study, it was not possible to determine the mechanisms of action of VB against ruminal microorganisms; to that end, studies will be conducted by our research group to verify possible interactions plant extracts and microbes.

The higher concentration of ruminal choline is probably due to the presence of ciliated protozoa. Investigators reported that the only source of choline (phosphatidylcholine) that is resistant to ruminal degradation is incorporated into the membrane of these ciliated protozoa (Neill et al. 1978; Neill et al. 1979; Dawson et al. 1981). Because the MBT indicated that microbiological activity was lower in the supplemented groups, we might suggest that there was an increase in protozoan production. Hypothetically, the increase in circulating phosphatidylcholine would stimulate the growth of this group of protozoa, consequently decreasing the concentration of ruminal bacteria, because protozoa are capable of bacterial engulfment (Hungate, 1966).

Choline has a fundamental role in lipid and glucose metabolism, and is involved in the synthesis of molecules responsible for the orientation and function of various intracellular signaling proteins (Zeisel et al., 2003; Vance and Tasseva, 2013). The supplemented groups had lower glucose levels than those of the control group, suggesting that there was an increase in insulin production; this was found in Suffolk lambs supplemented with rumen-protected choline (Bryant et al., 1999). However, Xu et al. (2005), when evaluating serum metabolic parameters of cows during the transition period, found increases in glucose values after calving. These findings suggest that, depending on the production phase or animal species, carbohydrate metabolism can be altered.

Total protein levels increased in the treated groups as a result of increased globulin levels. Age significantly influences this parameter; young animals have increased globulin levels, especially because, in addition to changes in food sources, there is also contact with various etiological agents that causes antibody production to increase. Sun et al. (2016) evaluated blood metabolic levels (TP, albumin, globulin, among others) in cows of the transition period, but found no changes in these parameters in animals receiving choline

supplements. We suspect that there is a mechanism by which VB stimulates the production of globulins, which are important proteins involved in immune responses. This hypothesis is based on our present findings, as well as results from other studies from our ruminant research group, where increased globulin levels are frequently found in clinical pathology (unpublished data).

ALT levels did not differ between treatments despite biocholine supplementation. Importantly, this enzyme is released when hepatocytes are damaged; and according to Zeisel et al. (1991) when there is choline deficiency in the organism, there are increased levels of this enzyme and other enzymatic markers such as the enzyme AST. In the present study, animals that received 8 g VB/day had lower AST activity, suggesting adequate liver function, because the animals are physiologically capable of lipid metabolism, one of the characteristic functions of choline. According to Rama Rao et al. (2001), liver fat levels are inversely related to dietary choline content. GGT levels were also lower in T8 animals. At 4 g VB/animal/day, we did not find any effect on GGT, as Aires et al. (2016) found when using protected choline. These same authors reported decreased liver fat deposition; protected choline supplementation gave rise to a moderate negative energy balance, with efficient fat mobilization and appropriate liver function in cows during the transition period (Aires et al., 2016). Effects on hepatic response parameters were expected when choline is used in animal diets, as results similar to the current study for AST and GGT activity were described by Koujalagi et al. (2018) in dairy cows supplemented with biocholine.

Choline is a donor of methyl groups that form methionine, an amino acid that is fundamental for maintaining antioxidant cellular defense systems that prevent oxidative stress and apoptosis (Saeed et al., 2017). This agrees with the results found in our experiment on antioxidant enzymatic activity. SOD and GST activities were higher in supplemented animals, suggesting greater capacity of the defense systems. Similar results were reported for the livers of healthy and aflatoxin-challenged Nile tilapia (Baldissera et al., 2019; Sousa et al., 2019; Souza et al., 2020). Koujalagi et al. (2018) reported similar results for SOD and GHS activity in biocholine-fed dairy cows who showed minimized oxidative stress. These results reinforced the notion that plant biocholine has an antioxidant effect.

Complementary studies should be performed to elucidate the mechanism of action of phosphatidylcholine in the rumen, and other parameters need to be verified to confirm the

effect of supplementation on VFA production so as to identify the optimal dose of administration for small ruminants. It is also important to highlight some limitations of this study: the use of animals with varying body weights and provision of concentrate once a day without correcting the VB concentrations for body weight. Another limitation of this experiment was the impossibility of measuring food intake; with silage detack that can alter rumen fermentation, possibly explaining our short chain fatty acid findings. Notwithstanding these limitations, our study provides important results that may direct new studies to clarify the metabolism of VB in sheep.

5. Conclusion

Supplementation with vegetable biocholine (phosphatidylcholine) had no effect on the performance in young female sheep to long-term (75 days), but in the first 30 days of ingestion potentiates weight gain. Ingestion of VB modified the ruminal fermentation profile, and decreased the concentration of volatile fatty acids. Biocholine supplementation stimulated antioxidant, immunological and hepatoprotective activities, all of which improve animal health.

Conflict of interest

The authors declare no conflict of interest.

Ethics committee

The project was approved by the Animal Research Ethics Committee of the State University of Santa Catarina, protocol number 8560130319.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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Table 1: Ingredients and chemical composition of the feeds used in our study.

Ingredients	Green matter (kg/animal/day)		Dry matter (kg/animal/day)	
Silage	2.00		65.84	
Concentrate[1]	0.400		35.4	
Chemical composition	Corn silage	Concentrate T0	Concentrate T4	Concentrate T8
Dry matter, %	32.9	88.5	88.5	88.9
Ash, % of DM	4.21	5.63	5.84	6.38
Crude protein, % of DM	8.01	15.9	15.91	15.81
Ethereal extract, % of DM	4.32	4.20	4.40	4.20
NDF, % of DM	33.0	8.64	10.5	9.83
ADF, % of DM	17.8	3.41	4.87	4.88

Note: 1 Total 100 kg of base concentrate was formulated with corn (70%), soy flour (25%) and nucleus (5%). The nucleus used in this study contained phosphorus (min 55 g/kg), calcium (215 g/kg, max 225 g/kg), sulfur (min 12 g/kg), sodium (min 80 g/kg), cobalt (min 60 mg/kg) chromium (min 12 mg/kg), iron (1420 mg/kg), iodine (min 14 mg/kg), magnesium (min 14 mg/kg), manganese (min 1550 mg/kg), selenium (min 22 mg/kg), vitamin A (2000 IU/kg), vitamin D (min 40000 IU/kg), vitamin E (min 550 IU/kg) and fluorine (max 550 mg/kg).

Table 2: Performance of lambs supplemented via plant biocholine concentrate (VB) in the rearing period.

Period	T1 (control)	T2 (4 g VB/kg)	T3 (8 g VB/kg)	P-value
Body weight (Kg)				
1	29.9 ±3.8	29.8 ± 5.5	29.8 ± 4.2	0.95
15	32.2 ± 2.9	33.4 ± 2.6	34.0 ±3.7	0.26
30	36.9 ± 4.9	37.4 ± 6.0	39 ± 5.1	0.21
45	40.1 ± 4.1	40.5 ± 8.4	41.7 ± 3.9	0.76
60	44.1 ± 5.1	42.5 ± 8.4	44.7 ± 3.9	0.68
75	46.9 ± 5.8	47.3 ± 7.6	47.1 ± 4.6	0.74
Weight gain (kg)				
1 to 30	7.0 ± 1.81^{b}	8.6 ± 2.10^{ab}	9.2 ± 1.32^{a}	0.03*
1 to 60	14.2 ± 2.0	12.7 ± 3.4	14.9 ± 1.6	0.54
1 to 75	17.0 ± 1.7	17.5 ± 1.9	17.3 ± 2.1	0.76
Average daily gain (g)				
1 to 75	226.6	233.3	230.6	0.81

Note: * $P < 0.05$ shows difference between groups. Different letters on the same line indicate the difference between the groups.

Table 3: pH, microbiological activity (methylene blue test - MBT) and short chain fatty acid profile in the ruminal fluid of lambs fed with vegetable biocholine (VB).

Period	**T0 (control)**	**T4 (4 g VB/kg)**	**T8 (8 g VB/kg)**	**P-value**
pH				
15	6.3 ± 0.3	6.14 ± 0.2	6.6 ± 0.3 ^{A}	0.21
45	6.4 ± 0.03^{a}	6.3 ± 0.09^{a}	6.0 ± 0.1^{Bb}	0.02*
75	6.1 ± 0.3	6.2 ± 0.3	6.3 ± 0.1^{AB}	0.16
P-value	0.18	0.32	0.01$^{\#}$	
MBT (minutes)				
15	6.51 ± 1.78^{b}	8.13 ± 0.23^{ab}	9.33 ± 1.15^{a}	0.05*
45	7.46 ± 0.92^{b}	8.20 ± 0.34^{b}	10.2 ± 0.25^{a}	0.01*
75	6.43 ± 3.50^{b}	9.66 ± 1.47^{ab}	10.56 ± 0.15^{a}	0.01*
P-value	0.47	0.20	0.52	
Acetic acid (mmol/100 mL^{-1})				
15	21.7 ± 2.0	21.9 ± 2.3 ^{A}	18.9 ± 4.9	0.35
45	22.3 ± 3.1^{a}	15.4 ± 4.41^{Bb}	19.78 ± 4.1 ^{ab}	0.01*
75	23.7 ± 3.26 ^{a}	15.8 ± 2.85 ^{Bb}	16.2 ± 2.79 ^{b}	0.01*
P-value	0.44	0.01$^{\#}$	0.35	
Propionic acid (mmol/100 mL^{-1})				
15	8.51 ± 3.42	7.55 ± 2.10 ^{A}	6.95 ± 1.29	0.23
45	8.07 ± 1.47 ^{a}	4.72 ± 2.49 ^{Bb}	7.86 ± 3.12 ^{a}	0.01*
75	6.49 ± 0.83 ^{a}	4.80 ± 1.99^{Bb}	7.74 ± 2.42 ^{a}	0.01*

P-value	0.06	0.01[#]	0.49	
Butyric acid (mmol/100 mL^{-1})				
15	4.16 ± 1.29	3.91 ± 0.14 [A]	3.49 ± 0.53 [A]	0.16
45	4.10 ± 1.44 [a]	2.60 ± 0.74 [Bb]	2.98 ± 0.1 [Bab]	0.01*
75	4.55 ± 0.47 [a]	2.28 ± 1.05 [Bb]	2.30 ± 0.59[Cb]	0.01*
P-value	0.84	0.01[#]	0.01[#]	
Total volatile fatty acids				
15	34.3 ± 3.41	33.3 ± 2.47[A]	29.3 ± 2.24	0.27
45	34.4 ± 2.96 [a]	22.7 ± 4.41 [Bb]	30.6 ± 3.30 [a]	0.01*
75	34.7 ± 3.74 [a]	22.8 ± 3.87 [Bb]	26.2 ± 3.02 [b]	0.01*
P-value	0.92	0.01[#]	0.27	
Acetate/propionate ratio				
15	2.54 ± 0.16 [Bb]	2.90 ± 0.17 [Ba]	2.71± 0.09 [Aab]	0.05*
45	2.76 ± 0.14 [Bb]	3.26 ± 0.11 [Aa]	2.51 ± 0.10 [Bb]	0.01*
75	3.65 ± 0.19 [Aa]	3.29 ± 0.14 [Ab]	2.09 ± 0.12[Cc]	0.01*
P-value	0.01[#]	0.01[#]	0.01[#]	

Note:

* P <0.05 shows difference between groups. Different lowercase letters on the same line indicate the difference between the groups.

[#] P <0.05 shows difference over time in each group. Differences between groups were illustrated by different capital letters in the same column.

Table 4: Biochemical profile of lambs supplemented with vegetable biocholine concentrate (VB) in the rearing period.

Period	T0 (control)	T4 (4 g VB/kg)	T8 (8 g VB/kg)	P-value
AST (U/L)				
1	150 ± 36.2	157.5 ± 56	145.8 ± 41.4^{A}	0.82
15	117.3 ± 34.5	137.3 ± 25.8	118.1 ± 35.1 ^{AB}	0.69
30	106.8 ± 22.7	145.6 ± 33.9	111.5 ± 53.8 ^{AB}	0.40
45	106.1 ± 17.3	115.6 ± 29.2	121.8 ± 26.4 ^{AB}	0.56
60	116.1 ± 12.8^{a}	118.5 ±20.5^{ab}	90.5 ± 18.8 ^{Bb}	0.05*
75	116.1 ± 11.6^{a}	111.6 ± 22.2^{ab}	94.1± 10.7 ^{Bb}	0.01*
P-value	0.08	0.15	0.03$^{\#}$	
ALT (U/L)				
1	12.2 ± 4.2 ^{B}	15.7 ± 5.8	21.5 ± 11.7 ^{AB}	0.39
15	19 ± 9.5 ^{AB}	14.1 ± 5.8	18.3 ± 9.7 ^{AB}	0.57
30	20.3 ± 4.6 ^{A}	19.6 ± 3.5	22.3 ± 6.7 ^{A}	0.80
45	14.6 ± 3.9 ^{AB}	15.6 ± 2.4	14.6 ± 3.5 ^{B}	0.89
60	18.3 ± 5.8 ^{AB}	14.1 ± 5.3	15 ± 2.5 ^{B}	0.29
75	17.6 ± 7.1 ^{AB}	18 ± 10.1	19.1 ± 4.3 ^{AB}	0.83
P-value	0.01$^{\#}$	0.39	0.04$^{\#}$	
GGT (U/L)				
1	126.3 ± 33.4	125 ± 29.8 ^{A}	110.8 ± 17.4 ^{A}	0.66
15	105.17 ± 10.0	125.3 ± 36.8 ^{AB}	88.1 ± 15.6 ^{AB}	0.11
30	94.8 ±19.1	95.8 ± 29.8 ^{AB}	88 ± 11.5 ^{AB}	0.58
45	89.1 ± 17.3	97.8 ± 24.5 ^{AB}	92.1 ± 17.6 ^{AB}	0.82
60	86.16 ± 16	91.1 ± 14.1 ^{BC}	73.3 ± 10.8 ^{B}	0.30
75	89.6 ± 10.6^{a}	77 ± 13.3 ^{Cab}	74.6 ± 8.7 ^{Bb}	0.05*
P-value	0.057	0.01$^{\#}$	0.01$^{\#}$	
Glucose (mg/dL)				
1	107.5 ± 53.2 ^{AB}	63.7 ± 32 ^{AB}	85.8 ± 22.2 ^{A}	0.20

15	109.3 ± 10.5^{Aa}	74.8 ± 10.2 ^{Ab}	60.6 ± 30.4 $^{AB\ b}$	0.04*
30	68.5 ± 25.8 ^{AB}	63.1 ± 8.9 ^{AB}	97.5 ± 39.5^{AB}	0.36
45	60.5 ±6.2 ^{B}	72.3 ± 31.4 ^{AB}	55.5 ± 5.9 ^{B}	0.50
60	53.5 ± 5.1 ^{B}	59 ± 6.8 ^{B}	57 ± 8 ^{B}	0.72
75	87.6 ± 12.4 ^{Aba}	56 ± 4.1 ^{Bb}	68.1 ± 3.7 ^{ABb}	0.01*
P-value	0.01$^{\#}$	0.01$^{\#}$	0.01$^{\#}$	
Total protein (mg/dL)				
1	6.2 ± 0.7	7.9 ± 5.5	6.6 ± 1.5	0.49
15	6.3 ± 2	6.7 ± 1.8	7.3 ± 1.6	0.15
30	5.9 ± 0.5	7 ± 1.5	6.5 ± 1.0	0.08
45	6.1 ± 0.3^{b}	6.8 ± 0.6^{a}	7.2 ± 1.06^{a}	0.01*
60	6.0 ± 0.9^{b}	7.4 ± 1.1^{a}	6.6 ± 0.3^{ab}	0.04*
75	5.9 ± 0.8^{b}	7 ± 0.5^{a}	7.2 ± 0.7^{a}	0.01*
P-value	0.65	0.52	0.30	
Albumin (mg/dL)				
1	2.0 ± 0.4 ^{B}	2 ± 0.3 ^{B}	2.5 ± 0.5 ^{B}	0.70
15	2.35 ± 0.6 ^{B}	2.2 ± 0.8 ^{AB}	1.9 ± 0.7 ^{B}	0.71
30	2.5 ± 0.5 ^{B}	2.3±0.5 ^{B}	2.2 ± 0.2 ^{B}	0.86
45	3.1 ± 0.5 ^{AB}	3.3 ± 0.8 ^{A}	3.2 ± 0.5 ^{AB}	0.94
60	3.35 ± 0.3 ^{A}	3.5 ± 0.3 ^{A}	3.2 ± 0.2 ^{A}	0.93
75	3.18 ± 0.4 ^{A}	2.9 ± 0.5 ^{AB}	3.2 ± 0.1 ^{A}	0.81
P-value	0.01$^{\#}$	0.01$^{\#}$	0.01$^{\#}$	
Globulin (mg/dL)				
1	4.2 ± 0.8 ^{A}	3.0 ± 1.7	4.2 ± 1.0	0.61
15	3.95 ± 1.2 ^{ABb}	4.5 ± 1.0^{ab}	5.3 ± 1.04^{a}	0.05*
30	3.4 ± 0.5 ^{ABb}	4.7 ± 1.1^{a}	3.1 ±3.1^{ab}	0.05*
45	2.9 ± 0.3 ^{Bb}	3.5 ± 1.2^{ab}	3.9 ±0.7^{a}	0.02*
60	2.7 ±0.9 ^{Bb}	3.9 ± 0.8^{a}	3.9 ± 0.29^{a}	0.01*
75	2.8 ± 0.6 ^{Bb}	4.0 ± 0.3^{a}	3.9 ± 0.7^{a}	0.01*
P-value	0.01$^{\#}$	0.19	0.11	

Urea (mg/dL)				
1	28.2 ± 6.2 ^{B}	22.8 ± 8.8 ^{B}	27.5 ± 6.4	0.24
15	30.8 ± 2.9 ^{B}	26.8 ± 9.9 ^{B}	33.1 ± 19.3	0.65
30	29 ± 1.7 ^{B}	33 ±7.2 ^{AB}	35.8 ±9.7	0.06
45	36.3 ± 9.5 ^{AB}	39.8 ± 9.8 ^{A}	41.1 ± 10.8	0.56
60	45.6 ± 14.1 ^{A}	47.3 ± 31.2 ^{AB}	31.5 ± 8.6	0.09
75	26.3 ± 10.3 ^{B}	29.6 ±11.0 ^{AB}	35.3 ± 8.8	0.33
	0.01$^{\#}$	0.01$^{\#}$	0.08	

Note: * P <0.05 shows difference between groups. Different lowercase letters on the same line indicate the difference between the groups. $^{\#}$ P <0.05 shows difference over time in each group. Differences between groups were illustrated by different capital letters in the same column.

Table 5: Oxidant and antioxidant status in the serum of lambs fed on vegetable biocholine (VB).

Period	T0 (control)	T4 (4 g VB/kg)	T8 (8 g VB/kg)	P-value
SOD (SOD IU/mg protein)				
1	11.7 ± 4.5 ^{A}	9.84 ± 4.12	12.9 ± 4.8 ^{A}	0.56
15	8.22 ± 1.29 $^{A\,b}$	7.85 ± 2.19^{b}	12.9 ± 2.6 ^{Aa}	0.01*
30	10.9 ± 4.33 ^{A}	10.8 ± 5.09	7.54 ± 5.53 ^{AB}	0.15
45	8.75 ± 2.92 ^{A}	5.98 ± 1.82	6.55 ± 1.42 ^{B}	0.07
60	2.66 ± 0.78 ^{Bb}	5.57 ± 2.55 ^{ab}	5.01 ± 1.89 ^{Ba}	0.04*
75	3.23 ± 1.18 ^{Bb}	5.51 ± 1.27^{a}	5.37 ± 1.07 ^{Ba}	0.05*
P-value	0.01$^{\#}$	0.13	0.01$^{\#}$	
GST (µmol CDNB/min/mg protein)				
1	2.30 ± 1.27	3.62 ± 1.78 ^{AB}	2.77 ± 1.10 ^{AB}	0.72
15	3.27 ± 1.40	2.72 ± 1.34 ^{AB}	3.60 ± 1.19 ^{AB}	0.43
30	3.65 ± 1.62	3.77 ± 1.80 ^{AB}	2.95 ± 0.68 ^{AB}	0.18
45	2.20 ± 0.40	2.51 ± 0.54 ^{AB}	2.32 ± 0.68 ^{B}	0.52
60	2.38 ± 1.03	1.64 ± 0.85 ^{B}	2.36 ± 0.80 ^{B}	0.08
75	2.64 ± 0.62 ^{b}	3.77 ± 0.82^{Aa}	4.06 ± 1.25 ^{Aa}	0.01*
P-value	0.25	0.01$^{\#}$	0.01$^{\#}$	

Note:

* P <0.05 shows difference between groups. Different lowercase letters on the same line indicate the difference between the groups.

$^{\#}$ P <0.05 shows difference over time in each group. Differences between groups were illustrated by different capital letters in the same column.

Supplementary Table 1: Validation of the methodology used to quantify short chain fatty acids in sheep ruminal fluid.

	Acetic acid	Propionic acid	Butyric acid
R^2	0.9971	0.9959	0.9958
Equation	y = 0.0261x - 0.0889	y = 0.0486x - 0.0798	y = 0.0747x - 0.0991
Linear range (mmol 100 mL^{-1})*	4.13 - 86.13	3.55 - 70.92	2.93 - 58.56
LOD (mmol 100 mL^{-1})	2.15	1.18	1.15
LOQ (mmol 100 mL^{-1})	4.13	3.55	2.93
Accuracy (%)	90.3	84.8	83.7
Repeatability (RSD)	1.78	1.84	0.86

* The linear range, LOD - limit of detection and LOQ - limit of quantitation expressed as mmol of SFA for 100 mL of ruminal fluid

2.3 MANUSCRITO III

Effects of soybean pre-cleaning residue on meat quality of feedlot lambs

Karoline Wagner Leal[a]; Davi F. Alba [a]; Luiza Ilha Borges [a]; Viviana Reboledo da Costa[b]; Juliana M. Silveira[c]; Sergio Carvalho[d]; José Laerte Nörnberg[e;] Julcemar Dias Kessler[f]; Aleksandro Schafer da Silva[f];

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ABSTRACT

The sheep industry requires intensification of production; this has spurred the search for alternative foods designed to enhance lamb growth. The objective of the present study was to determine whether the qualitative characteristics and lipid profiles of lamb meat would be influenced by the presence of soybean pre-cleaned residue (SPCR) as a substitute for conventional foods. We allocated 32 male lambs from the fixed cross between Texel and Ile de France, weaned at 60 days of age, in a completely randomized design, i.e., four groups with eight replications, where each animal represented an experimental unit. Treatments consisted of various levels of replacement of sorghum silage with SPCR (0%, 33.5%, 66.5% and 100%) as roughage based on dry matter. The roughage: concentrate ratio of 45:55 was used, with the concentrate containing milled corn, soybean meal and mineral core. The animals remained confined until they reached adequate body condition for slaughter. After carcass cooling, the longissimus thoracis et lumborum muscles were collected for meat analysis. We found that inclusion of SPCR modified indices related to meat color. Yellow tones (b *) and chromaticity (C *) were higher at T100 than at T0. In general, the inclusion of SPCR increased saturated fatty acid content, while polyunsaturated fatty acid content decreased. The ratio of unsaturated fatty acids to saturated fatty acids was lower at T66 than at T0. Similarly, omega (n-3 and n-6) content was lower at T66 than at T0. Nevertheless, the atherogenicity index increased at T66. This suggests that SPCR did not negatively influence meat parameters. Despite the fact that our findings for meat lipid profile agree with most of the parameters mentioned in the literature, we observed important changes in the fatty acid profile that may impact consumer health, depending on the level of inclusion of the SPCR, suggesting that there are limitations when using this agroindustrial byproduct.

Keywords: Sheep. Meat quality. Agroindustrial byproduct.

1. Introduction

The use of alternative feed in ruminant nutrition provides producers with greater food availability and lower costs (Nascimento et al., 2004). In fact, the expansion of soybean

cultivation in Brazil has made available a substantial amount of byproducts (bran, grains, husks and crop residues) that can be used to feed ruminants (Townsend et al., 1997).

The cost of sheep production has increased as a result of the competition for ingredients in the feed; therefore, there is a need for new feed sources for ruminants that to maintain livestock sustainability (Gôes et al., 2008). Agroindustrial residues have high bromatological variation; however, they require studies to verify their potential use (Meneghetti et al., 2008; Silva et al., 2014). From a nutritional point of view, soybean hulls, for example, are considered an energy food. They possess large amounts of digestible fiber that can be used as both bulk and concentrated foods (Silva et al., 2004).

The feasibility of using soybean processing residues was investigated, as well as the characterization of this residue, treatment methods, nutritional value and storage systems (Babilônia et al., 2000; Oliveira et al., 2006; Goes et al., 2011; Carvalho et al., 2012). However, the purpose of production is to reach the consumer market, so the consequences of these residues on the production of lamb deserve investigation, in order to determine the influences on meat and their impacts on consumer health. Specialized herds for meat production need attention regarding nutritional management, as the feeding system reflects directly on lamb development (Motta, 2018). In addition, the intake of energy foods can accelerate rumen development, allowing rapid finishing and obtaining carcasses with characteristics suitable for consumption (Monteiro et al., 2014). In view of short-term termination, feedlot and total diet have been widely used in post-weaning lambs (Monteiro et al., 2014). Nevertheless, the cost of feedlot food makes the activity unprofitable (Ziguer et al., 2011). According to Babylon et al. (2000) the use of soybean processing residues may be an alternative to lower production costs, however research should be carried out to verify the potential use of these alternative foods in face of meat characteristics. Whereas differences in dietary fiber levels alter the ruminal fermentation profile resulting in modifications in the production of volatile fatty acids; therefore, absorption and utilization of nutrients may reflect in meat composition (Motta, 2018).

Therefore, the objective of this work was to determine whether the quality and lipid profile of lamb meat can be affected by the inclusion of soybean pre-cleaned residues in feedlot lambs.

2. Materials and Methods

2.1 Characterization of soy pre-cleaned residue - SPCR

The residue used in this experiment was purchased from the CAMNPAL Cooperative, located in the state of Rio Grande do Sul. 2,000 kilos were used. After processing in the agroindustry, the residue was saved. Formed by pods, stalks, shells, broken grains and other seeds, then material was homogenized on a tarp for later use. Samples were collected from all portions to verify bromatological composition. We measured protein content (PC) of 9.6%, with ethereal extract (EE) values of 2.8%, neutral detergent fiber (NDF) of 60.51%, total digestible nutrients (TDN) of 68.77%, in addition to high dry matter (DM) content, 88.78%, allows the material to be easily stored (Table 1).

2.2 Animals, treatments and experimental management

We used 32 non-castrated male lambs, weaned at 60 days of age, maintained native pasture until weaning time. All lambs were clinically evaluated, dewormed and vaccinated against clostridiosis. Soon after, they were confined to individual stalls in a covered shed (2 m^2 per animal) with slatted floors, and were provided with feeders and drinking fountains.

The concentrate: roughage ratio of the diets was 55:45, based on dry matter (DM). The concentrate food consisted of milled corn, soybean meal and mineral core. The mineral core contained calcium 145 g, phosphorus 65 g, sulfur 18 g, magnesium 7 g, sodium 125 g, iodine 80 ppm, manganese 1400 ppm, selenium 20 ppm, zinc 4000 ppm, copper 60 ppm and molybdenum 100 ppm. The bulk fraction of the treatments consisted of different levels of soybean pre-cleaning residue inclusion (0%, 33.5%; 66.5%; 100%) to replace sorghum silage. All ingredients used for the formulation of total diets were chemically analyzed for their proximate composition (Table 1). The methodology for determining bromatological composition was performed as described by AOAC (2005). Table 2 displays the proportions of ingredients and the estimated chemical composition of the experimental diets in the same way that we analyzed the lipid profile of the dietary ingredients (Table 3).

To adapt the animals to management, facilities and diet, the experimental period was preceded by 10 days. The percentages of soybean pre-cleaning residue and concentrate from the treatments were determined at equal intervals. Subsequently, the animals remained in

confinement until each lamb reached slaughter weight, defined according to Butterfield (1988), as 60% of the live weight at maturity of their mothers.

The diet was provided ad libitum twice a day, 8:30 am and 5:30 pm. The leftovers were observed daily, to adjust the amount of food provided. This represented only 20% of the food provided on the previous day, in order to ensure the maximum voluntary consumption. When the lambs reached farm live weight, they were fasted for solids and liquids for 12 hours, weighed again to obtain the fasting live weight and then sent to slaughter.

2.4 Sample collection

Prior to slaughter, blood samples from the jugular vein were collected with needles and vacuolated tubes without anticoagulants to obtain blood serum and perform biochemical analyses. Immediately after collection, the tubes were refrigerated at 10º C. To obtain the serum, the samples were centrifuged at 5,100 RPM for 10 minutes. The samples were properly identified, stored in a freezer at –20 ºC and kept frozen until analysis.

After blood collection, the animals were sent to slaughter at a local slaughterhouse. The methodology used followed the rules of the federal inspection service (SIF) with technical supervision of the responsible veterinarian. The animals were properly stunned with a captive dart pistol positioned in the nuchal ligament towards the muzzle. Within 60 seconds, bleeding was performed, followed by skinning and evisceration.

After slaughter, a cross section was performed between the 12^{th} and 13^{th} rib, with cross section of the longissimus thoracis et lumborum muscle. From this section, the color evaluation was performed using a colorimetric measuring instrument, a Minolta Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan) calibrated to the white standard. Results were expressed with the coordinates L* (brilliance, luminosity), a* (red index) and b* (yellow index). The calculation to determine chromaticity (C*) based on the following formula, $C^* = [(c^*)^2+(b^*)^2]^{1/2}$, tonality (h*) was calculated according the equation $h^* = \tan^{-1}(b^*/a^*)$ (Ramos and Gomide, 2017)

The longissimus thoracis et lumborum muscle was removed whole, on both sides, then sectioned into three parts, separately vacuum packed (Cryovac, SP, Brazil) in low permeability polyamide packages, then the samples were stored at 4 ºC ± 2 ºC, to perform the meat quality analyses. The muscle portion between the 6^{th} and 10^{th} thoracic vertebrae were

intended for chemical analysis, water retention capacity by pressure, moisture, fatty acids and the portions between the 11th and 13th thoracic vertebrae were used for texture analysis. Shear force was measured as described by Selaive and Osório (2014), adapted from Sañudo et al. (2000).

2.5 Serum biochemical assays

Biochemical tests were performed to measure serum levels of total proteins (TP), albumin, glucose, cholesterol, triglycerides, urea, alanine aminotransferase (ALT), gamma glutamyltransferase (GGT). The serum was evaluated using a Bio-2000 semi-automatic analyzer (BioPlus®) and commercial kits (Analisa®), and the methodology followed the manufacturer's recommendations. Globulin values were calculated as the difference between total protein and albumin.

2.6 Meat quality analysis

To determine meat quality, analyses were performed after thawing. The samples were weighed in a semi-analytical balance, after being wrapped in aluminum foil and placed in a preheated electric oven, they remained in the oven until they reached an internal temperature of 71 °C. This temperature was measured using a meat-specific digital thermometer. As soon as the sample returned to room temperature, it was re-weighed to determine cooking loss (Felício, 1999).

Sequentially, the samples were cut into approximately 1.3 cm^3 cubes to determine the texture. We used a TAXT.plus texturometer with a probe coupled to the equipment. Data were collected using the Texture Expert Exponent program (Stable MicroSystems Ltd., Surrey, England). We followed the methodology of Huidobro et al. (2005), where the apparatus was calibrated for compression, for test, pre-test and post-test speeds and cycle time. The shear force measurement similarly used the texturometer, operated with a Warner-Bratzler blade, to the maximum force, expressed as kilogram force (Kgf).

According to the methodology described by AOAC (2005), the centesimal composition in the longissimus thoracis muscle, which corresponds to moisture, crude protein, total lipids and mineral matter for these analyses, used portions between the 6th and 10th thoracic vertebrae. The samples were lyophilized for 48 hours at 40 °C under vacuum.

They were then desiccated and weighed to obtain dry weight. The moisture content was obtained after drying in an oven at 105 ºC for at least 8 hours. To determine ash content, incineration was performed in a muffle furnace at 600 ºC for four hours. Following the Kjeldahl technique (AOAC, 2005), meat protein was defined as a percentage of natural matter. To determine the water retention capacity (WRC) we followed the methodology of Osório et al. (1998).

Derivatization, transesterification to methyl ester for lipid extraction was performed according to Christie's methodology (1982), and the determination of the various fatty acids followed the methodology described by Hartman and Lago (1973), using gas chromatography. At high resolution (Kramer et al., 1997), meat samples were ground in a Turrax to ensure complete sample homogeneity. Extraction of total lipids was performed according to Bligh and Dyer (1959). The atherogenic index (AI) was calculated according to Ulbricht and Southgate (1991): AI = (C12:0 + 4 × C14:0 + C16:0) / (MUFA + PUFA).

2.7 Statistical analysis

First, the data were tabulated and subjected to descriptive analysis. Subsequently, we performed the Shapiro–Wilk normality test, and non-normal data were transformed to logarithms for normalization. Analysis of variance was applied for comparison between groups. To verify the accuracy of the data we performed the Tukey test. $P < 0.05$ indicated statistical significance. Total fatty acid profile variables were subjected to regression analysis when they differed in the mean test.

3. Results

3.1. Fatty acid profiles of diet ingredients

The fatty acids found in the dietary ingredients are shown in Table 3. In general, 26 fatty acids were identified, corresponding to 86.5% of the total: tridecylic acid (C13:0), palmitic acid (C16:0), elaidic acid (C18:1N9T), oleic acid (C18:1N9C) and folic acid (C19:0); expressive values above 15% in only three: C13 (30%), C18:1N9T (18.6%) and C19:0 (20.8%).

3.2. Qualitative meat analysis

The centesimal composition of meat showed no significant differences between treatments (P <0.05) (Table 4). Similarly, the pH, cooking loss (CP), water retention capacity (CRA) and shear force (KgF/cm²) analyses of longissimus thoracis et lumborum muscle showed no significant differences between treatments. We performed the following texture tests: Flexibility, cohesiveness, resilience, hardness, fracturability and adhesiveness. Regarding the brightness (L*), red (a*) and hue (h*) indices, no significant differences were observed. However, the intensity of yellow tones (b*) and chromaticity (C*) showed similar results, higher at T100 than at T0 (Table 5).

3.3. Lipid profiles of meat

Fatty acids were organized into their respective groups: saturated fatty acids (SFAs), monounsaturated fatty acids (SFAIs), polyunsaturated fatty acids (SFAIs) and unsaturated fatty acids (SFAIs) (Table 9). Regarding total FAs, levels at T66 were higher than at T0 (Figure 1A). Significant differences were found only in C21:0 fatty acid. T100 presented higher levels than the other treatments. Total MUFA showed no significant difference between the groups. However, vaccenic acid showed higher levels in T100 compared to T0. When analyzing the total PUFA, T0 presented higher concentration than did T66 and T100 (Figure 1B). Separately, arachidonic acid (C20: 4N6C) showed high levels at T0 compared to T100. Nevertheless, the total unsaturated fatty acids showed no significant differences between the groups.

The ratio of UFA:SFA was higher at T0, mean 1.36, compared to T66, mean 1.09 (Figure 1C). As for the total average of omegas 3 (n:3), T0 presented a higher average than T66, 0.73 and 0.38 respectively (Figure 2A). When the total omega 6 (n:6) was analyzed, T0 had higher concentrations than T66 or T100 (Figure 2B). Relationships n3:n6 and n6:n3 showed no significant differences between groups. The atherogenicity index in T66 was higher than in T0, 0.82 and 0.60 respectively (Figure 2C).

3.4. Serum biochemistries

Levels of aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) did not differ between treatments. Total protein levels were higher in the group with 100% inclusion of soybean pre-cleaning residue (SPCR), just as the globulin results showed higher concentration in T100. Significant differences were not observed in albumin, glucose, urea

and cholesterol levels. However, triglyceride levels were elevated in T100 compared to T0 and T33 (Table 4).

4. Discussion

Meat is one of the most nutritious of human foods; as a protein source it possesses high biological value. Because of the growing importance of the global market for meat, it is essential to determine the effects of animal nutrition on the final product. In the present study, we observed no influence of the experimental diet on the centesimal composition of lamb meat. Our results are similar to those of demonstrated Leão et al. (2011), who used different concentrate and roughage ratios, and found that the composition remained unchanged. However, in our study we observed that protein levels are higher (24.0%) than those found by Leão et al. (2011) (17.0%). The explanation for the discrepancy may be related to the age of the animals, because the percentage of protein in the meat is influenced by age (Pellegrini et al., 2015). However, Zeola et al. (2004) found that, in lamb semimembranosus muscles, there were variations in protein levels dependent on the level of concentrate in the diet. Variations in centesimal composition may also be attributed to slaughter weight and the particular muscle used for analysis (Zeola et al., 2004).

Meat pH is considered an indicator of final quality. In the present study, we did not observe differences between treatments, in agreement with the literature, between 5.2 and 5.7 respectively (Silva Sobrinho et al., 2005; Costa et al., 2015). Abreu et al. (2019) used different levels of cactus in the diet and found similar pH results in the longissimus lumborum muscle. In general, meat pH is related to pre- and post-slaughter factors. It is undoubtedly one of the most important instrumental characteristics of meat, because it has a marked influence on water retention capacity, and consequently on juiciness (Osório et al., 2014).

We found low cooking losses (26.87%) independent of SPCR level. Other researchers measured this parameter in different breeds and found higher averages than we did (38.41%) (Silva Sobrinho et al., 2005; Costa et al., 2015); This parameter is related to the amount of fat deposition that ‘waterproofs’ membranes and decreases water losses (Silva Sobrinho et al., 2005). We suggest that the amount of fat deposited was adequate to reduce water elimination.

Proportionally, the water retention capacity was high (78.94%); experiments with Texel and Corriedale lambs showed similar values (77.86–80.33%) (Bonacina et al., 2011).

Our measured shear force was 3.2 KgF; According to the literature, this parameter is entirely linked to consumer-perceivable softness; thus, values below 5.1 KgF are considered very soft (Bickerstaffe et al., 2001).

Color is the first characteristic to be evaluated by the consumer. Red meats are most appreciated at the time of purchase. Myoglobin and hemoglobin are color pigments in meat; the former retains oxygen in the muscle and the second transports oxygen in the bloodstream. In fresh meat, myoglobin is found in three basic forms and the color varies depending on the relative proportion and distribution of these pigments: reduced myoglobin or deoxymyoglobin (purple red); oxymyoglobin (bright red) and metmyoglobin (brown) (Osorio et al., 2014; Abreu et al., 2019).

We evaluated the color of meat according to the reflected light fraction. Our results were within the range cited by Sañudo et al. (2000), with variations from 30.03 to 49.47 for L*, 8.24 to 23.53 for a* and 3.38 to 11.10 for b*; despite the fact that we found differences for b*, the values are within the previously published ranges. Grandis et al. (2016) used soybean cake at various levels to feed lambs and found chromaticity values higher than the results obtained in this experiment, 18.34 and 13.70 respectively. Chromaticity (c*) is related to color concentration, representing a quantitative attribute for intensity. Brighter meat is associated with ante-mortem factors such as age, race and diet; Hue (h*) is related to the qualitative attribute of color, chemical status of the pigment, associated with post-mortem factors such as freshness of the cut (Osório et al., 2014; Ferreira and Spricigo, 2017).

Regarding the lipid profile of lamb meat, 33 fatty acids (FA) were identified. Values higher than 1% were found in ten FAs, representing an average of 86.03% of the total. It is noteworthy that the expressive values were observed in only three FAs: oleic acid (34%), palmitic acid (21.08%) and stearic acid (17.08%) representing 72.07% of the total fatty acids, on average. These results are consistent with those reported in the literature; this fatty acid composition is characteristic of sheep meat, as it agrees with most studies of meat quality (Madruga et al., 2005; Leão et al., 2011; Manzoni, 2019; D Alessandro et al., 2019).

The type of fatty acid present in meat is of great interest to the consumer; because cardiovascular diseases have been associated with the consumption of foods rich in saturated

fatty acids (Gillingham et al., 2011). The composition of FA is directly responsible for influencing meat quality and oxidation (Senegalhe et al., 2014). The results on the use of SPCR showed increased SFA levels. It is known that the fatty acid profile of meat depends on the type of diet (Brito et al., 2017). It is noteworthy that studies that used soybean hulls at various levels for finishing lambs found no differences in lipid profiles (Costa et al., 2012; Wommer, 2013).

The group without inclusion of SPCR showed higher concentrations of PUFA, n-3 and n-6, essential fatty acids that are beneficial to human health. The decrease of these fatty acid levels may be linked to the dietary profile. Leão et al. (2011) compared the effect of diets with high or low roughage:concentrate ratios and found no differences in lipid profile. SPCR is likely responsible for this result, because the proportion of concentrate was similar between treatments. Nevertheless, further research should be conducted to verify this effect.

The ratio of unsaturated fatty acids:saturated fatty acids was lower at T66 compared to T0 (1.09 and 1.36, respectively). In ruminants, this ratio is around 1.0 because of the biohydrogenation of the unsaturated fatty acids from the rumen diet by ruminal microorganisms. As a result, there is a higher absorption of saturated fatty acids in the intestine, resulting in higher concentrations of these acids in lamb meat (Costa et al., 2012;). The saturated fatty acids:unsaturated fatty acid ratio found in our study is in accordance with published parameters (Costa et al., 2012; Carvalho et al., 2015). The determination of the ratio of these acids identifies risk factors with respect to the increase in blood cholesterol levels in humans (Arruda et al., 2012). Leão et al. (2011) used two sources of roughage and different roughage:concentrate ratios; they found that the AGI:AGS ratio did not differ significantly. These authors reported lower values than those found in our study, suggesting that the SPCR influenced this parameter.

The atherogenicity index (AI) was higher at T66 than at T0 (0.82 and 0.60 respectively). According to Castro (2016), the lower the AI, the greater the amount of anti-atherogenic fatty acids present in food, with these Fas being responsible for preventing the onset of coronary diseases. AI is an important mathematical tool for understanding the nutritional value of fats. Considering that the inclusion of SPCR influenced the lipid profile, we evaluated the recommended levels for AI. Ulbricht & Southgate (1991) proposed an ideal

value of a maximum of 0.72 to guarantee the consumer good health. In general, the inclusion of SPCR presented numerically atherogenic indices higher than 0.75, higher than desired.

According to Contreras et al. (2000) sheep herds are affected by various factors that limit their production. One of them is the presence of nutritional imbalances that can cause metabolic diseases. Analyzing serum biochemical profiles is therefore very important because there are variations in the blood concentration of a metabolite that may be caused by excesses or deficiencies of a nutrient. Observing the results of total protein and globulins obtained in this experiment, we found significant differences with high levels compared to the results found by Contreras et al. (2000). As explained by Souza et al. (2014), the increase in the concentration of globulins may be related to the initial development of the hepatic metabolic process for protein synthesis; this is because, hypothetically, the feeding change influenced the metabolic profile. The levels of albumin, glucose and urea values we observed were comparable to those reported in the literature (Contreras et al., 2000; Madureira et al., 2013).

There were no significant differences with respect to enzymatic tests. Contreras et al. (2000) explained that such results suggest that the animals did not have liver overload or liver and muscle injuries. AST levels were within normal range, although GGT levels are higher than those reported in the literature. Madureira et al. (2013) explain that the enzyme GGT may be influenced by the age of the animals, and their results were higher values in animals under 12 months of age. According to this information Souza et al. (2014) stated that, during the neonatal period, GGT activity should not be used as a marker of liver disease, although it can be used in older animals.

We did not observe differences in cholesterol levels. However, the increase of triglycerides may be explained by the relationship between the concentrations of triglycerides and free fatty acids in the circulation, as reported by Souza et al. (2014). We found that the experimental diet modified the lipid profile of the meat of these animals; in similar fashion, we believe that the diet influenced metabolism.

5. Conclusion

The soybean pre-cleaned residue (SPCR) used in this experiment did not change the intrinsic characteristics of lamb meat; however, we detected some influence of SPCR on lipid

profiles of the meat. Nevertheless, the atherogenicity index was high in the groups with addition of SPCR. Given that there are many bromatological differences in residues from soybean processing, and that these are determining factors for consumer health, further studies are recommended

Ethics committee

This study was approved by the Animal Ethics Committee of Universidade do Estado de Santa Catarina (CEUA/UDESC), protocol number 9541281118.

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Table 1: Chemical composition of the foods used to formulate diets.

	Milled corn	*Soy flour*	*Sorghum sileage*	*SPCR*
MS	86.86	88.3	33.45	88.78
MO	98.72	93.17	91.44	90.76
PB	8.87	49.2	5.83	9.6
EE	4.92	1.85	4.54	2.86
FDN	14.12	16.54	59.16	60.51
FDA	2.97	5.69	36.2	41.66
NDT	87.24	81.54	57.23	68.77
MM	6.83	6.83	8.56	9.24
Ca	0.02	0.3	0.34	0.44
P	0.21	0.69	0.17	0.14

Note: SPCR = Soybean pre-cleaned residue.

Table 2: Pre-cleaning residue content of experimentaldiets, proportion of ingredients based on dry matter (%DM).

	SPCR content			
	0	33.5	66.5	100
Proportion of Ingredients (%DM)				
Sorghum sileage	45.00	29.93	15.08	0.00
SPCR	0.00	15.08	29.93	45.00
Milled corn	25.45	27.05	28.55	30.07
Soybean flour	28.31	26.87	25.46	24.03
Calcitic limestone	1.24	1.08	0.99	0.90
Bromatological composition (%DM)				
MS	64.3	72.29	80.18	88.19
MO	92.65	92.78	92.85	92.92
PB	18.81	18.81	18.81	18.81
EE	3.82	3.62	3.42	3.21
FDN	34.9	35.09	35.27	35.45
FDA	18.66	19.45	20.22	21.01
CHT	71.26	71.43	71.61	71.80
CNE	36.36	36.34	36.34	36.35
NDT	71.04	73.00	74.87	76.77
CIN	6.11	6.14	6.16	6.18
Ca	0.66	0.62	0.60	0.58
P	0.33	0.31	0.30	0.29

Table 3: Fatty acid percentage found in diet ingredients

Ingredients	Sorghum sileage	SPCR	Soybean flour	Milled corn
C8:00	0.70	0.95	ni	ni
C10:00	2.73	0.64	ni	ni
C11:00	1.92	0.80	ni	ni
C12:00	2.42	0.83	ni	ni
C13:00	57.48	36.60	3.29	26.55
C14:00	0.60	1.06	0.20	ni
C15:00	0.66	0.77	ni	ni
C16:00	3.40	9.86	11.79	2.97
C16:1	1.34	0.61	0.13	ni
C17:00	ni	Ni	0.12	ni
C17:1	ni	Ni	0.05	ni
C18:00	0.81	4.83	4.34	ni
C18:1N9T	ni	9.86	50.15	14.52
VACENIC	0.66	0.18	0.07	8.61
C18:1N9C	3.80	13.26	19.04	ni
C18:1N7* **	0.23	0.87	1.51	ni
C18:2N6C	0.21	Ni	0.05	ni
C18:3N6C	1.77	0.65	Ni	ni
C18:3N3	1.04	1.05	6.35	ni
C19:00	18.17	16.58	2.49	46.31
CLA1	ni	0.44	ni	ni
C20:1N9	1.45	Ni	0.14	ni
C20:2	ni	Ni	0.05	ni
C20:3N6	0.51	Ni	0.12	ni
C22:00	ni	Ni	ni	ni
C24:1	ni	Ni	ni	1.01
Sum	100	100	100	100

Note: SPCR= Soybean pre-cleaned residue; ni = not identified

Table 4: Centesimal analysis of whole lamb meat samples.

VARIABLE	T0	T33	T66	T100	P-VALUE
MOISTURE	72.9 ± 1.24	71.54 ± 3.19	71.16 ± 5.83	74.89 ±2.42	0.45
ASH	1.37 ± 0.28	1.64 ± 0.8	1.47 ± 0.33	1.13 ± 0.10	0.24
CRUDE PROTEIN	24.72 ± 1.45	26.23 ± 3.79	25.84 ±5.19	22.58 ± 2.21	0.16
TOTAL LIPIDS	0.69 ± 0.32	0.57 ±0.29	0.76 ± 0.42	0.43 ± 0.09	0.27
SUM	99.67 ± 0.61	99.98 ± 1.73	99.23 ± 2.5	99.05 ±1.28	-

Note: P>0.05 suggests no significant difference.

Table 5: Qualitative analysis of lamb meat.

VARIABLE	T0	T33	T66	T100	P-VALUE
L*	43.33 ± 2.62	42.57 ± 2.57	42.95 ± 1.88	43.28 ± 3.5	0.81
A*	10.08 ± 1.52	10.61 ± 1.25	11.09 ± 1.14	11.36 ± 0.51	0.51
B*	7.49 ± 1.12^{b}	8.57 ± 0.96^{ab}	8.15 ± 0.64^{ab}	9.42 ± 0.56^{a}	0.01*
C*	12.63 ± 1.24^{b}	13.66 ± 1.41^{ab}	13.77 ± 1.15^{ab}	14.77 ± 0.59^{a}	0.03*
H*	0.64 ± 0.12	0.61 ± 0.05	0.64 ± 0.05	0.69 ± 0.03	0.15
PH	5.66 ± 0.06	5.62 ± 0.05	5.65 ± 0.06	5.64 ± 0.02	0.74
PC	26.99 ± 5.6	27.98 ± 3.7	25.79 ± 3.4	26.72 ± 4.9	0.68
CRA	80.56 ± 7.6	80.03 ± 2.4	76.32 ± 5.7	78.85 ± 4.5	0.57
KGF	3.35 ± 1.57	3.45 ± 0.43	2.95 ± 1.9	3.35 ± 0.60	0.40
FLEXIBILITY	0.53 ± 0.05	0.47 ± 0.02	0.52 ± 0.04	0.52 ± 0.05	0.24
COHESIVENESS	0.52 ± 0.05	0.54 ± 0.04	0.54 ± 0.02	0.53 ± 0.03	0.82
RESILIENCE	0.24 ± 0.04	0.28 ± 0.05	0.26 ± 0.02	0.26 ± 0.04	0.40
DURABILITY ($X10^3$)	16.9 ± 6.9	19.4 ± 9.7	16.4 ± 6.3	15.8 ± 7.1	0.36
FRACTURABILITY ($X10^3$)	23.0 ± 16.1	12.9 ± 11.7	21.4 ± 20.0	23.9 ± 16.8	0.49
ADHESIVENESS	- 6.23 ± 10.1	- 14.2 ± 10.6	- 11.9 ± 19.1	-11.9 ± 8.23	0.42

Note 1: P <0.05 (*) and different letters on the same line show difference between groups.

Note 2: L* = luminosity; a* = red intensity; b* = yellow intensity; C* = chromaticity and h* = tonality. pH = hydrogen potential; PC = cooking losses; CRA = water holding capacity. KgF = Shear force, WBSF kg fore.

Table 6: Fatty acids found in meat.

FATTY ACID	T0	T33	T66	T100	P-VALUE
C6:00	0.172 ± 0.12	0.014 ± 0.25	0.15 ± 0.21	0.11 ± 0.04	0.25
C8:00	0.175 ± 0.11	0.064 ± 0.05	0.16 ± 0.20	0.06 ± 0.06	0.19
C10:00	0.149 ± 0.05	0.130 ± 0.05	0.15 ± 0.08	0.15 ±0.05	0.85
C12:00	0.189 ± 0.11	0.280 ± 0.13	0.32 ± 0.24	0.32 ±0.13	0.35
C14:00	3.000 ± 3.4	2.598 ± 1.24	2.99 ± 1.6	2.95 ± 0.97	0.74
C15:00	0.255 ± 0.10	0.294 ± 0.14	0.34 ± 0.14	0.37 ± 0.14	0.13
C16:00	19.065 ± 3.07	22.35 ± 4.55	23.73 ± 3.1	22.17 ± 1.48	0.26
C17:00	0.647 ± 0.15	0.708 ± 0.13	0.72 ± 0.10	0.77 ± 0.16	0.77
C18:00	16.871 ± 4.0	17.82 ± 3.32	17.16 ± 2.4	16.46 ± 1.88	0.80
C21:00	0.453 ± 1.2^{b}	0.176 ± 0.18^{b}	0.09 ± 0.12^{b}	2.57 ± 1.09^{a}	0.001*
TOTAL STF	40.97 ± 2.6^{b}	44.1 ± 1.6 ^{ab}	46.1 ± 1.13^{a}	45.95 ± 3.55 ^{bb}	0.033*
C14:1	0.12 ± 0.05	0.09 ± 0.03	0.08 ± 0.04	0.09 ± 0.03	0.82
C15:1	0.15 ± 0.09	0.19 ± 0.20	0.20 ± 0.21	0.12 ± 0.03	0.50
C16:1	1.07 ± 0.24	0.98 ± 0.29	1.07 ± 0.21	1.03 ± 0.28	0.78
C17:1	0.32 ± 0.03	0.30 ± 0.68	0.29 ± 0.07	0.31 ± 0.10	0.72
C18:1N9T	0.35 ± 0.13	0.45 ± 0.12	0.5 ± 0.35	0.51 ± 0.16	0.07
VACÊNICO	0.88 ± 0.44^{b}	1.22 ± 0.44^{ab}	1.8 ± 1.42^{ab}	1.9 ± 0.6^{a}	0.001*
C18:1N9C	33.93 ± 5.4	37.86 ± 7.32	37.81 ± 5.5	38.09 ± 8.4	0.28
C18:1N7	1.17 ± 0.33	1.07 ± 0.36	0.98 ±0.29	0.91 ± 0.28	0.75
C20:1N9	0.11 ± 0.07	0.20 ± 0.32	0.07 ± 0.05	0.08 ± 0.05	0.44
TOTAL MUFA	38.08 ± 1.75	42.05 ± 3.37	42.90 ± 1.7	43.09 ± 2.7	0.079
CLA 1	0.28 ± 0.22	030 ± 0.24	0.42 ± 0.30	0.35 ± 0.33	0.41
C20:3N6	0.30 ± 0.21	0.22 ± 0.19	0.19 ± 0.18	0.12 ± 0.80	0.29
C20:4N6	4.48 ± 2.28^{a}	2.86 ± 1.42^{ab}	1.88 ± 1.7^{ab}	1.89 ± 0.80^{b}	0.001*
C20:5N3	0.25 ± 0.15	0.15 ± 0.13	0.12 ± 0.16	0.07 ± 0.05	0.20
C22:6N3	0.17 ± 0.14	0.11 ± 0.12	0.09 ± 0.11	0.07 ± 0.06	0.09
TOTAL PUFA	5.95 ± 1.29 ^{a}	3.24 ± 1.0 ^{ab}	2.70 ± 1.12^{b}	2.53 ± 0.32 ^{b}	0.025*

TOTAL UFA	51.6 ± 1.57	51.1 ± 2.27	50.2 ± 1.87	51.3 ± 2.74	0.74
UFA/SFA RATIO	1.36 ± 0.20 [a]	1.17 ± 0.17 [ab]	1.09 ± 0.11 [b]	1.14 ± 0.14 [ab]	0.046*
S N-3	0.73 ± 0.04 [a]	0.53 ± 0.08 [ab]	0.38 ± 0.03 [b]	0.45 ± 0.09 [ab]	0.031*
S N-6	12.5 ± 4.74 [a]	8.29 ± 1.10 [ab]	6.73 ± 1.85 [b]	6.92 ± 2.52 [b]	0.023*
N-6/N-3	17.2 ± 2.4	15.6 ± 1.98	18.7 ± 2.04	15.7 ± 1.46	0.31
N-3/N-6	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.01	0.30
ATHEROGENICITY	0.60 ± 0.08 [b]	0.75 ± 0.12 [ab]	0.82 ± 0.06 [a]	0.76 ± 0.09 [ab]	0.039*

Note: P <0.05 (*) and different letters on the same line show difference between groups.

Table 7: Biochemical analysis of the day of slaughter of feedlot lambs fed different levels of soybean pre-cleaning residue replacement.

Variable	**T0**	**T33**	**T66**	**T100**	**P-value**
AST (u/l)	161.5 ± 39.85	151.5 ±51.69	106.43 ± 59.35	155.33 ± 2.51	0.14
GGT (u/l)	126 ± 37.50	101.5 ± 48.96	116.5 ± 49.91	168.33 ±35.64	0.09
Total protein (g/dl)	7.75± 1.89^{ab}	6.33 ± 1.25^{b}	6.76 ± 2.17 ^{ab}	8.67 ± 1.4^{a}	0.01*
Albumin (g/dl)	2.83 ± 0.25	2.85 ± 0.36	2.90 ± 0.85	3.20 ± 0.86	0.54
Globulin (g/dl)	4.47 ± 1.76^{ab}	3.48 ± 0.90^{b}	3.86 ± 1.4^{ab}	5.47 ±0.61^{a}	0.001*
Glucose (mg/dl)	175.5 ± 74.16	122.5 ± 22.39	119.57±25.78	151.33 ± 28.67	0.07
Cholesterol (mg/dl)	47 ± 6.69	47 ± 6.69	63.57 ± 18.31	43 ± 10.44	0.25
Triglycerides (mg/dl)	35.17 ± 7.13^{b}	38.5 ± 17.47^{b}	46.43 ± 14.63^{ab}	72.67 ± 24.44^{a}	0.001*
Urea (mg/dl)	54.33 ± 22.45	59.33 ± 19.13	65.43 ± 14.08	67.67 ± 21.38	0.32

Note: P <005 (*) and different letters on the same line show difference between groups.

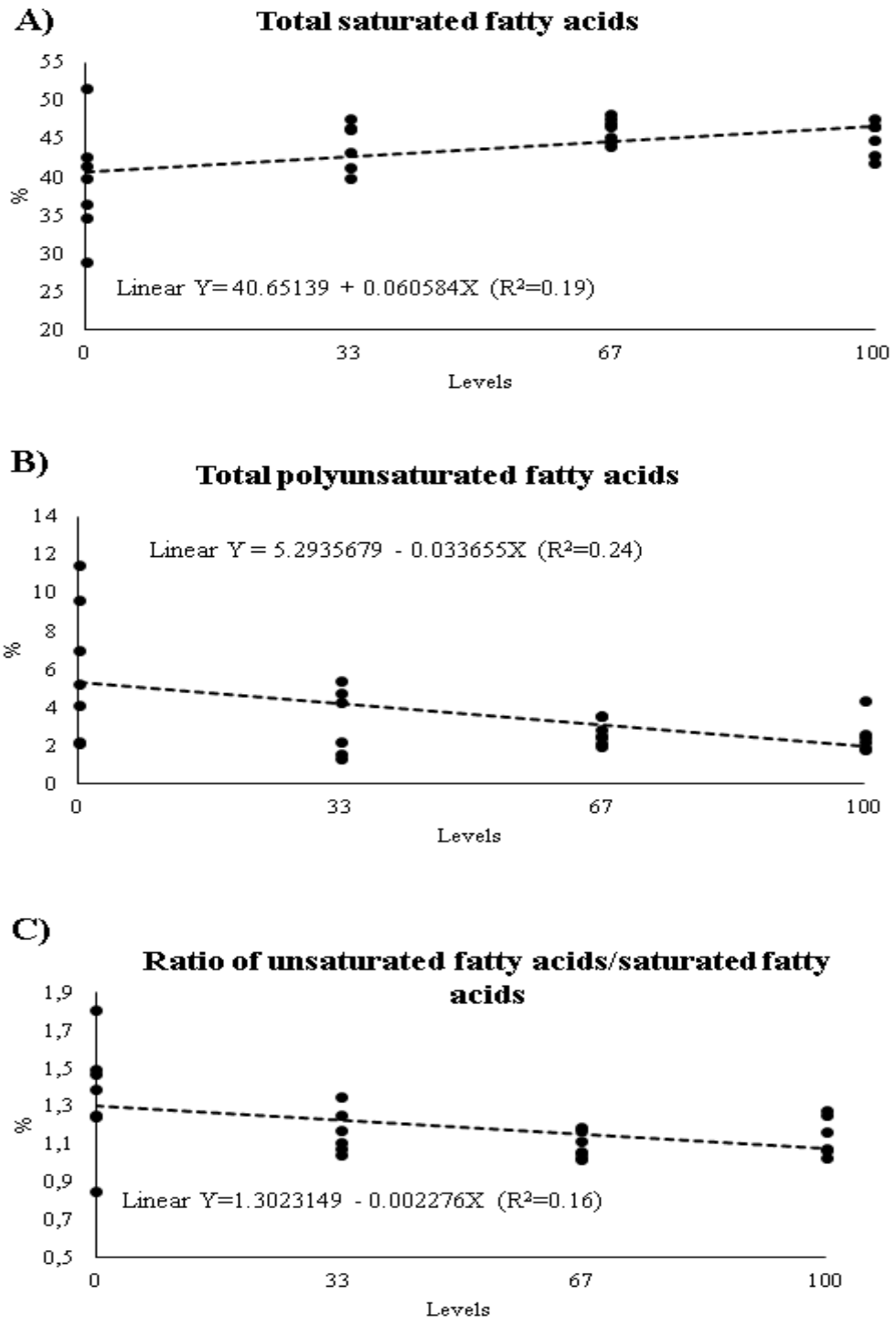

Figure 1: (A) Total saturated fatty acids; (B) total polyunsaturated fatty acids; (C) Ratio of unsaturated fatty acids: saturated.

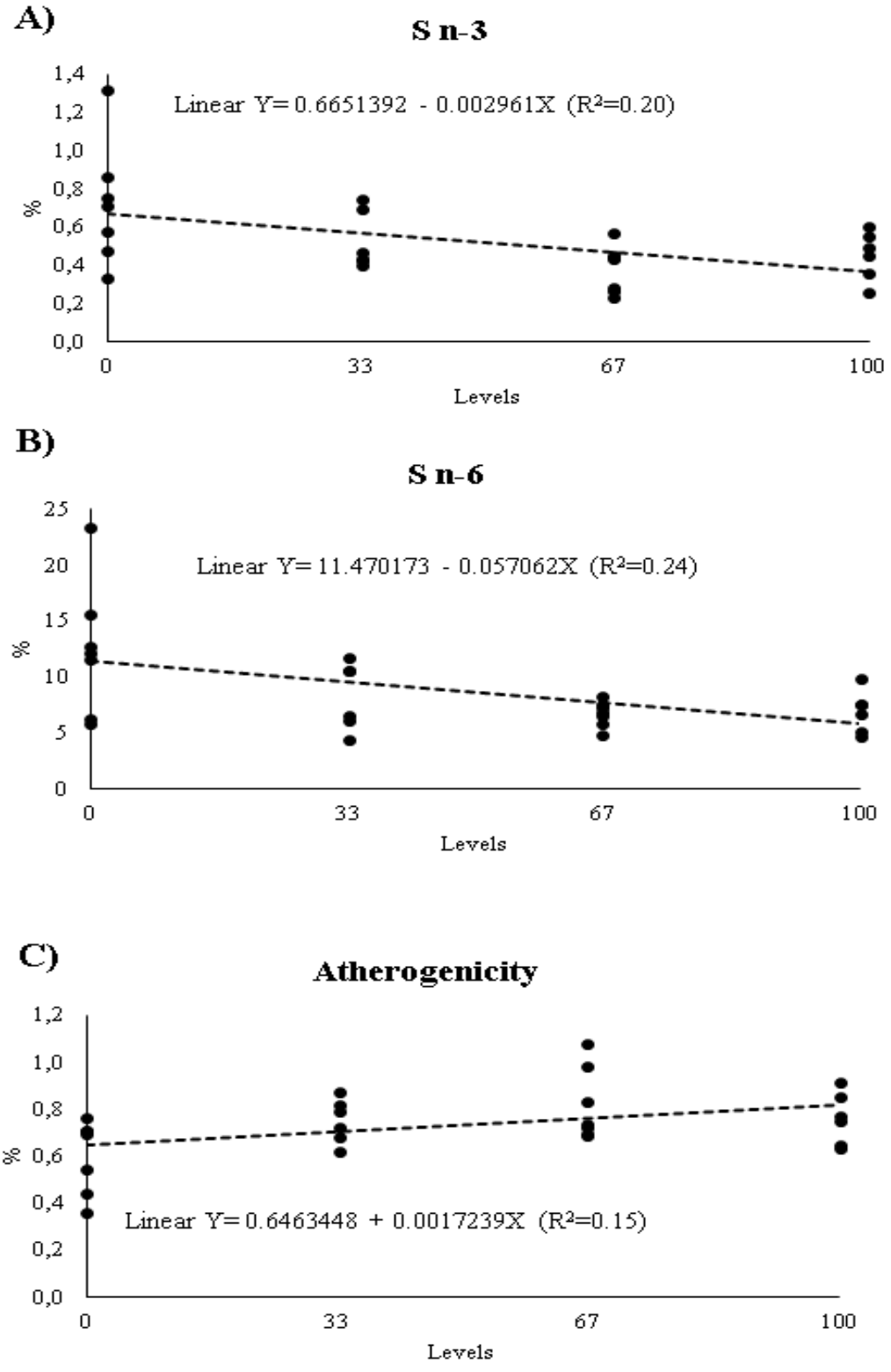

Figura 2: (A) Total de ômegas; (B) total de ômegas 6; (C) índice de aterogenicidade.

3. CONSIDERAÇÕES FINAIS

Mudanças na alimentação e no ambiente durante a fase de cria podem interferir positiva ou negativamente no crescimento e no desenvolvimento animal. No experimento I os animais foram separados das mães logo após a ingestão do colostro; contávamos com aleitamento artificial nos primeiros 30 dias de experimento, ação fundamental para a sobrevivência do cordeiro, já que esse estava em fase de transição alimentar. Sendo a biocolina vegetal (BV) responsável pela síntese de diversas moléculas que mantém a integridade do organismo, acredita-se que a taxa de crescimento dos animais aumentaria. Nossos resultados apontaram que a BV foi benéfica para os cordeiros pois melhorou o desempenho dos animais e demonstrou equilíbrio considerável no status oxidante e antioxidante, assim cumpriu o propósito de melhorar a saúde dos animais frente aos desafios do período de transição alimentar.

No experimento II a suplementação de biocolina vegetal para as borregas na recria teve como objetivo manter a saúde e bem-estar das fêmeas. Já que nossos resultados não apresentaram aumento no ganho de peso, o que pode ter relação com a dose. No entanto, a ingestão de BV modificou o perfil de fermentação ruminal e diminuiu a concentração de AGV, por isso hipotetizamos que não seria adequado aumentar a dosagem; em contrapartida evidenciamos que a suplementação apresentou ação antioxidante, imunológica e hepatoprotetora. Nossos resultados chamam a atenção para questões importantes quanto a utilização da BV, e estudos complementares devem ser realizados para sanar dúvidas quanto a influência do aditivo nos parâmetros ruminais e assim eleger a dose ideal nessa fase.

Por fim, o experimento III ressalta os efeitos da alimentação animal na produção de carne. A utilização do resíduo de pré-limpeza de soja (RPLS) não influenciou os parâmetros qualitativos da carne, no entanto o mercado consumidor está cada vez mais exigente e focado na produção sustentável. Não somente na utilização de fontes alimentares alternativas e de menor custo, mas também na produção de carnes funcionais que beneficiem a saúde humana, agregando valor de mercado no produto. Nossos resultados indicaram alterações no perfil lipídico dependentes do nível de inclusão do RPLS; efeitos negativos, pois aumentou acidos graxos saturados que são indesejáveis para consumo humano. Portanto, os resíduos de pré-

limpeza de soja devem ser utilizados com cautela, pois as alterações encontradas podem refletir na qualidade da carne e no índice de aterogenicidade, que são critérios a ser considerados em relação do perfil lipidico e a prevenção de doenças coronarianas em humanos.

Por fim a criação de ovinos de diferentes categorias mostra a importância do aprimoramento dos sistemas de produção. Após a execução desses experimentos os resultados demonstraram a notável resposta que os ovinos oferecem quando há variação do manejo nutricional, relacionado a qualidade da alimentação utilizada. Desta forma, percebe-se a necessidade de um planejamento alimentar nas propriedades que busque sanar as exigências nutricionais de cada categoria. A suplementação de BV foi interessante na cria e na recria de ovinos Lacaune. A utilização de ingredientes alternativos requer cuidados, pois o RPLS apresentou efeito negativo quanto ao perfil lipídico da carne. Embora o foco da pesquisa seja o aumento do desempenho zootécnico e da qualidade da carne, vale ressaltar que nos três experimentos observamos os cuidados que as propriedades tiveram quanto ao manejo reprodutivo e sanitário que da mesma forma visam a saúde dos rebanhos e o aumento dos índices produtivos.

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CARTA DE APROVAÇÃO DO CEUA

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 "Características da carne de cordeiros terminados em confinamento, alimentados com diferentes níveis de resíduo de pré-limpeza de soja.", protocolada sob o CEUA nº 9541281118 (ID 000789), sob a responsabilidade de **Julcemar Dias Kessler** - 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 12/12/2018.

We certify that the proposal "Meat characteristics of lambs finished in confinement, fed with different levels of soybean pre-cleaning residue.", utilizing 32 Ovines (32 males), protocol number CEUA 9541281118 (ID 000789), under the responsibility of **Julcemar Dias Kessler** - 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 12/12/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 12/2018 a 12/2019 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Machos	idade:	60 a 120 dias	N:	32
Linhagem:	Cruzamento Texel e Ile de France			Peso:	15 a 35 kg		

Local do experimento: Propriedade Rural do sudoeste do Rio Grande do Sul. A definir.

Lages, 29 de setembro de 2019

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

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

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 "Efeitos da adição de colina vegetal na dieta de pequenos ruminantes em fase de crescimento para avaliação de desempenho e saúde do fígado", protocolada sob o CEUA nº 8560130319 (ID 000866), 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 12/04/2019.

We certify that the proposal "Effects of plant choline addition on the diet of growing small ruminants for performance and liver health evaluation", utilizing 100 Ovines (males and females), protocol number CEUA 8560130319 (ID 000866), 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 04/12/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2019 a 12/2019 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Machos e Fêmeas	idade:	30 a 90 dias	N:	100
Linhagem:	lacaune			Peso:	12 a 30 kg		

Local do experimento: A pesquisa será realizada na Cabanha Chapecó, criadores de ovelhas da raça Lacaune, localizada no município de Chapecó- SC. Serão montados dois experimentos, conforme descrito a seguir.

Lages, 07 de novembro de 2019

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

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