UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

AVALIAÇÃO DO BAGAÇO DE UVA NA DIETA DE OVELHAS NATURALMENTE INFECTADAS COM NEMATOIDES GASTRINTESTINAIS E DE CORDEIROS INFECTADOS ARTIFICIALMENTE COM *Haemonchus contortus*

MATEUS OLIVEIRA MENA

Botucatu-SP
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Tese apresentada junto ao Programa de Pós-Graduação em Medicina Veterinária para obtenção do título de Doutor.

Orientador: Prof. Dr. Alessandro Francisco Talamini do Amarante
Coorientadores: Prof. Dr. Ricardo Velludo Gomes de Soutello e Profa. Dra. Ana Cláudia Alexandre de Albuquerque

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Impacto Potencial desta Pesquisa

Impacto científico: O bagaço de uva na dieta de ovinos reduz as consequências do parasitismo por *Haemonchus contortus* e melhora parâmetros hematológicos, oferecendo uma alternativa sustentável complementar no controle de nematoides.

Impacto social: Solução acessível para produtores rurais, alinhada aos ODS 2 (Agricultura Sustentável) e 12 (Produção Responsável), ao valorizar resíduos agroindustriais e promover saúde animal.

Impacto econômico: Reduz custos com ração e medicamentos, beneficiando pequenos produtores e fomentando a economia circular.

Potential Impact of This Research

Scientific impact: Grape pomace in the diet of sheep reduces the consequences of parasitism by *Haemonchus contortus* and improves hematological parameters, offering a complementary sustainable alternative in the control of nematodes.

Social impact: Eco-friendly solution for farmers, aligned with SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption), by repurposing agro-waste and improving livestock health.

Economic impact: Reduces costs with feed and medicines, supporting smallholders and circular economy practices.

Nome do Autor: Mateus Oliveira Mena

Título: AVALIAÇÃO DO BAGAÇO DE UVA NA DIETA DE OVELHAS NATURALMENTE INFECTADAS COM NEMATOIDES GASTRINTESTINAIS E DE CORDEIROS INFECTADOS ARTIFICIALMENTE COM *Haemonchus contortus*.

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RESUMO

O controle de nematoides gastrintestinais em ovinos representa um desafio global devido à resistência anti-helmíntica crescente e aos impactos econômicos e ambientais associados ao uso excessivo de fármacos. Esta tese explorou o uso do bagaço de uva da variedade Syrah (*Vitis vinifera* L.), um subproduto agroindustrial rico em compostos bioativos, como alternativa nutracêutica para o controle de parasitoses e a melhoria do desempenho animal. Foram conduzidos dois experimentos complementares: (1) 18 cordeiros Santa Inês recém-desmamados, infectados artificialmente com *Haemonchus contortus*, com um grupo suplementado com 20% de bagaço de uva e um controle, sem o suplemento; e (2) 18 ovelhas lactantes naturalmente infectadas com nematoides gastrintestinais recebendo a mesma suplementação. No primeiro experimento, observou-se redução significativa na contagem de ovos por grama de fezes (OPG) ($P<0,05$) e redução no tamanho dos parasitas fêmeas e na fecundidade dos mesmos, além de incremento nos valores de volume globular (VG) e proteínas plasmáticas totais (PPT). No segundo experimento, a suplementação durante o pico de lactação reduziu temporariamente os valores de OPG ($P<0,05$), melhorando parâmetros hematológicos como VG e eosinófilos. As ovelhas suplementadas mantiveram melhores condições imunológicas durante este período crítico. Em ambos os casos, a inclusão do bagaço de uva na dieta demonstrou efeito positivo no controle parasitário, sem prejuízo ao desempenho zootécnico. Os resultados demonstraram que a inclusão de bagaço de uva na dieta de ovinos é uma estratégia viável para o controle integrado de nematoides, sem comprometer o desempenho zootécnico. Além de contribuir para a sustentabilidade na produção animal, valoriza um subproduto agroindustrial, promovendo a economia circular.

Palavras-chave: imunidade, nutracêuticos, ovinos, ruminantes, verminose.

ABSTRACT

The control of gastrointestinal nematodes in sheep represents a global challenge due to increasing anthelmintic resistance and the economic and environmental impacts associated with the excessive use of pharmaceuticals. This thesis explored the use of grape pomace from the Syrah variety (*Vitis vinifera L.*), an agroindustrial byproduct rich in bioactive compounds, as a nutraceutical alternative for parasite control and improved animal performance. Two complementary experiments were conducted: (1) 18 newly weaned Santa Inês lambs, artificially infected with *Haemonchus contortus*, with one group supplemented with 20% grape pomace and a control group without the supplement; and (2) 18 lactating ewes naturally infected with gastrointestinal nematodes receiving the same supplementation. In the first experiment, a significant reduction in fecal egg count (FEC) ($P<0.05$) was observed, along with a reduction in the size of female parasites and their fecundity, as well as an increase in packed cell volume (PCV) and total plasma protein (TPP) values. In the second experiment, supplementation during peak lactation temporarily reduced FEC values ($P<0.05$), improving hematological parameters such as PCV and eosinophils. The supplemented ewes maintained better immunological conditions during this critical period. In both cases, the inclusion of grape pomace in the diet demonstrated a positive effect on parasite control without compromising zootechnical performance. The results showed that the inclusion of grape pomace in sheep diets is a viable strategy for the integrated control of nematodes, without compromising zootechnical performance. In addition to contributing to sustainability in animal production, it valorizes an agroindustrial byproduct, promoting a circular economy.

Keywords: immunity, nutraceuticals, ruminants, sheep, worms.

CAPÍTULO 1

1. INTRODUÇÃO

O desenvolvimento da criação de pequenos ruminantes é gravemente afetado por inúmeros fatores, dentre eles, perdas significativas nos rebanhos decorrentes de patologias, como infecções causadas por nematoides gastrintestinais, que levam à perda de peso dos animais, queda na produtividade e, em casos mais graves, ao óbito, causando considerável impacto econômico no setor do agronegócio (IDRIS et al., 2012; CHAGAS et al., 2013; AMARANTE, 2014; TOSCANO et al., 2018).

Durante o período do desenvolvimento desses animais, existem algumas categorias em que eles são mais suscetíveis às infecções por nematoides gastrintestinais. O desmame de cordeiros é considerado uma das categorias mais críticas, pois, além do estresse da troca de alimentação e da separação da mãe, ocorre uma abrupta redução no aporte proteico proveniente do leite materno, combinada com o aumento do pastejo. Essa transição nutricional, associada à maior exposição a pastagens contaminadas, resulta não apenas em maior ingestão de larvas infectantes, mas também em menor disponibilidade de nutrientes essenciais para o desenvolvimento e manutenção da resposta imune (AMARANTE, 2014; CAMPBELL et al., 2021; HÖGBERG et al., 2023).

O terço final de gestação e a lactação também são períodos críticos para as matrizes. Nesta fase, ocorre não apenas uma mudança de prioridade na utilização de nutrientes – direcionados para o desenvolvimento fetal e produção de leite em detrimento do sistema imunológico – mas também uma redução significativa no consumo voluntário de alimento devido à compressão abdominal pelo crescimento do útero, que reduz o volume ruminal e limita a capacidade de ingestão, especialmente em dietas volumosas. Essa restrição física, somada às elevadas demandas metabólicas, pode comprometer a capacidade imunológica da ovelha, tornando-a particularmente suscetível a infecções por nematoides gastrintestinais (TAYLOR, 1935; FORBES, 1970; O'SULLIVAN & DONALD, 1970, 1973; HOUDIJK et al., 2003; BRICARELLO et al., 2004; ROCHA et al., 2004, 2011; FLAY, 2022).

Os gêneros de nematoides gastrintestinais de maior ocorrência em ovinos no estado de São Paulo são *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum* spp. e *Strongyloides* spp. (WILMSEN et al., 2014; ALBUQUERQUE et al., 2017; BELLO et al., 2022; BASSETTO et al., 2024; SCHMIDT et al., 2024).

Haemonchus contortus, nematoide hematófago do abomaso, é considerado a espécie mais relevante devido à sua alta patogenicidade, além de apresentar vários relatos de resistência a diferentes classes de anti-helmínticos (ALMEIDA et al., 2010; REINIGER et al., 2017; BASSETTO et al., 2024). Trata-se de espécie altamente prolífica, com capacidade de produzir diariamente cerca de 5.000 ovos que são eliminados para o ambiente nas fezes do hospedeiro (URQUHART et al., 1998; EMERY et al., 2016). Outra espécie relevante é *Trichostrongylus colubriformis,* que forma túneis nas vilosidades do intestino delgado, onde se alimenta do conteúdo das células epiteliais necrosadas. O parasitismo por *T. colubriformis* pode provocar atrofia das vilosidades, erosão epitelial, com alteração no processo de digestão e absorção de nutrientes (CARDIA et al., 2011). Em infecções severas causa grave enterite (ANDRONICOS et al., 2012). Os efeitos patogênicos da infecção por *Cooperia* e *Strongyloides* são similares aos de *T. colubriformis* (AMARANTE, 2005). Já *Oesophagostomum columbianum* penetra profundamente na mucosa do intestino grosso, desencadeando uma resposta inflamatória que resulta na formação de nódulos. Em casos de reinfecção, essa resposta se intensifica, com formação de nódulos, contendo pus esverdeado rico em eosinófilos e uma larva de quarto estágio (L4). A emergência da L4 pode levar à ulceração da mucosa (TAYLOR et al., 2016).

O controle dessas helmintoses é majoritariamente realizado através da administração de anti-helmínticos, tais como albendazol, closantel, levamisol, lactonas macrocíclicas e monepantel (ALMEIDA et al., 2010; MARTINS et al., 2017).

No início da década de 1980, com o lançamento das lactonas macrocíclicas, endectocidas de amplo espectro (avermectinas e milbemicinas), houve uma revolução no mercado de produtos veterinários para o controle de helmintos em que estes começaram a ser largamente utilizados em animais domésticos (GEARY et al., 2005). Porém, com o tempo, várias formulações contendo avermectinas foram liberadas no mercado com preço reduzido levando ao uso indiscriminado e, consequentemente, à seleção de populações resistentes de endo e ectoparasitos (PAIVA et al., 2001). Almeida et al. (2010) demonstraram que os isolados de *H. contortus* e *T. colubriformis* apresentaram resistência anti-helmíntica ao albendazol, levamisol, moxidectina, ivermectina, closantel e triclorfon. Bassetto et al. (2024) evidenciaram que o status da resistência anti-helmíntica em ovinos de rebanhos do estado de São Paulo está em situação alarmante, com nematoides resistentes a múltiplas moléculas anti-helmínticas.

Sendo assim, novas pesquisas têm sido realizadas na busca por controles alternativos, como é o caso de estratégias baseadas em nutrição animal, que visem a redução na utilização de anti-helmínticos, estimulem ou melhorem a resposta imunológica e não causem danos diretos ao ambiente (SYKES e COOP, 2001; KNOX et al., 2006; TORRES-ACOSTA e HOSTE, 2008).

A utilização de resíduos da indústria alimentícia como suplemento na alimentação animal, como o bagaço de uva, pode se constituir em estratégia econômica e sustentável, para a melhoria na qualidade do alimento e como nutracêutico, utilizado na prevenção e controle de infecções parasitárias (ÁVILA et al., 2021; HOSTE et al., 2022). Sua inclusão em dietas para ovinos tem se mostrado viável como substituto parcial de volumosos, mantendo o desempenho animal enquanto oferece benefícios antiparasitários, o que o torna uma alternativa promissora no contexto do controle integrado de nematoides e da produção sustentável (ÁVILA et al., 2021; HOSTE et al., 2022).

Devido à necessidade da busca de alternativas que minimizem o uso de anti-helmínticos tradicionais, enfrentando a crescente resistência dos helmintos aos anti-helmínticos e promovendo a sustentabilidade na produção animal, o presente estudo teve como objetivo avaliar os eventuais benefícios para a saúde e desempenho de ovelhas naturalmente infectadas com nematoides gastrintestinais e de cordeiros infectados artificialmente com *H. contortus* suplementados com bagaço de uva Syrah (*Vitis vinifera L.*).

2. REVISÃO DE LITERATURA

2.1 IMPACTO DAS PARASITOSES NA PRODUÇÃO DE OVINOS

Uma das principais causas de perdas de produção em ovinos são as infecções causadas por nematoides gastrintestinais, contribuindo para a diminuição do ganho de peso, menor desempenho reprodutivo, aumento das demandas nutricionais devido à resposta imunológica necessária para combater os parasitas e aumento das taxas de mortalidade (ŠTRBAC et al., 2023; GEDDES et al., 2024).

Além disso, as infecções podem reduzir significativamente a disponibilidade de proteína, exacerbando ainda mais os desafios em atender às necessidades nutricionais de ovelhas durante períodos fisiológicos críticos, como o final da gestação e lactação (LIU et al., 2003).

Ovelhas infectadas contaminam as pastagens, agravando o problema para cordeiros, que são particularmente vulneráveis a estes parasitas (WILLIAMS et al., 2024).

Animais infectados artificialmente com *H. contortus* e *T. colubriformis* apresentaram redução de 13,7% no consumo de alimentos, redução de 21,3% no ganho de peso corporal diário e redução de 16,6% no peso da carcaça fria, em comparação com os controles não infectados (CARVALHO et al., 2021).

Em relação ao ganho de peso de cordeiros criados em pastagem e que recebiam suplementação, cordeiros que recebiam tratamento supressivo com anti-helmíntico e eram mantidos livres de infecção por nematoides gastrintestinais apresentaram ganho de peso corporal diário 17,1% maior que os grupos infectados naturalmente e que eram tratados seletivamente. Nesse mesmo estudo, em animais mantidos com suplementação basal, a diferença foi de 26,7% no ganho de peso corporal diário (STARLING et al., 2019).

Além da perda de peso, animais infectados com nematoides gastrintestinais tendem a apresentar queda nos níveis de hematócrito devido à espoliação sanguínea, principalmente em infecções por *H. contortus*, o que está diretamente correlacionado com a redução da produtividade (ALBERS et al., 1990).

A abordagem terapêutica convencional geralmente envolve a aplicação de anti-helmínticos, frequentemente sem prescrição de um médico veterinário e sem avaliação dos animais ou investigações parasitológicas (CASTAGNA et al., 2021; ŠTRBAC et al., 2022). A falta de assistência técnica induz ao não seguimento das boas práticas clínicas e preventivas, gerando um grande problema, pois a intervenção humana visa reduzir substancialmente a carga parasitária, utilizando grandes quantidades de substâncias químicas, muitas vezes em tratamentos desnecessário, as quais causam sérios impactos ambientais e riscos à saúde pública.

O uso excessivo desses anti-helmínticos levou à resistência, complicando os esforços de controle e aumentando os custos de produção (ŠTRBAC et al., 2023).

Estudos de Geddes et al. (2024) na Escócia indicam que das nove fazendas de ovinos que participaram do teste de eficácia anti-helmíntica, todas apresentaram resistência a pelo menos um grupo anti-helmíntico estudado, destacando a necessidade de novas estratégias. Similar ao observado no estado de São Paulo, que ao avaliar 15 fazendas de criação de ovinos, observou-se que a eficácia dos diferentes grupos químicos anti-helmínticos (albendazol, ivermectina, levamisol, monepantel, closantel e moxidectina) variou entre as propriedades, porém com todas apresentando resistência a dois ou mais princípios ativos. Dentre os medicamentos testados, apenas o monepantel demonstrou redução na contagem de OPG, com eficácia ≥95% em cinco das 15 fazendas avaliadas. Essa maior eficácia do monepantel se deu provavelmente devido ao seu uso limitado no Brasil, devido ao alto custo que restringiu sua adoção (BASSETTO et al., 2024), reforçando a relação entre a pressão de seleção pelo uso recorrente e o desenvolvimento de resistência.

No Brasil, Chagas et al. (2022) avaliaram o impacto econômico de nematoides gastrintestinais em ovinos Morada Nova e observaram que o tratamento rotineiro (a cada 42 dias) aumentou o ganho de peso diário (0,101 kg/dia) e a receita bruta (US$ 1.946,92 por grupo) em comparação ao tratamento seletivo ou ao grupo controle sem tratamento cujos animais alcançaram pesos finais de 22,28 kg e 20,94 kg, respectivamente.

No entanto, o tratamento rotineiro acelerou o desenvolvimento de resistência ao levamisol, inviabilizando sua sustentabilidade. Em contraste, o tratamento seletivo, que tratou apenas 27,2% dos animais (com base no desempenho zootécnico), manteve resultados econômicos próximos (diferença de 1,9% na estação seca) e reduziu a pressão de seleção para resistência reforçando sua adoção em sistemas de produção intensivos.

Embora os nematoides gastrintestinais representem desafios significativos, a principal forma de controle utilizada atualmente é o uso de antiparasitários (STARLING et al., 2019). No entanto, existem outras estratégias alternativas, como:

- Vacinas: alternativa promissora para reduzir a dependência de anti-helmínticos, estimulando a resposta imune do hospedeiro. Estudos com a vacina Barbervax® (BASSETTO et al., 2018) mostraram que a suplementação nutricional em ovelhas vacinadas potencializou a proteção, reduzindo a necessidade de tratamentos durante o período periparto.

- Suplementação proteica: melhora a resistência e resiliência de ovinos às infecções. Dietas com 19% de proteína bruta reduziram significativamente a contagem de OPG e a carga parasitária ($P < 0,05$) em cordeiros Santa Inês durante a estação chuvosa, além de aumentar o ganho de peso (LOUVANDINI et al., 2006).

- Tratamento seletivo: visa tratar apenas animais com sinais clínicos ou com valor de OPG alto, reduzindo o uso de anti-helmínticos e a pressão de seleção para resistência. Critérios como escore de condição corporal e sistema Famacha© podem ser utilizados quando não é possível realizar o exame de OPG para identificar ovelhas que necessitam de tratamento, especialmente em ovelhas periparturientes (WILLIAMS et al., 2024; DE CARVALHO et al., 2023).

- Seleção genética: identificação de ovinos resistentes por meio de marcadores genéticos. Recentemente, Niciura et al. (2024) identificaram regiões genômicas associadas à resistência a *H. contortus* em ovinos Morada Nova, incluindo loci nos cromossomos 2 e 11 que abrigam genes candidatos envolvidos na resposta imune, adaptação e peso corporal (como ALOX15, CXCL16 e DHRS9), reforçando o potencial de marcadores genéticos para seleção de animais resistentes.

- Controle biológico: fungos nematófagos, como *Duddingtonia flagrans* (Bioverm®), reduziram em 74,6% a contagem de OPG e em 89,3% as larvas nas pastagens, além de melhorar o ganho de peso (RODRIGUES et al., 2022).

- Preparações botânicas: extratos de plantas como *Moringa oleifera* e *Azadirachta indica* apresentaram atividade anti-helmíntica significativa contra nematoides gastrintestinais em ovinos (RAFIQUE et al., 2022). Além disso, bioativos como taninos condensados, taninos hidrolisáveis e saponinas foram identificados como eficazes no controle de nematoides em pequenos ruminantes (OLANREWAJU et al., 2023).

Conforme destacado por Štrbac et al. (2023), a combinação dessas estratégias em uma abordagem integrada é essencial para o controle sustentável de nematoides gastrintestinais, reduzindo a dependência de anti-helmínticos e mitigando o impacto na produção ovina.

2.2 IMPORTÂNCIA DA NUTRIÇÃO PARA O DESENVOLVIMENTO DO SISTEMA IMUNOLÓGICO

O manejo nutricional tem sido cada vez mais reconhecido como uma ferramenta crucial para o controle eficiente das infecções por nematoides gastrintestinais. A nutrição adequada aprimora a capacidade dos ovinos de debelar a infecção por nematoides gastrintestinais, ao fortalecer a função imunológica (CRILLY, 2023).

A criação de ovinos é predominantemente realizada em sistemas de produção extensivos, dependendo do uso de pastagens para a alimentação animal, porém, uma vez que as larvas de terceiro estágio (L3) estão presentes nas pastagens esse tipo de sistema de produção favorece infecção por nematoides gastrintestinais (HOSTE et al., 2016; GEDDES et al., 2024).

Cordeiros sob dieta basal, que receberam apenas uma pequena quantidade de concentrado, pastejam em média 1,7 horas a mais que os cordeiros suplementados. Consumindo mais forragem, eles possivelmente ingerem um número maior de larvas infectantes que os cordeiros suplementados. Portanto, ao fornecer dieta de melhor qualidade associada a menor exposição a infecções, a suplementação de concentrado favorece o desempenho de cordeiros, que atingem, antes de completarem seis meses de idade, peso médio de 40 kg, considerado a meta de peso corporal ideal para a raça Dorper (STARLING et al., 2019).

Infecções mistas por *H. contortus* e *T. colubriformis* causam diminuição do consumo de alimentos e redução do desempenho produtivo, independentemente da dieta, entre cordeiros jovens; e dietas ricas em nutrientes não só aumentam a resiliência dos animais como também permitem reduzir o tempo necessário para que os cordeiros atinjam pesos ideais de carcaça

(CARVALHO et al., 2021).

Em cordeiros submetidos a diferentes tipos de dietas, com quantidades de concentrados diferentes, foi possível observar que animais que receberam maior quantidade de concentrado apresentaram respostas imunes mais robustas, com eosinófilos sanguíneos e produção de imunoglobulinas G anti-L3, o que influenciou na redução da carga parasitária (CARVALHO et al., 2023). Já em borregas com idade inicial de 8 a 12 meses naturalmente infectadas com *Haemonchus* spp., a melhoria nas condições da pastagem levou a estas aos mesmos benefícios, além de níveis mais altos das citocinas: interleucina-6 (IL-6), fator de necrose tumoral beta (TNF-β), interferon-gama (IFN-γ) (TOSCAN et al., 2017).

A suplementação rica em proteínas demonstrou aumentar a produção de imunoglobulinas (IgA e IgG) em ovelhas lactantes, indicando resposta imune superior contra nematoides gastrintestinais (CHAUDHARY et al., 2023).

O sistema imunológico dos ovinos combate infecções por nematoides gastrintestinais por meio da imunidade humoral e celular, mediada pela resposta de células Th2. Essa resposta envolve a ativação de linfócitos, produção de interleucinas (como IL-4, IL-5 e IL-13), imunoglobulinas (IgE, IgA e IgG), além do recrutamento de células efetoras como mastócitos, leucócitos globulares e eosinófilos para o local da infecção. A resposta humoral, particularmente a produção de IgG, desempenha um papel crucial na defesa, sendo influenciada por fatores como suplementação nutricional. A eficiência dessa resposta é também modulada pelo estado nutricional, idade, raça e condição fisiológica do hospedeiro (HENDAWY, 2018; ALBUQUERQUE et al., 2019; GONZÁLEZ-GARDUÑO et al., 2021; LINS et al., 2022; 2023; STEAR et al., 2023). Estudos recentes demonstraram que ovinos resistentes, como os da raça Santa Inês, desenvolvem uma resposta humoral robusta e uma ativação precoce de mecanismos celulares, incluindo a apresentação de antígenos e a proliferação de células B, que contribuem para a redução da carga parasitária (LINS et al., 2025).

2.3 NUTRACÊUTICOS

O termo “nutracêutico” é uma junção das palavras “nutrição” com “farmacêutico”. Também é descrito como "alimento funcional", que foi introduzido pela primeira vez no contexto da saúde humana (HASLER, 1998). Na ciência veterinária o nutracêutico pode ser definido como alimento que combina valor nutricional com benefícios para a saúde animal, além da nutrição básica (HOSTE, 2015).

O uso de nutracêuticos é favorecido devido à relação custo-benefício, segurança e facilidade de acesso em comparação com medicamentos sintéticos (GUPTA et al., 2021). No entanto, desafios como controle de qualidade e supervisão regulatória persistem, impactando sua eficácia e segurança (FINNO, 2020).

A maioria das pesquisas até hoje focou na atividade anti-helmíntica de determinadas plantas que contêm taninos condensados (HOSTE et al., 2012; 2015; QUIJADA et al., 2015), embora existam outros compostos secundários como saponinas, flavonoides, terpenóides e alcaloides (SPIEGLER et al., 2017; VÁRADYOVÁ et al., 2018; MRAVČÁKOVÁ et al., 2019).

Esses compostos funcionam por meio de vários mecanismos de ação, incluindo a estabilização de comunidades microbianas comensais ou probióticas tanto no rúmen quanto no trato gastrintestinal inferior, melhorando a resposta imunológica da mucosa e a função de barreira do trato gastrintestinal, ligando ou adsorvendo patógenos ou toxinas, melhorando a capacidade antioxidante, atividade antimicrobiana direta e aumentando ou diminuindo as respostas imunológicas sistêmicas (BALLOU et al., 2019).

Alguns trabalhos mostraram que saponinas e taninos são compostos que demonstraram potencial para melhorar a eficiência reprodutiva e controlar nematoides gastrintestinais (ROCHFORT et al., 2008); suplementos alimentares microbianos, como por exemplo, *Streptococcus bovis* e *Bacillus subtilis,* melhoram a saúde do rúmen, estabilizam o pH, melhoram a digestibilidade de fibras e nutrientes (AZZAZ et al., 2015).

Bactérias fibrolíticas como *Fibrobacter succinogenes* e *Ruminococcus flavefaciens* são particularmente importantes para a digestão de fibras, enquanto *Archaeas metanogênicas* estão associadas à produção de metano (HUWS et al., 2018). Ku-Vera et al. (2020) destacam que metabólitos secundários de plantas, como taninos, saponinas, óleos essenciais e flavonoides, reduzem as emissões de metano em ruminantes ao modular o microbioma ruminal. Esses compostos inibem arqueias metanogênicas, reduzem protozoários e redirecionam hidrogênio para a produção de ácido propiônico. Leguminosas tropicais, como *Leucaena leucocephala*, mostraram reduções de até 50% nas emissões de metano. Adicionalmente, compostos bioativos, como óleos essenciais e peptídeos, têm sido investigados por sua capacidade de inibir bactérias metanogênicas e promover bactérias produtoras de propionato, como *Prevotella* **e** *Selenomonas*, que competem por substratos com os microrganismos produtores de metano.

Uma revisão sistemática no México identificou 13 espécies de plantas com compostos secundários benéficos que melhoram a nutrição e reduzem infecções parasitárias em pequenos ruminantes (TORRES-FAJARDO et al., 2021).

Um aditivo alimentar poli-herbal composto por *Achyrantes aspera*, *Trachyspermum ammi*, *Azadirachta indica* e *Citrullus colocynthis* melhorou efetivamente a saúde e a produtividade de pequenos ruminantes ao melhorar o ganho de peso diário, a produção de leite e os indicadores metabólicos sanguíneos do metabolismo de proteínas (proteína total, globulinas e ureia) (MENDOZA-MARTÍNEZ et al., 2024). De maneira similar, os óleos essenciais têm se mostrado nutracêuticos eficazes, não apenas impulsionando o ganho de peso e a produção de leite, mas também aprimorando a qualidade da carne de pequenos ruminantes. Além disso, esses óleos ajudam a reduzir o estresse oxidativo e as emissões de metano, contribuindo para uma produção mais sustentável e com menor impacto ambiental (DORANTES-ITURBIDE et al., 2022).

Os subprodutos agroindustriais consistem em resíduos de culturas agrícolas ou indústrias de processamento de vegetais, apresentando um sério desafio para o meio ambiente na sua grande maioria (GARCÍA-RODRÍGUEZ et al., 2019). Segundo o Relatório de Desperdício de Alimentos de 2021 do Programa das Nações Unidas para o Meio Ambiente (PNUMA), cerca de 17% da produção anual de alimentos é desperdiçada, sendo que 45 a 60% desse total ocorre em decorrência do processamento industrial e das atividades pós-colheita (PNUMA, 2021).

Nesse contexto, a reutilização desses resíduos surge como uma alternativa sustentável. Um exemplo promissor é o estudo de Domingues et al. (2013), que avaliou o resíduo industrial do abacaxi (*Ananas comosus*) no controle de *H. contortus* em ovinos. Os resultados demonstraram uma eficácia de 42,2% na redução de ovos por grama de fezes (OPG) *in vivo*, além de destacar o potencial desse resíduo para diminuir a contaminação ambiental quando reaproveitado na alimentação animal. Essa abordagem não apenas mitiga o desperdício, mas também oferece uma solução economicamente viável e ecologicamente consciente para o setor agropecuário.

Muitos subprodutos não são transformados em produtos de valor agregado, levando ao desperdício em vez da otimização de recursos (STEWART et al., 2024). A redução de resíduos industriais é uma tendência mundial que tem levado a estudos sobre o uso deste material na alimentação animal (DE CASTRO et al., 2023).

Embora esses nutracêuticos sejam promissores, é essencial considerar os riscos potenciais associados ao seu uso, como a necessidade de mais pesquisas sobre a segurança de partículas ultrafinas e os efeitos de longo prazo dos aditivos fitobióticos na saúde e produtividade animal (AHMED et al., 2024; LUTKOVSKAYA et al., 2024).

2.4 USO DO BAGAÇO DE UVA

O bagaço de uva, um subproduto da indústria vinícola, tem recebido crescente atenção como um ingrediente alternativo. Sua utilização é promissora não apenas para reduzir custos de alimentação, mas também para promover a sustentabilidade, ao reaproveitar resíduos que, de outra forma, seriam descartados (KALLI et al., 2018; TAYENGWA e MAPIYE, 2018).

Diferentes cultivares de uva apresentam perfis polifenólicos e atividades antioxidantes distintos. Por exemplo, as uvas tintas, como *Cabernet Sauvignon* e *Merlot*, demonstram conter níveis mais elevados de compostos fenólicos específicos em comparação com bagaços de uvas brancas, como a *Muscat Ottonel* (ONACHE et al., 2022). A cultivar “Vinhão”, de Portugal, destaca-se pelo alto teor de fibras alimentares e níveis significativos de compostos fenólicos, tornando-a adequada para enriquecimento nutricional (MACHADO et al., 2024).

A incorporação do bagaço de uva seco e moído em alimentos e rações é uma alternativa viável para reduzir o impacto ambiental e adicionar valor ao subproduto (MACHADO et al., 2024). O método de extração influencia o rendimento e a qualidade dos compostos bioativos, sendo que diferentes técnicas são otimizadas para perfis fenólicos específicos (LOPES et al., 2025). Tecnologias simples ou até mais avançadas, como a extração, podem recuperar compostos fenólicos valiosos do bagaço de uva, que podem ser usados em produtos alimentícios, aumentando seu valor nutricional (ALMANZA-OLIVEROS et al., 2024). A origem geográfica e as condições climáticas, frequentemente denominado “efeito da safra”, também desempenham um papel crucial na determinação da composição química do bagaço de uva. Esses fatores podem levar a variações nas propriedades antioxidantes e no teor de polifenóis do bagaço (GUAITA et al., 2023).

Ele é constituído por sementes, polpa residual e engaços dos cachos que são ricos em fibras alimentares (49,37%) e compostos fenólicos (35,35 mg de ácido gálico equivalente por grama (AGE/g)) (MACHADO et al., 2024). Adicionalmente, representa fontes ricas de compostos bioativos com propriedades antioxidantes, a saber, carotenoides e polifenóis (POPOVICI et al., 2024). Também é rico em fenólicos, com um teor total de 3,95 mg/g (equivalente de ácido tânico) em 100 mg de bagaço seco, e taninos com um teor total de 1,92 mg/g (equivalente de ácido tânico) em 100 mg de bagaço seco. O extrato hidroalcoólico demonstrou possuir atividade anti-helmíntica *in vitro*, inibindo com sucesso a eclosão, o desenvolvimento e a motilidade de estágios de vida livre de *H. contortus* (SOARES et al., 2018).

Os taninos e polifenóis do bagaço de uva reduzem a produção de metano ao inibir arqueas metanogênicas e protozoários ruminais, além de modular a fermentação ruminal para aumentar a produção de propionato, um ácido graxo energeticamente mais eficiente. Essa mudança metabólica não compromete a produtividade, pois a energia poupada é direcionada para o crescimento animal, como observado na melhoria da conversão alimentar (MOATE et al., 2014; GRAZZIOTIN, 2017;). O bagaço de uva seco melhora a digestibilidade de nutrientes em ovinos, sem afetar negativamente o perfil bioquímico sanguíneo (JURÁČEK et al., 2021).

A substituição do feno por bagaço de uva em até 300 g/kg MS na dieta não afetou negativamente o consumo de ração ou o desempenho de cordeiros em crescimento (AMARAL et al., 2019). Além disso, a menor quantidade de fibra detergente neutro (FDN) e a maior digestibilidade da dieta com bagaço de uva reduziram o tempo de ruminação, liberando mais tempo para repouso (ÁVILA et al., 2021). Esse efeito está associado à composição do subproduto, que, por ser menos fibroso e mais rico em compostos bioativos (ex.: polifenóis e taninos), promove uma fermentação ruminal mais eficiente, com maior produção de propionato e redução de metano, favorecendo a saúde digestiva e o equilíbrio energético dos animais (MOATE et al., 2014).

Economicamente, a combinação de resíduos de uva desidratados com fontes de energia produziu uma análise de custo/benefício com indicadores financeiros favoráveis, com o melhor desempenho associado à farinha de forragem de palma combinada com bagaço de uva (BARROSO et al., 2007).

Assim, seu aproveitamento na pecuária alia benefícios ambientais, econômicos e sanitários, especialmente em regiões com alta disponibilidade do subproduto, como o Sul do Brasil (IBGE, 2023). Dessa forma, o aproveitamento desse coproduto agroindustrial alinha-se tanto aos princípios da economia circular quanto à necessidade de estratégias inovadoras no controle de parasitos resistentes.

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Evaluation of Grape Pomace Supplementation in Lamb Diets to Mitigate *Haemonchus contortus* Infection

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Abstract: This study aimed to evaluate the potential benefits and feasibility of adding dried and ground grape pomace to the concentrate fed to lambs experimentally infected with *Haemonchus contortus*. Eighteen male Santa Inês lambs, recently weaned, were housed in individual pens and were allocated into two groups based on their body weight. The lambs in the supplemented group ($n = 9$) initially received a diet composed of 50% ground hay and 50% concentrate. Subsequently, these animals were gradually adapted to grape pomace until its final inclusion in the concentrate reached 20%. The lambs in the control group received a concentrate without grape pomace. Both groups of lambs were artificially infected with 4000 infective larvae of *H. contortus*, and 28 days later, the lambs were euthanized for quantification of the nematodes present in the abomasum. The following variables showed no statistical differences ($p > 0.05$) between the groups: worm burden, packed cell volume, total plasma protein, blood eosinophil count, and daily weight gain. Regarding anti-*Haemonchus* IgG plasma levels, there was a significant time * treatment interaction ($p = 0.0099$) with higher values in the supplemented group. At the two final samplings, the supplemented group showed significantly lower values of eggs per gram of feces than the control group ($p < 0.05$). The supplemented group showed female worms shorter and with less eggs in utero than those of the control group, with significant difference for these variables ($p < 0.05$). In conclusion, grape pomace can be included in the diet, as it promotes more sustainable animal production, and, additionally, it can cause a reduction in *H. contortus* fecundity, benefiting haemonchosis prophylaxis.

Keywords: nutraceutical; nematodes; nutrition; ruminants; sheep

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1. Introduction

Significant economic losses in sheep production result from infections caused by gastrointestinal nematodes (GINs). The weaning period for lambs is considered one of the most critical stages, as it involves not only the stress of dietary transition but also separation from the mother and an increase in grazing activity. These factors lead to a higher ingestion of infective larvae [1–3]. Consequently, animal development is impaired, and in some cases, an increased mortality rate is observed [1,4–6].

Among GIN, *Haemonchus contortus* stands out as the most significant species due to its hematophagic nature and high biotic potential, which confer high pathogenicity. This species is highly prolific, capable of producing between 5000 and 15,000 eggs per day, which are excreted into the environment where they give rise to infective third-stage larvae [7,8].

Control of these helminthiases is primarily achieved through the administration of anthelmintics such as albendazole, closantel, levamisole, macrocyclic lactones, and monepantel [9,10]. However, due to indiscriminate use, anthelmintic resistance has increased over recent decades, with frequent reports of flocks harboring nematode populations resistant to multiple anthelmintic compounds worldwide [11–15].

The genetic diversity and rapid life cycle of *H. contortus* facilitate the selection and dissemination of resistant genotypes, further complicating control efforts [16]. This resistance not only reduces treatment efficacy but also increases the economic losses on farmers due to production reduction and the need for alternative management strategies. Addressing this issue requires integrated approaches, including targeted selective treatment, pasture management, and the development of novel anthelmintics or vaccines [17].

Therefore, alternative control measures are necessary to reduce anthelmintic usage, minimize environmental residue discharge, and enhance or stimulate the immune response [17–19]. The current research status of other natural anthelmintic methods comprises a variety of innovative strategies aimed at controlling gastrointestinal nematodes. These methods include the use of herbal treatments [20], biological control agents [21,22], and integrated management practices [22], which collectively offer promising alternatives to traditional chemical treatments. In this context, approaches involving improved nutrition to intensify the resistance and resilience of animals to GIN infections have been explored.

Grape pomace, a by-product of the winemaking industry, has gained increasing attention as an alternative ingredient in ruminant feed formulations. Its use is promising not only for reducing feeding costs but also for promoting sustainability by repurposing waste that would otherwise be discarded [23,24]. The incorporation of dried and ground grape pomace into feed and rations is a viable strategy to reduce environmental impact and add value to this by-product [25]. A study reports on the application of grape pomace in animal nutrition, where they found that grape pomace is safe and can be added to ruminant feed at rates of up to 100 g/kg/day as a good nutritional source [26].

Grape pomace consists of seeds, residual pulp, and stems, which are rich in dietary fiber (49.37%) and phenolic compounds (35.35 mg of gallic acid equivalents per gram (GAE/g)) [25]. Additionally, it is a rich source of bioactive compounds with antioxidant properties, including carotenoids and polyphenols [27]. Grape pomace from the IAC 138-22 'Máximo' variety, produced in São Paulo State, was found to be rich in phenolics, with a total content of 3.95 mg/g (tannic acid equivalent) per 100 mg of dried pomace, corresponding to 4.2% total phenolic content in the dry matter, and tannins with a total content of 1.92 mg/g (tannic acid equivalent) per 100 mg of dried pomace, corresponding to 2.03% total tannin content in the dry matter. The hydroalcoholic extract of this grape pomace demonstrated in vitro anthelmintic activity, successfully inhibiting the hatching, development, and motility of *H. contortus* free-living stages [28].

Given the need to identify alternatives that reduce the reliance on conventional anthelmintics to prevent anthelmintic resistance, and promote sustainability in livestock production, the present study aimed to evaluate the potential benefits of adding ground grape pomace to the feed of newly weaned lambs artificially infected with *H. contortus*. By

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exploring the potential use of grape pomace, this study seeks not only to improve the health, welfare, and performance of small ruminants but also to add value to an agro-industrial by-product frequently discarded.

2. Materials and Methods

2.1. Location, Animals, and Experimental Design

All procedures involving animals in this study comply with international ethical standards and were approved by the Animal Ethics Committee of FMVZ/UNESP (0542/2023).

The experiment was conducted in the experimental area of the Veterinary Helminthology Laboratory, Institute of Biosciences (IBB), São Paulo State University (UNESP), located in the city of Botucatu, São Paulo, Brazil (22°53′16″ S and 48°29′57″ W, altitude of 884 m), during the period from October 2023 to December 2023 (spring/summer).

Eighteen male Santa Inês lambs used in this study remained with their mothers from birth until weaning in paddocks with pastures consisting of *Urochloa decumbens* and *Cynodon* spp. The paddocks were equipped with water troughs and feeders for ad libitum mineral salt supplementation and concentrate feed, which the ewes received daily in amounts equivalent to 2% of their live weight. The lambs had access to the feeders alongside their mothers and began consuming small amounts of feed as weaning approached. At 60 days of age, the lambs were weaned and placed in individual pens where they remained throughout the experiment. The pens were equipped with water troughs and feeders for daily supplementation with concentrate and ground hay.

Initially, all animals were fed the same diet for 10 days based on their live weight, consisting of 2% ground hay and 2% concentrate. Additionally, the animals had free access to mineral salt provided in a separate feeder. The concentrate feed was composed of 70% ground corn and 30% soybean meal, and the daily ration was divided into two portions, one offered in the morning and the other in the afternoon, following the Nutrient Requirements of Small Ruminants (NRC) recommendations [29].

The lambs were evenly distributed into two groups of 9 animals each, based on body weight, in a completely randomized design. On day −27 (D−27) all animals were housed and treated with levamisole (10 mg/kg; Ripercol® L solution; Zoetis, Campinas, Brazil) and albendazole (20 mg/kg; Valbazen® 10; Zoetis, Campinas, Brazil) for three consecutive days to eliminate natural nematode infections (Table 1).

Table 1. The timeline of the animal experiment with lambs artificially infected with *Haemonchus contortus* and supplemented or not with grape pomace.

	Days									
	−27	**−26**	**−25**	**−17**	**0**	**7**	**14**	**21**	**28**	**29**
Anthelmintic treatment	✓	✓	✓							
Starting of grape pomace supplementation				✓						
Artificial infection					✓					
Sampling days					✓	✓	✓	✓	✓	
Euthanasia										✓

Eight days after the last administration of anthelmintics (D−17), the supplemented group was gradually adapted to grape pomace until the final amount represented 20% of the concentrate. The dried and ground grape pomace was mixed with the concentrate (Table 2) and provided to the animals. Lambs in the control group received concentrate

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(Table 2) without grape pomace. Diets were formulated with distinct proportions of different ingredients to ensure similar protein-energy intake among animals.

Table 2. The composition of the concentrate supplied to lambs in the supplemented group with grape pomace and the control group.

Group	Ingredient (%)		
	Corn	Soybean Meal	Grape Pomace
Supplemented	34.4	45.6	20
Control	53.0	47.0	0

After the diet adaptation period (D0), all lambs were artificially infected with 4000 L3 larvae of *H. contortus* isolate via oral administration.

From day zero (D0), animals of both groups received ground hay ad libitum. Voluntary feed and ground hay intake for each animal was measured daily by calculating the difference between the weight of the diet offered and the weight of the leftovers collected the following morning.

2.2. Preparation of Dried and Ground Grape Pomace

Syrah (*Vitis vinifera* L.) grapes intended for wine production were harvested in September 2022. The grape pomace was obtained from a winery in São Roque, São Paulo State, Brazil (23°35′41.2″ S and 47°09′41.6″ W, altitude 880 m). The pomace was dried in an air-circulation oven (50 °C) for approximately 24 h, ground, and stored in a freezer (−20 °C) until use.

2.3. Production of Infective Haemonchus contortus Larvae

Two Santa Inês lambs were housed in a pen with no access to pasture and fed concentrate and ground hay. To eliminate natural nematode infections, both were treated with levamisole (10 mg/kg; Ripercol® L solution; Zoetis, Campinas, Brazil) and albendazole (20 mg/kg; Valbazen® 10; Zoetis, Campina, Brazil) for three consecutive days. Fourteen days after the last anthelmintic treatment, nematode elimination was confirmed through eggs per gram of feces (EPG) using the McMaster technique [30]. The lambs were experimentally infected via the oral administration of 4000 third-stage larvae (L3) of an *H. contortus* isolate susceptible to albendazole, ivermectin, and levamisole [31]. After 21 days, feces were cultured [32] at 26 °C for 7 days, and L3 larvae were recovered, stored at room temperature, and used for artificial infection.

2.4. Measurement of Total Phenolics and Tannins

The concentrations of total phenols (TP) were measured using the Folin–Ciocalteu reagent method, as described by Makkar [33]. Total tannins (TT) were quantified by calculating the difference between TP concentrations before and after treatment with insoluble polyvinylpolypyrrolidone, following the protocol of Makkar [33,34], with tannic acid serving as the standard. The levels of condensed tannins (CT) were assessed using the butanol-HCl method, also outlined by Makkar [33], with leucocyanidin employed as the reference standard.

2.5. Fecal Collection

The first fecal sample was collected on D−17, and subsequent collections occurred every 7 days starting from D0. Samples were obtained directly from the rectum of each animal for fecal egg counts (FECs) following Ueno and Gonçalves [30].

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2.6. Hematological Examinations

Blood samples were collected on D−17 and every 7 days thereafter, via jugular vein puncture into EDTA-coated vacutainer tubes. Packed cell volume (PCV) was determined by centrifugation in microhematocrit tubes. Total plasma protein (TPP) was measured via refractometry (SPR-Atago). Eosinophil counts were conducted using a Neubauer chamber after staining with Carpentier's solution [35] and expressed as the number of eosinophils per µL of blood. Plasma samples were stored at −20 °C for further analysis.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA)

Specific Immunoglobulin G (IgG) against *H. contortus* L3 was quantified in stored plasma samples using a soluble extract from L3 larvae, as described by Amarante et al. [36]. ELISA procedures were carried out according to Albuquerque et al. [37]. Negative control samples were obtained from worm-free animals [38], and positive controls were obtained from sheep naturally infected with *H. contortus* and *T. colubriformis*. Results were expressed as the optical density (OD) ratio of the sample to the positive control [39].

2.8. Bromatological Analyses

Samples (500 g) of ground hay, concentrate, and grape pomace were collected and stored in a freezer until analysis. The composition analysis included dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash content, following the Association of Official Analytical Chemists [40] procedures.

2.9. Body Weight Gain

Animals were weighed on D−17 and every 7 days in the morning, before being fed, to monitor productive performance and calculate average daily weight gain until the end of the study.

2.10. Determination of Parasitic Load and Measurement of Worms

All animals were humanely slaughtered on D29 at a slaughterhouse. The abomasum was removed and opened, and its contents were collected in graduated buckets. Ten percent aliquots of the total content were preserved with 5% formaldehyde. Helminths were quantified and identified based on their developmental stage [30].

Five female and five male specimens of *H. contortus* were randomly collected from each animal for the measurement of adult parasite length and the egg count of females. The measurement of adult parasites was performed using a stereomicroscope and a ruler. To quantify the eggs inside the females, each specimen was placed in a polyethylene microtube containing 800 µL of deionized water and 200 µL of sodium hypochlorite solution with 0.25% active chlorine. The tube was agitated twice for 30 s using a vortex mixer, with a 2 min interval between agitations. The number of eggs per mL was estimated by observing 10 drops of 10 µL each, distributed on a slide, and examined under 100× magnification using a microscope. The total count was extrapolated from this observation.

2.11. Statistical Analysis

Statistical analyses were performed using the Statistical Analysis System version 9.4 (SAS Institute, Inc., Cary, NC, USA). Variables with repeated measures were analyzed using the PROC MIXED procedure. Averages were considered significantly different when $p < 0.05$. Data were tested for normality and transformed (log10 (x + 1)) when necessary (EPG, blood eosinophil counts, ELISA data). Means of variables with a single measurement (worm burden, worm length, and number of eggs per female worm) were compared using a T-test at a 5% significance level. To simplify interpretation, the Results section presents the arithmetic means of the non-transformed data (±standard error). Only significant interactions between time and treatment are presented.

3. Results

A total of 91 kg of wet grape pomace was obtained, which decreased to 28 kg after drying, representing a 31% reduction. Regarding grape pomace consumption, only one lamb (No. 9) from the supplemented group did not consume the entire concentrate mixed with grape pomace during the first two days. On the first day, 57% (0.235 kg) of the mixture (concentrate + grape pomace) remained in the trough, and on the second day, 25% (0.105 kg) was left. Although the supplemented group consumed more ground hay weekly compared to the control group, no significant difference was observed (Tables 3 and S1, Supplementary Materials).

Table 3. Average daily consumption in grams of ground hay (±standard error) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Days	Supplemented (*n* = 9)	Control (*n* = 9)	*p*-Value
D−17–D−11	277.3 ± 27.72	220.5 ± 44.26	0.2924
D−10–D−4	359.5 ± 23.06	292.8 ± 46.55	0.2172
D−3–D0 *	454.6 ± 33.25	360.6 ± 56.71	0.1497
D1–D7	418.0 ± 41.85	326.1 ± 47.44	0.2314
D8–D14	543.9 ± 46.55	443.9 ± 72.53	0.1930
D15–D21	555.6 ± 32.69	482.6 ± 63.68	0.3398
D22–D28	516.1 ± 46.70	491.7 ± 64.93	0.7488

Legend: * Artificial infection with 4000 infective larvae of *Haemonchus contortus*.

Despite anthelmintic treatments, some animals continued shedding eggs in their feces, although with low EPG values. On D−17, two animals in the supplemented group presented 100 EPG; one animal from each group presented 100 EPG on D7; and on D14, two animals in the control group exhibited 100 EPG.

In the first sampling, corresponding to the onset of the patent period of artificial infection (Day 21), all animals shed eggs in their feces, with a mean of 5167 (±1313.18) in the supplemented group and a mean of 8656 (±1851.81) in the control group (p = 0.0012). A similar result was observed on day 28; the supplemented group mean was lower (7722 ± 1272.77) than in the control group (12,311 (±2450.93) (p = 0.0358) (Table 4). There was a significant interaction between time * treatment (p = 0.0384), with a more pronounced increase in EPG values in the control group.

Table 4. Mean (±standard error) eggs per gram of feces (EPG) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Day	Supplemented (*n* = 9)	Control (*n* = 9)	*p*-Value
−17	22.2 ± 14.70	0	0.4419
0 *	0	0	-
7	11.1 ± 11.11	11.1 ± 11.11	1.0000
14	0	11.1 ± 11.11	0.7002
21	5166.7 ± 1313.18	8655.6 ± 1851.81	0.0012
28	7722.2 ± 1272.77	12,311.1 ± 2450.93	0.0358

Legend: * Day of artificial infection with 4000 infective larvae of *Haemonchus contortus*.

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The weight gain in lambs supplemented with grape pomace did not show significant differences ($p > 0.05$) compared to the control group at any collection point. The mean weight gain post-infection (from D0 to D28) for the supplemented lambs was 3.7 kg (±0.47), while it was 3.8 kg (±0.30) for the control group. The average daily weight gain was 0.131 kg (±0.02) in the supplemented group and 0.137 kg (±0.01) in the control group ($p > 0.05$) (Table 5).

Table 5. Mean body weight (±standard error) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Day	Supplemented (n = 9)	Control (n = 9)	p-Value
−17	19.1 ± 1.16	19.2 ± 1.78	0.9643
0 *	22.1 ± 1.32	21.9 ± 2.33	0.9546
7	21.6 ± 1.34	21.7 ± 2.17	0.9870
14	23.5 ± 1.50	23.7 ± 2.40	0.9416
21	25.6 ± 1.59	25.8 ± 2.53	0.9513
28	25.8 ± 1.76	25.8 ± 2.50	0.9935
WG	3.7 ± 0.47	3.8 ± 0.30	0.7545
ADWG	0.131 ± 0.02	0.137 ± 0.01	0.7545

Legend: WG: weight gain, post-infection; ADWG: average daily weight gain, post-infection. * Day of artificial infection with 4000 infective larvae of *Haemonchus contortus*. Body weight values are in kg.

The initial PCV means were 36% (±1.12) in the supplemented group and 34% (±1.47) in the control group. From D7 onwards, these means showed a progressive decline until D21, when the lowest values were recorded: 25% (±1.08) in the supplemented group and 24% (±1.02) in the control group.

Total plasma protein concentrations fluctuated throughout the experiment but remained within the normal range for sheep (6.0–7.9 g/dL) [41]. The highest mean values observed in the supplemented group were 6.6 g/dL (±0.17) on D21, and on the same day, the highest mean value of the control group was 6.8 g/dL (±0.06). No influence of grape pomace supplementation was observed on the mean PCV or TPP concentrations ($p > 0.05$) at any collection point (Figures 1 and 2). Nevertheless, there was a significant time * treatment interaction for TPP ($p = 0.0089$).

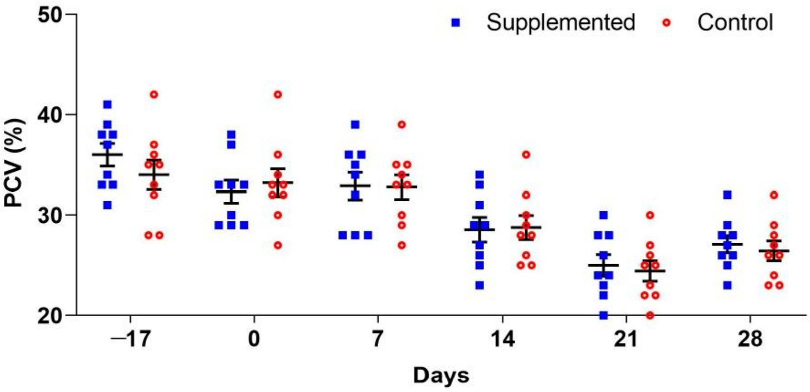

Figure 1. Mean (±standard error) packed cell volume (PCV) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

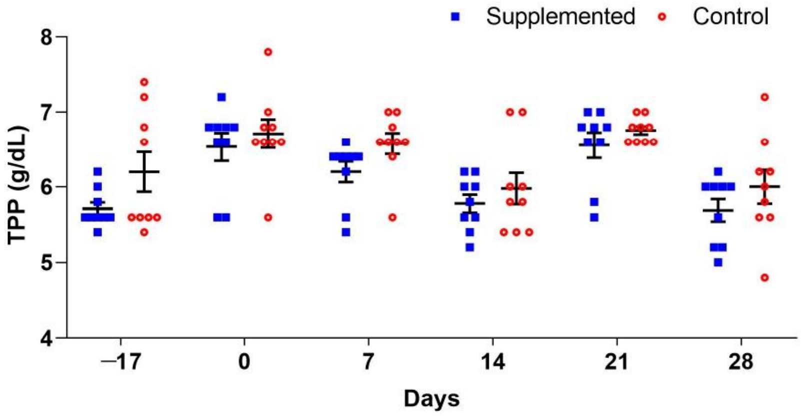

Figure 2. Mean (±standard error) total plasma protein (TPP) concentrations (g/dL) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Blood eosinophil counts varied throughout the experiment, peaking seven days post-infection, with averages of 611 (±171.26) and 454 (±114.47) in the supplemented and control groups, respectively ($p > 0.05$). Subsequent collections showed a marked decrease in the average for both groups (Figure 3).

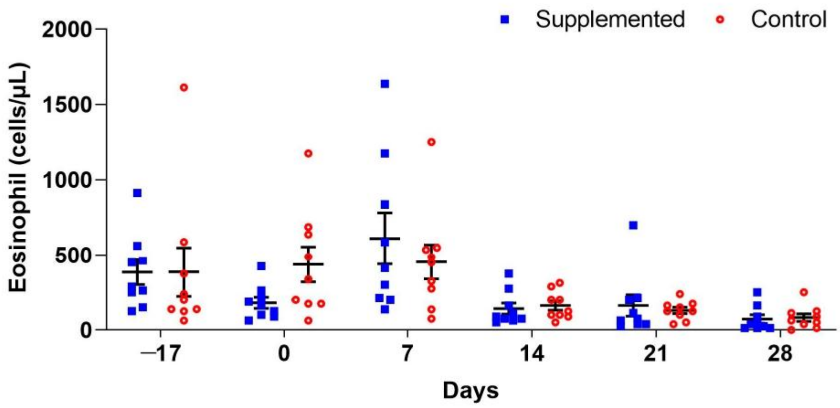

Figure 3. Mean (±standard error) eosinophil counts (cells/µL) of Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

ELISA analyses showed a significant time * treatment interaction ($p = 0.0099$) with IgG levels consistently higher in the supplemented group. On D0, the supplemented group presented its highest mean 41.5 (±19.33) and the control group 16.5 (±3.11), but without presenting a significant difference ($p = 0.0596$) (Figure 4).

Regarding adult *H. contortus* worms, the average female burden was 1157 (±88.43) in the supplemented group and 1049 (±85.56) in the control group, with no significant difference ($p = 0.3940$). Similarly, the male burdens were 1003 (±103.95) in the supplemented group and 1009 (±84.83) in the control group ($p = 0.9675$).

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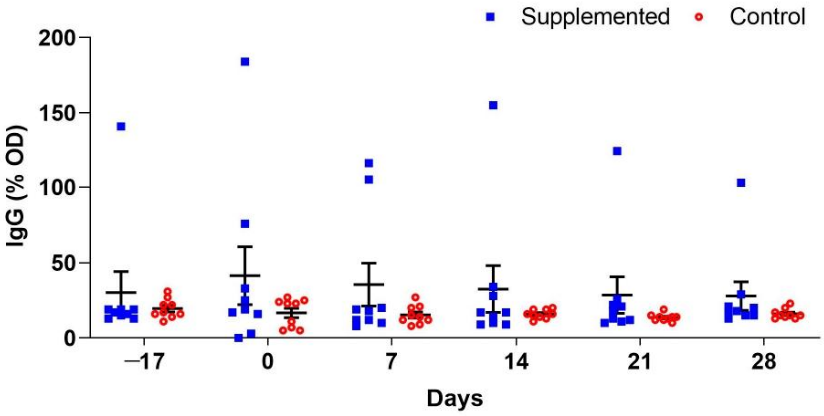

Figure 4. Mean (±standard error) IgG levels (OD: optical density percentage relative to control) against *H. contortus* L3 in Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Three immature *H. contortus* (L5 early-stage) worms in total were found in two lambs of the control group. Additionally, two male specimens of *Trichostrongylus colubriformis* were found—one in Lamb 9 (supplemented) and another in Lamb 18 (control).

Regarding length measurements, male and female parasites from the supplemented group were smaller compared to the control group; with a significant group difference ($p = 0.0003$) observed in the female measurements. The average number of eggs quantified within the uterus of each female was 343 for the supplemented group and 585 for the control group ($p < 0.0001$) (Table 6).

Table 6. Averages (±standard error) of worm burden, measurements, and the number of eggs of *H. contortus* specimens obtained from Santa Inês lambs (five male and five female worms per animal) supplemented with grape pomace or non-supplemented controls, at 29 days post-infection with 4000 L3 larvae.

Variables	Group		*p*-Value
	Supplemented (*n* = 9)	Control (*n* = 9)	
Adult Male	1003.3 ± 103.95	1008.9 ± 84.83	0.9675
Adult Female	1156.7 ± 88.43	1048.9 ± 85.56	0.3940
Male length in cm (*n* = 45)	1.38	1.40	0.2313
Female length in cm (*n* = 45)	1.96	2.10	0.0003
Number of eggs/female (*n* = 45)	342.9	584.9	<0.0001

The bromatological composition of grape pomace, ground hay, and concentrated feed provided to the lambs of the two groups are shown in Table 7.

The grape pomace presented total phenolic compounds value of 42.21 in equivalent grams of tannic acid/kg dry matter; total tannin value of 39.06 in equivalent grams of tannic acid/kg dry matter; and condensed tannin value of 2.89 in equivalent grams of leucocyanidin/kg dry matter. The average amount consumed per day per animal can be seen in the Supplementary Material, Table S2.

Table 7. Bromatological composition of grape pomace, ground hay, and concentrate ration supplied to Santa Inês lambs supplemented with grape pomace or non-supplemented controls.

Variables (%)	Grape Pomace	Hay	Supplemented Group Concentrate	Control Group Concentrate
DM	94.66	95.51	88.19	88.43
MM	9.52	5.77	4.22	4.17
CP	15.57	6.44	30.56	30.35
EE	13.47	0.59	1.65	1.64
NDF	55.83	81.23	11.17	15.53
ADF	63.00	44.67	21.51	18.74

Legend: DM—dry matter; MM—mineral matter; CP—crude protein; EE—ether extract; NDF—neutral detergent fiber; ADF—acid detergent fiber.

4. Discussion

In the present study, grape pomace administered daily to lambs demonstrated an effect against *H. contortus* infection. The supplemented group showed significantly lower FECs, shorter worms, and female worms with less eggs in utero in comparison to the control group. The effect of grape pomace on the parasite fecundity may have been a direct effect of the compounds against the worms and/or an indirect effect due to improved immune response. The metabolites produced by the fermentation of phenolic compounds in the rumen can create an unfavorable environment for parasitic nematodes. Metabolites produced by the fermentation of phenolic compounds can create an unfavorable environment for parasitic nematodes. Metabolites, such as short-chain fatty acids, and other bacterial metabolites are believed to be important mediators of microbiota-related health effects [42,43]. Polyphenols and tannins in grape pomace have been demonstrated to interfere with the reproductive processes of *H. contortus* by disrupting egg development and hatching. Tannins, in particular, can bind to proteins and glycoproteins in the parasite's reproductive tract, inhibiting essential enzymatic activities required for egg production and embryogenesis [44]. This binding may also compromise the structural integrity of the parasite's cuticle and reproductive organs, leading to a reduction in egg production [45]. By reducing fecundity, the elimination of eggs is consequently decreased, leading to fewer third-stage (infective) larvae in the pasture. This, in turn, contributes to reduced reinfection rates and a lower parasitic burden.

Condensed tannins are known for their ability to bind dietary proteins, protecting them from ruminal degradation. This process increases the flow of proteins and improves amino acid absorption in the small intestine, thereby enhancing the immune response against gastrointestinal nematodes [44]. In the present study, such a benefit was confirmed by the higher levels of IgG-anti-*H. contortus* in the supplemented group. Higher dietary protein levels in naturally infected sheep are associated with increased eosinophil, IgE, IgA, and IgG levels, leading to a stronger immune response against nematodes [46]. Despite the higher levels of IgG-anti-*H. contortus* in the supplemented group, we did not observe a significant difference in eosinophil counts because this parameter is related to the innate immune response against incoming larvae. Since the infection was a single event and the eosinophilia was not intense, no significant differences were detected.

Grape pomace is rich in bioactive compounds, such as phenolic acids, proanthocyanidins, anthocyanins, and stilbenes, with significant health-promoting potential, including antioxidant activity, anti-inflammatory properties, antitumor effects, neuroprotection, and gut health benefits. These functions are largely attributed to metabolites derived from phenolic compound degradation in the digestive tract, along with phase II metabolic enzyme-mediated transformations that inactivate toxins for efficient excretion [47]. The phenolic compounds found in grape pomace significantly influence both the gastrointestinal microbiota and the immune system. These compounds interact with gut bacteria, promoting beneficial microbial populations and generating bioactive metabolites that enhance health outcomes. The gut microbiota metabolizes phenolic compounds, leading to the production of bioactive metabolites that can be more readily absorbed and exert systemic health benefits [42,43]. Phenolic compounds have been shown to exhibit anti-inflammatory effects, which may strengthen immune responses [43]. Naturally infected, grazing lambs supplemented with an organic extract of Pinot Noir grape (*Vitis vinifera*), containing a condensed tannin concentration of 0.20 mg/mL and a combined procyanidin and prodelphinidin level of 0.0007 mg/mL with 15.5% galloylated tannins, exhibited lower EPG compared to the control group [48]. Galloylated tannins are a type of hydrolyzable tannin primarily composed of gallic acid units, which are structurally diverse secondary plant metabolites with potential applications in pharmaceuticals and phytomedicines. These include gallotannins and ellagitannins, which are abundant in fruits and medicinal plants. Known for their ability to chelate iron and inhibit enzymes such as the DNA repair enzyme ALKBH2, galloylated tannins also offer significant health benefits [49–51].

In agreement with our results, six-month-old Santa Inês lambs, artificially infected with and subjected to treatment with an aqueous extract of banana leaves (*Musa* spp.), showed a 33% reduction in FEC. Phytochemical analyses identified the presence of flavonoids, saponins, catechins, and condensed and gallic tannins [52]. The total polyphenol content of banana (*Musa x paradisiaca*) foliage was reported to be high (80 g/kg DM), whereas the condensed tannin content was negligible (0.007 g/kg DM), suggesting that other compounds contribute to the observed nematicidal activity when provided to lambs [53]. A study by Lima [54] demonstrated that lambs infected with nematodes and treated with *Acacia mearnsii* extract (15 g/animal daily for 50 days), containing 577 g/kg DM of total tannins and 279 g/kg DM of condensed tannins, exhibited reduced FEC.

Significant reductions in the parasitic load of *T. colubriformis* and *Cooperia* sp. were observed in naturally infected lambs supplemented with 18 g of powdered black wattle (*A. mearnsii*) bark, containing 18% condensed tannins (18 g of the extract or 3.24 g of condensed tannins), once weekly for 13 weeks [55]. The extract used in the present experiment also contained tannins, albeit in lower quantities than those reported for *A. mearnsii*. This suggests that relatively high levels of tannins may be required to cause a reduction in worm burden. One possible reason for the lower tannin content may be related to the processing of grape pomace, as drying and milling processes significantly influence the retention of bioactive compounds in this by-product of winemaking. These processes can affect the stability, solubility, and overall bio-accessibility of phytochemicals, particularly phenolic compounds, which are known for their health benefits. Higher drying temperatures may lead to the degradation of sensitive bioactive compounds [56]; moisture levels during drying affect the stability of these compounds, with lower moisture content generally enhancing their retention [57,58]. Milling can increase the surface area of grape pomace, potentially improving the extraction of bioactive compounds during subsequent processing. However, excessive milling may result in the loss of volatile compounds [58]. While drying and milling can enhance the extraction and retention of bioactive compounds, it is crucial to balance these processes to prevent degradation. Conversely, improper handling can lead to significant losses of these valuable compounds, highlighting the need for optimized processing techniques.

Despite the expected decline following artificial infection with *H. contortus*, a hematophagous nematode, PCV and TPP values remained, respectively, above 20% and

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5 g/dL, without the need for precautionary anthelmintic treatment during the study. Alterations in these hematological variables are often linked to parasitic infections and nutritional deficiency. Our findings indicate that the addition of grape pomace in the diet did not negatively impact lamb health, based on those indicators. This aligns with other studies using by-products in lamb diets without altering hematological parameters [59–61].

Regarding animal performance, grape pomace did not negatively affect the lambs' average daily weight gain. These findings corroborate those of Cheng [62], who used grape pomace at 8% of dietary DM without affecting lambs' average daily weight gain. Other studies report that agricultural by-products, such as corn cobs and peanut hulls, can effectively replace Napier grass (*Pennisetum purpureum*) in lamb diets, enhancing weight gain without compromising growth and development [63]. However, higher inclusion levels of grape pomace (150, 300, and 500 g/kg DM) reduced average daily weight gain due to slower passage rates caused by increased NDF and lignin. This resulted in lower energy and protein intake, can limit nutrient availability, and highlights the need for balanced dietary formulations [64]. Despite the significant differences in EPG counts, there was no significant difference in body weight gain due to the short duration of the experiment. If the period had been longer, we might have observed some differences. For this reason, new studies should be carried out to evaluate longer periods of the action of grape pomace in the diet of sheep.

In our study, the inclusion of grape pomace at a 20% proportion in the concentrate was palatable for the lambs, as only one lamb from the supplemented group did not consume the entire concentrate mixed with grape pomace during the first two days. Subsequently, this lamb consumed all the feed offered. Most crop residues and agro-industrial by-products are reasonably palatable based on the acceptability classification scale (1 = low; 2 = medium; 3 = good; and 4 = very good). Palatable and highly palatable residues (with average scores of 3 and 4, respectively) represent 56%, while unpalatable residues account for only 8% of 14 crop residues and 11 agro-industrial by-products studied [65].

The improper disposal of hundreds of millions of tons of agro-industrial by-products generated annually can result in significant environmental impacts [66]. The reuse and inclusion of this by-product may thus be a viable strategy to enhance feed efficiency, particularly in production systems facing cost challenges, while contributing to sustainable animal production. The UNEP 2024 Food Waste Report [67] highlights significant food waste, and incorporating by-products like grape pomace can mitigate waste and its environmental impacts.

The global trend toward reducing industrial waste has driven studies on using these materials in animal feed [68]. These findings highlight the complexity of interactions between diet and gastrointestinal nematode infections, emphasizing the importance of considering both nutritional effects and overall animal health when evaluating new dietary ingredients.

5. Conclusions

Grape pomace can be added to the diet of lambs, as it promotes more sustainable practices in animal production. Additionally, it can cause a reduction in *H. contortus* fecundity, which may be beneficial for the prophylaxis of haemonchosis in sheep.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15030341/s1, Table S1: Average daily consumption of feed components and corresponding nutritional levels; Table S2: Average daily consumption of grape pomace and total phenols of the supplemented lambs.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to new analyses are undergoing.

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CAPÍTULO 3: Effect of grape pomace inclusion in the diet of ewes naturally infected with gastrointestinal nematodes during lactation

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Article

Effect of Grape Pomace Inclusion in the Diet of Ewes Naturally Infected with Gastrointestinal Nematodes During Lactation

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Abstract: This study evaluated the feasibility of including grape pomace in the diet of Santa Inês ewes during lactation, aiming to reduce the effects of gastrointestinal nematode (GIN) infections and improve productive performance. Grape pomace, which contains phenolic compounds and tannins, was provided as a dietary supplement, replacing 20% of the concentrate over 28 days of lactation, starting on day 7 after lambing. A total of 18 ewes were used, divided into two groups: supplemented (n = 8) and control (n = 10). The supplemented group showed significant reduction in fecal egg count on day 14 of lactation ($p < 0.05$) in comparison with the control group. Lambs from the supplemented group had higher weaning weights ($p < 0.05$). Additionally, the supplemented group showed higher values for packed cell volume and circulating eosinophils, indicating greater resilience to infections. Analysis of anti-L3 IgG against *Haemonchus contortus* revealed no significant differences between the groups. It is concluded that grape pomace supplementation demonstrated potential to improve hematological and productive parameters in lactating ewes, with more evident effects during early lactation. Although the results suggest a possible complementary role in controlling gastrointestinal nematodes, future studies are needed to confirm and optimize this nutritional strategy.

Keywords: lactating ewes; nutraceuticals; sheep; animal production; agro-food residues

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1. Introduction

Small ruminant farming is severely affected by numerous factors, among which infections caused by gastrointestinal nematodes (GINs) stand out, leading to significant economic losses due to increased mortality rates and reduced herd productivity [1–3].

There are specific phases during which sheep are more susceptible to GIN infections. The final third of gestation and lactation are considered critical periods as nutrient allocation priorities shift toward fetal development and milk production, often at the expense of the immune system [4–10].

In tropical regions, *Haemonchus contortus*, a hematophagous nematode of the abomasum, is considered the most relevant species due to its high prevalence and pathogenicity [11]. This highly prolific species has females capable of producing between 5000 and 15,000 eggs per day, which are released into the environment through the host's feces resulting in significant pasture contamination with infective larvae [12]. Another important species is *Trichostrongylus colubriformis*, a parasite of the small intestine that forms tunnels in the intestinal villi to feed on the contents of necrotic epithelial cells. The damage and inflammation caused by this parasite result in villous atrophy, epithelial erosion, and subsequent impairment of nutrient digestion and absorption [13]. In intense infections, they can cause severe enteritis [14]. Similar pathogenic effects are observed in *Cooperia* and *Strongyloides* infections [15]. Infections by *Oesophagostomum* spp. can cause macroscopic lesions with large fibrotic nodules in the intestine, potentially compromising the health and productivity of sheep [16].

The handling of GIN infections is predominantly accomplished through the use of anthelmintics, including albendazole, closantel, levamisole, macrocyclic lactones, and monepantel. Nevertheless, the indiscriminate application of anthelmintics has led to a rise in resistance over recent decades, resulting in a growing prevalence of herds harboring multi-drug-resistant worms [17]. This has motivated the search for alternative control methods that reduce the reliance on anthelmintics, particularly approaches that stimulate the immune response and enhance animal resilience, such as nutritional strategies [18–20].

The use of food byproducts such as grape pomace, a residue generated by the wine industry, has gained attention as an alternative ingredient. Its application is promising not only for reducing feeding costs but also for promoting sustainability by repurposing materials that would otherwise be discarded [21,22]. The inclusion of desiccated and milled grape pomace into animal feed and rations offers a feasible method to reduce the environmental effect and enhance the value of this residue [23].

Grape pomace comprises seeds, leftover pulp, and stems, which are abundant in fiber (49.37%) and phenolic chemicals (35.35 mg of gallic acid per gramme), including tannins, carotenoids, and other bioactive antioxidants [23,24]. The dried pomace has a significant concentration of phenolics, measuring 3.95 mg/g (tannic acid equivalent), and tannins, totaling 1.92 mg/g (tannic acid equivalent), per 100 mg. The hydroalcoholic extract of grape pomace has in vitro anthelmintic activity, significantly reducing the hatching, development, and motility of free-living stages of *H. contortus* [25].

Santa Inês lambs artificially infected with *H. contortus* and supplemented with grape pomace, replacing 20% of the concentrate provided to the animals, showed significantly lower fecal egg counts (FECs) compared to the group that did not receive this supplement. It was also observed that these animals harbored *Haemonchus* females with shorter lengths and fewer eggs in the uterus [26]. Furthermore, grape pomace contained 15.57% crude protein, allowing supplemented animals to achieve weight gains similar to those receiving concentrate without the supplement, highlighting the potential of this byproduct as a dietary supplement (ibidem).

The benefits of including grape pomace in the diet of lambs [26] motivated this study, which aimed to evaluate the feasibility of including this product in the diet of Santa Inês ewes during lactation, with a focus on the nutraceutical benefits of the product, particularly in enhancing resistance/resilience against gastrointestinal nematode infections.

2. Materials and Methods

2.1. Location, Animals, and Experimental Design

All procedures involving animals in this study comply with international ethical standards and were approved by the Animal Use Ethics Committee of the Institute of Biosciences at São Paulo State University (IBB/UNESP), under protocol CEUA n° 7450220324 (ID 000729).

The experiment was carried out in the Veterinary Helminthology Laboratory's experimental site, IBB (Institute of Biosciences), UNESP (São Paulo State University), situated in Botucatu, SP, Brazil (22°53′16″ S and 48°29′57″ O, altitude 884 m), from July 2023 to April 2024.

In July 2023, 2 rams were placed in a paddock with 27 multiparous Santa Inês ewes, with an average age of three years. All ewes had pre-existing natural infections with gastrointestinal nematodes (GINs), confirmed by fecal egg counts (FECs) prior to the experiment. In September 2023, after natural mating, pregnancy was confirmed in 25 ewes by ultrasound, of which 20 were selected for the experiment. Throughout the experiment, the sheep were kept in a 3840 m^2 area paddock containing *Urochloa decumbens*, which maintained natural larval contamination and ensured continuous GIN exposure. The sheep were provided with water troughs, mineral salt feeders (ad libitum), and concentrate feed (70% ground corn and 30% soybean meal), supplied daily in collective troughs at a rate equivalent to 2% of the ewes' body weight. The selected ewes lambed over 29 days, between 7 December 2023 and 5 January 2024.

As the ewes lambed, they were evenly distributed into two groups based on the birth of single or twin lambs, in a completely randomized design, with 10 ewes in each group.

However, in the supplemented group, data from two ewes and their respective lambs were excluded: one died 55 days after lambing due to an undetermined cause and the other developed mastitis. Therefore, the supplemented group consisted of eight ewes.

Seven days after lambing (D7), each ewe was placed in an individual pen with water ad libitum where they received daily concentrate feed, as outlined in Table 1. Immediately after confirming the complete consumption of the concentrate, the animals from both groups were released into the same paddock where they grazed together. This procedure was carried out over 28 days, starting on day 7 after lambing and ending on day 35 after lambing (D7 to D35).

Table 1. Amount in kilograms (kg) of concentrate and grape pomace provided individually to ewes from day 7 postpartum to day 35.

Groups	Type of Birth	Concentrate (kg)	Grape Pomace (kg)
Supplemented (n = 8)	Single (n = 5)	0.8	0.2
	Twin (n = 3)	1.0	0.2
Control (n = 10)	Single (n = 6)	1.0	0
	Twin (n = 4)	1.2	0

Concentrate composed of 70% ground corn and 30% soybean meal.

After this period, the animals continued to be fed in collective troughs with the same diet as at the beginning of the experiment. The lambs accessed the feeders with their mothers and started ingesting little quantities of feed as weaning neared.

When the lambs reached 60 days of age, they were weaned. The ewes were moved to a separate paddock away from the lambs, where they did not receive concentrate feed to facilitate milk drying.

2.2. Grape Pomace Preparation

Syrah grapes destined for wine production were harvested in August 2023. The grape pomace was collected from a winery located in São Roque, São Paulo, Brazil (23°35′41.2″ S 47°09′41.6″ W, altitude 880 m). The material was dried in a forced-air oven at 50 °C for approximately 24 h, then ground and stored at −20 °C until use.

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2.3. Measurement of Total Phenolics and Tannins in Grape Pomace

Total phenolic (TP) concentrations were determined using the Folin–Ciocalteu reagent method as described by Makkar [27]. Total tannins (TTs) were measured as the difference between TP concentrations before and after treatment with insoluble polyvinylpolypyrrolidone, following Makkar's [28] protocol with tannic acid as standard. Condensed tannins (CTs) were quantified using the butanol–HCl method [27], with leucocyanidin as reference standard.

2.4. Fecal Sample Collection and Coprological Exams

Fecal samples were collected directly from the rectal ampulla of each animal, placed in pre-labeled polyethylene bags, and refrigerated (4 °C) until processing. Collections followed Table 2's schedule, beginning at least 38 days before each lamb's birth and concluding 28 days post-weaning, for FECs and coprocultures. FECs and coprocultures were performed according to Ueno and Gonçalves [29], including the identification of infective larvae. As lamb births occurred on different dates, weekly coprocultures (processed every Friday) contained refrigerated (4 °C) samples collected from Saturday to Friday, with two separate cultures processed per treatment group.

Table 2. Experimental timeline.

Procedures	**Days**													
	−38 *	**−17 ***	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**77**	**91**
Prepartum sampling	X	X												
Parturition day			X											
Lactation sampling **				X	X	X	X	X	X	X	X	X		
Post-weaning sampling													X	X
Grape pomace feeding				X	X	X	X	X						

* Approximate days. ** Blood and fecal sampling plus body weight measurements.

2.5. Hematological Analyses

Blood samples were collected per Table 2's schedule via jugular venipuncture into EDTA vacutainers. Packed cell volume (PCV) was determined by microhematocrit centrifugation. Total plasma protein (TPP) was measured by refractometry (SPR-Atago). Eosinophils were quantified using a Neubauer chamber after Carpentier's solution staining [30], expressed as cells/µL blood. Samples were centrifuged at 1000× *g* for 15 min to obtain plasma, which was stored at −20 °C for subsequent analyses.

2.6. Anti-H. contortus L3 Immunoglobulin G (IgG)

To determine IgG levels against L3-soluble extract of *H. contortus*. The L3 extract (antigen) production followed earlier instructions [31]. Following a method previously established to measure the parasite-specific plasma IgG levels [32], with some modifications: In short, the plates were coated with 2 µg of antigen/mL, washed three times, rotated 180 degrees, and then washed three times more. Following the addition of diluted plasma and subsequent washing, the plates were incubated with a 1:20,000 diluted peroxidase-conjugated rabbit anti-sheep IgG, washed, and incubated with OPD (o-Phenylenediamine, Ref: P5412, Sigma) after 15 min the plate was read at 405 nm. The negative control (NC) sample used was from a worm-free animal, previously described [33], and the plasma positive control (PC) sample used was from an ewe naturally infected. Results were expressed as the percentage of the PC plasma sample's optical density (OD) value [34].

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2.7. Bromatological Analyses

Seven pasture samples were collected during paddock rotations (sampling the new paddock on transfer days). Concentrate and grape pomace samples were collected and frozen until analysis. Samples underwent bromatological analyses for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and mineral matter (MM) following AOAC [35] protocols. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined via sequential washing using a fiber analyzer (TE-149 Tecnal, Piracicaba-SP) and F57 filter bags (Ankom Technology Corp., Macedon, NY, USA), following Van Soest, Robertson, and Lewis [36] as modified by Mertens [37].

2.8. Weight Gain

Ewes were weighed per Table 2 schedule each morning before concentrate feeding. Lambs were weighed at birth and weaning.

2.9. Meteorological Data

Daily mean temperature and rainfall were recorded. Rainfall data came from an on-site pluviometer, while temperature data were obtained from UNESP's Lageado Experimental Farm weather station (7.7 km from the experimental site).

2.10. Statistical Analysis

Data were analyzed using Statistical Analysis System version 9.4 (SAS Institute, Cary, NC, USA). Repeated measures were evaluated with PROC MIXED. When the data did not present a normal distribution according to the normality test, they were transformed by $\log10(x + 1)$. Means were compared by *t*-test ($\alpha = 0.05$). The results section presents arithmetic means ($\pm$standard error). Only significant time $\times$ treatment interactions are reported.

3. Results

3.1. Dietary Nutrients

A total of 227 kg of grape pomace was obtained, which was reduced to 101 kg after drying, representing a 44% reduction. Regarding consumption in the grape pomace-supplemented group, from the first day (D7) until the last day of supplementation (D35) all ewes in the supplemented group consumed the provided concentrate. Ewes of the control group also consumed all the concentrate.

Averages of the seven pasture samples collected from the paddocks grazed by sheep, together with the bromatological composition of the grape pomace and concentrate and the measurement of total phenolics, total tannins, and condensed tannins, are shown in Table 3.

Table 3. Nutritional composition of grape pomace, pasture, and concentrate provided to ewes supplemented with grape pomace or control diet and measurement of total phenolics and tannins in grape pomace.

Variables (%)	Grape Pomace	Concentrate	Pasture
DM	93.59	91.16	86.02
CP	12.61	18.71	10.13
NDF	45.42	17.17	72.03
ADF	66.50	12.06	46.85
EE	5.71	3.19	2.55
MM	10.89	3.38	8.59

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Table 3. *Cont.*

Variables (%)	Grape Pomace	Concentrate	Pasture
Total phenolic compounds [a]	47.87	-	-
Total tannins [a]	42.21	-	-
Condensed tannins [b]	2.63	-	-

Legend: DM—dry matter; MM—mineral matter; CP—crude protein; EE—ether extract; NDF—neutral detergent fiber; ADF—acid detergent fiber. [a]—Values expressed in equivalent gram of tannic acid/kg dry matter. [b]—Values expressed in equivalent gram of leucocyanidin/kg dry matter.

3.2. Fecal Examination

Fecal egg count (FEC) means varied between the supplemented and control groups throughout the experiment (Table 4). At study initiation (D-38, prepartum), both groups showed low FEC means. The supplemented group averaged 75 (±36.60) EPG, whilst controls had 260 (±195.62) EPG, with no significant difference (p = 0.7507). Subsequently, values increased and at day 7 (D7) during lactation the supplemented group mean peaked reaching 1663 (±551.60) EPG, while the control averaged 1790 (±381.65) EPG (p = 0.7943).

Table 4. Mean (±standard error) fecal egg counts (FECs) in ewes supplemented with grape pomace versus controls.

Period	Day	Supplemented (n = 8)	Control (n = 10)	p-Value
Prepartum	−38 #	75.0 ± 36.60	260.0 ± 195.62	0.7507
	−17 #	1625.0 ± 649.38	520.0 ± 174.99	0.0864
Lactation (Weekly sampling)	7 *	1662.5 ± 551.60	1790.0 ± 381.65	0.7943
	14 *	975.0 ± 413.50	2960.0 ± 1192.87	0.0087
	21 *	725.0 ± 372.13	1970.0 ± 921.96	0.0856
	28 *	762.5 ± 402.64	1370.0 ± 805.13	0.4305
	35 *	475.0 ± 256.87	1200.0 ± 516.61	0.2520
	42	437.5 ± 155.77	1300.0 ± 587.08	0.1894
	49	237.5 ± 98.08	670.0 ± 255.63	0.4570
	56	337.5 ± 129.47	500.0 ± 197.77	0.7816
	63	250.0 ± 96.36	320.0 ± 121.84	0.9019
Post-Weaning (Biweekly sampling)	77	550.0 ± 281.58	440.0 ± 166.13	0.8616
	91	437.5 ± 186.07	540.0 ± 260.00	0.8749

Legend: # Approximate days; * supply of grape pomace for the supplemented group.

By the next collection (D14), the supplemented group showed a marked FEC reduction (975 ± 413.5 EPG), while the control increased to 2960 (±1192.87) EPG, demonstrating significant differences between groups (p = 0.0087). This latter value represented the highest mean observed in the control.

From D7 onward, the supplemented group means remained lower than the control until study completion, except at D77 when supplemented ewes averaged 550 (±281.58) EPG versus 440 (±166.13) EPG in the control (p = 0.8616).

3.3. Coproculture

The coproculture analysis revealed a predominance of *Haemonchus* spp. in both groups (supplemented and control), followed by *Oesophagostomum* spp., *Trichostrongylus* spp., and *Cooperia* spp. Regarding monthly distribution, *Haemonchus* spp. predominated in

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coprocultures during the initial months of the experiment, with a mean percentage of 90%. However, by the study's conclusion there was a significant increase in the proportion of *Oesophagostomum* spp., reaching 100% in one control group sampling. *Trichostrongylus* spp. and *Cooperia* spp. appeared in intermediate samplings with mean percentages of 11% and 13%, respectively.

Mean ewe body weights are presented in Figure 1. No significant differences ($p > 0.05$) were observed between the supplemented and control groups at any evaluation period. Post-weaning weight reduction occurred in all ewes, attributable to the discontinuation of concentrate supplementation to facilitate milk drying and prevent mastitis incidence.

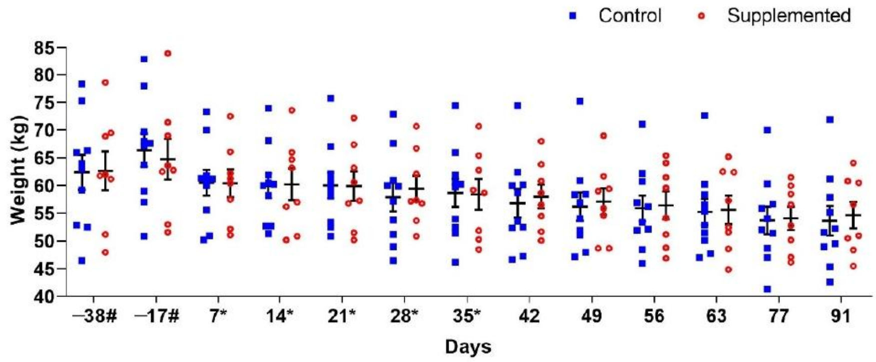

Figure 1. Mean body weight (±standard error) of ewes supplemented with grape pomace versus the control group during the experimental period. # Approximate days. * Supply of grape pomace for the supplemented group.

3.4. Packed Cell Volume and Total Plasma Protein

Significant differences ($p < 0.05$) between groups were only observed in PCV values on D14 when the supplemented group showed a significantly higher PCV ($p = 0.0399$). On this day, mean PCV values were 32% (±0.98) for the supplemented group and 28.9% (±0.98) for the control group (Figure 2).

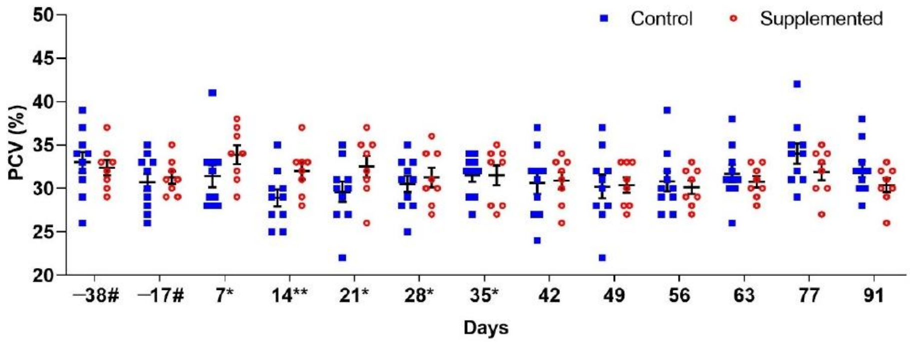

Figure 2. Mean packed cell volume (PCV) (±standard error) in ewes supplemented with grape pomace versus the control group. # Approximate days. * Supply of grape pomace for the supplemented group. ** Statistically significant difference.

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The results regarding TPP values were similar, showing no significant differences between the groups over time (Figure 3).

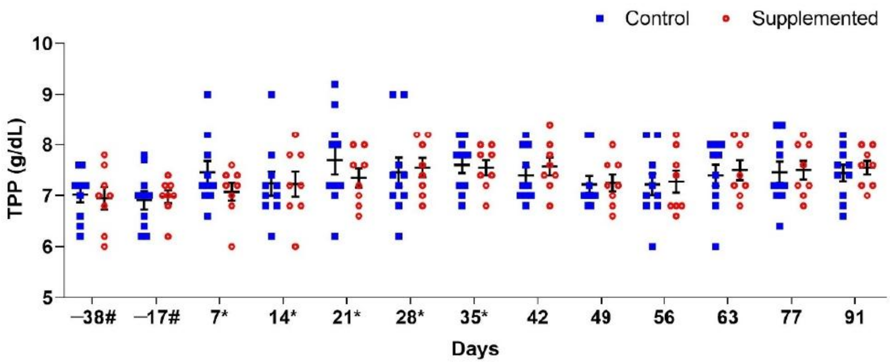

Figure 3. Mean total plasma protein (TPP) (±standard error) concentrations (g/dL) in ewes supplemented with grape pomace versus the control group. # Approximate days. * Supply of grape pomace for the supplemented group.

3.5. Blood Eosinophil Count and Anti-H. contortus L3 IgG Analysis

Blood eosinophil counts showed no significant differences ($p > 0.05$) between groups. However, the supplemented group generally exhibited higher mean values compared to controls, except at D35 when the supplemented group averaged 2319 (±498.91) eosinophils/µL versus 2418 (±582.87) eosinophils/µL in controls (Figure 4).

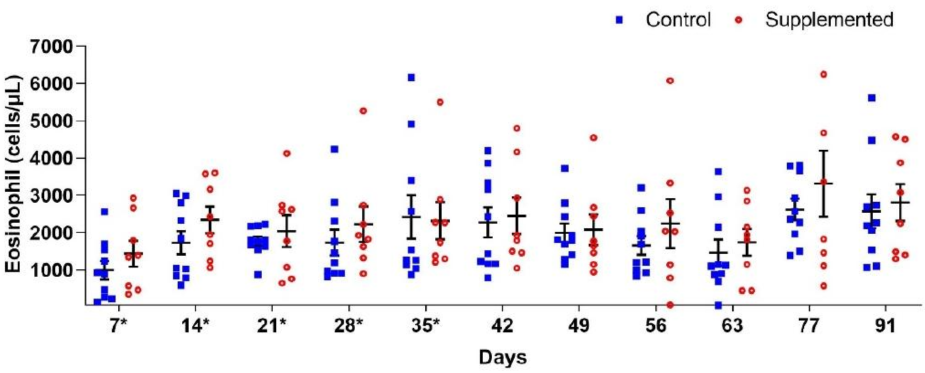

Figure 4. Mean eosinophil counts per µL of blood (±standard error) in ewes supplemented with grape pomace versus the control group. * Supply of grape pomace for the supplemented group.

Anti-*H. contortus* L3 IgG analysis revealed fluctuating antibody levels between supplemented and control groups throughout the study, though without significant differences ($p > 0.05$) (Figure 5).

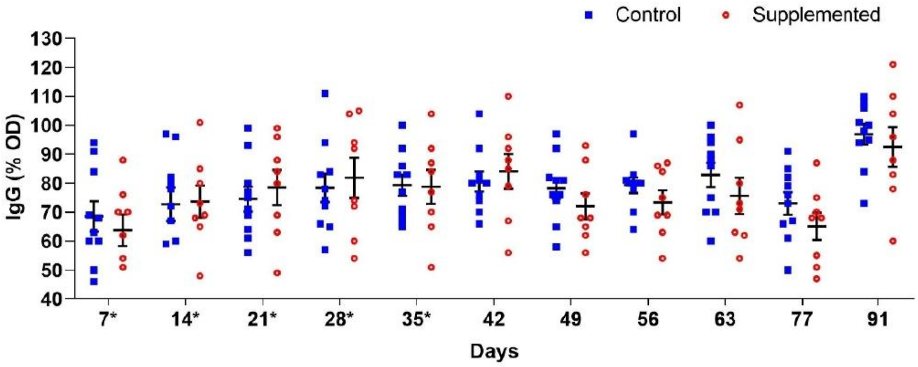

Figure 5. Mean anti-*H. contortus* L3 IgG levels (percentage of optical density, OD) (±standard error) in the serum of ewes supplemented with grape pomace versus the control group. * Supply of grape pomace for the supplemented group.

3.6. Body Weight of Lambs

Single-born lambs showed a higher birth weight compared to twins (p = 0.0193), with no significant difference between the experimental groups (p = 0.2675). At weaning, weight was higher in lambs from supplemented ewes, with a significant effect of birth type (p = 0.0008) and significant interaction between birth type and group (p < 0.0001). Weight gain was higher in lambs from supplemented ewes. There was a significant effect of birth type (p = 0.0029) and a tendency toward significance for supplementation (p = 0.0539) (Table 5).

Table 5. Body weight of lambs (±standard error) born to ewes supplemented with grape pomace or in the control group.

Weight	Supplemented (n = 11)		Control (n = 12)		*p*-Value		
	Single (5)	Twin (6)	Single (7)	Twin (5)	Type of Birth	Group	Type of Birth × Group
Birth	4.2 ± 0.42	3.4 ± 0.24	4.6 ± 0.33	3.8 ± 0.24	0.0193	0.2675	0.7602
Weaning	19.5 ± 1.29	15.2 ± 1.65	18.2 ± 0.73	11.8 ± 1.62	0.0008	0.0942	<0.0001
Weight gain	15.2 ± 0.94	11.8 ± 1.60	13.6 ± 0.77	8.0 ± 1.83	0.0029	0.0539	0.4394

3.7. Meteorological Data

The recording of meteorological data began seven days after the first lambing (D7), which was on 14 December 2023, and continued until 5 April 2024, the day of the last collection, coinciding with the summer and autumn seasons. The daily average rainfall was 2.71 mm, and the average temperature was 24.03 °C. Of the 17 days with observations in December, a total of 19.38 mm of rain was recorded, with a daily average temperature of 25.57 °C. This was the month with the highest temperatures. The month with the highest rainfall was January, with a total of 182.50 mm of rain and an average daily temperature of 23.46 °C (Figure 6).

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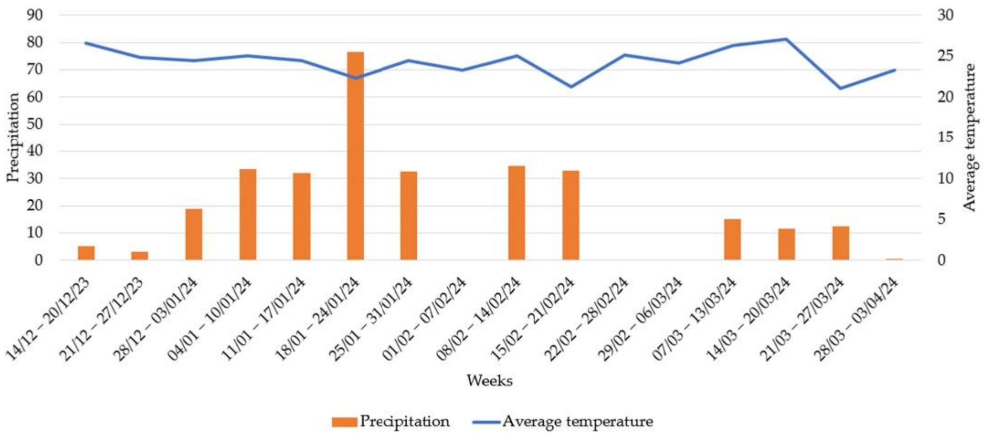

Figure 6. Meteorological data of weekly mean temperature (°C) and weekly accumulated precipitation (mm).

4. Discussion

The results of this study indicate that although grape pomace supplementation did not provide significant protection against GINs it showed potential to reduce FECs during the periparturient relaxation of immunity without compromising the productive performance of the ewes. In vitro studies by Soares et al. [25] demonstrated the anthelmintic activity of grape pomace extract against *H. contortus* eggs and larvae, likely due to the presence of phenolic compounds. In 100 g of dried pomace, the total phenolic content and total tannins in dry matter were 3.95 mg/g and 1.92 mg/g (tannic acid equivalent), respectively.

The relaxation of the immune response due to the peripartum phenomenon was evident in both groups, which showed elevated FECs between D17 and D14. The supplemented and control groups reached their highest mean FEC on D7 and D14, respectively, followed by a progressive decline throughout the experiment. Even ewes supplemented with high levels of metabolizable protein exhibit periparturient relaxation of immunity, leading to increased *H. contortus* egg shedding [9]. López-Leyva et al. [38] reported that lactating ewes, regardless of diet, showed a significant increase in FEC (2709 EPG). However, those fed high-protein diets (15% crude protein) had reduced FEC values compared to those receiving low amounts of protein (8% CP). Beasley et al. [39] linked periparturient relaxation of immunity to suppressed eosinophils and local antibodies in Merino ewes. Our results reinforce that lactation is the primary driver of periparturient relaxation of immunity as grape pomace supplementation (rich in tannins) mitigated but did not eliminate the FEC rise. This highlights the need for combined strategies (nutrition, management, and genetics) for effective periparturient relaxation of immunity control.

Tannins have direct anthelmintic activity by inhibiting egg hatching, larval motility, and adult nematode fertility, consequently reducing FECs [25,40]. The tannins in grape pomace may also enhance ruminant immune responses. They can bind to dietary proteins, forming complexes that protect them from ruminal degradation, increasing amino acid availability in the small intestine and improving nutrient absorption [41]. These indirect effects may have contributed to the greater resilience of supplemented ewes, reflected in lower FECs and higher PCVs and eosinophil counts in the supplemented group. Similar results were observed in lambs artificially infected with *H. contortus* and supplemented with grape pomace for 28 days, which had significantly lower FECs than the control group [26], reinforcing the benefits of grape pomace in sheep nutrition.

It is important to note that this study spanned spring, summer, and autumn—seasons where frequent rainfall and warm temperatures favor the development and survival of L3 larvae [42]. The predominance of *Haemonchus* spp. in coprocultures aligns with previous observations by Wilmsen et al. [43], who reported its high prevalence in sheep from the same region. Despite its high pathogenicity and potential to cause anemia, no clinical cases of haemonchosis were observed, even in animals with a high FEC (e.g., one control ewe with 12,300 EPG and 25% PCV on D14). This suggests that the diet enhanced resilience.

Regarding hematological parameters, while most values remained within normal ranges without significant differences between groups, the supplemented group showed a significantly higher PCV on D14. A similar outcome was reported in a study using 10% fresh Arabica coffee byproduct feed, which also did not significantly reduce FECs in infected lambs [44] despite containing 13.95 g/100 g total phenolics and 2.28 g/100 g condensed tannins [45]. This suggests tannin efficacy may vary by source and concentration.

While grape pomace supplementation did not affect ewes' productive performance, lambs from supplemented ewes had higher mean weaning weights, possibly due to improved milk production. This aligns with findings by Dove et al. [46], where lambs from beet-pulp-supplemented ewes gained 40–60 g/day more than the controls. Undernutrition or overnutrition during gestation can influence lamb muscle development and lifelong growth capacity [47,48].

In this study, grape pomace did not significantly enhance immunological variables (e.g., eosinophil counts, IgG levels), likely due to the short supplementation period. However, the absence of negative effects supports its use as a partial feed replacement without compromising health or performance.

The use of byproducts like grape pomace is a promising alternative for ruminant nutrition, particularly amid rising anthelmintic resistance. Beyond reducing feed costs by up to 33.8% [49], it promotes sustainable livestock production by repurposing waste [50]. Further research should investigate mechanisms of action, optimal dosages, and supplementation timing, especially during critical phases like lactation.

5. Conclusions

Grape pomace supplementation demonstrated potential to improve hematological and productive parameters in lactating ewes, with more evident effects during early lactation. Although the results suggest a possible complementary role in controlling gastrointestinal nematodes, future studies are needed to confirm and optimize this nutritional strategy.

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CAPÍTULO 4

1. DISCUSSÃO GERAL

A resistência aos anti-helmínticos em nematoides gastrintestinais, especialmente *H. contortus*, representa um desafio crescente para a ovinocultura mundial. Estudos recentes demonstram que a eficácia das principais classes de anti-helmínticos, incluindo benzimidazóis, lactonas macrocíclicas e imidazotiazóis, tem declinado significativamente, com relatos de resistência múltipla em mais de 80% dos rebanhos avaliados no estado de São Paulo (BASSETTO et al., 2024). Este cenário é agravado pelo fato de que, na prática, os tratamentos são frequentemente realizados sem a devida avaliação da eficácia dos princípios ativos disponíveis. Em grande parte das propriedades, testes prévios não são realizados para identificar qual anti-helmíntico seria mais adequado frente ao perfil de resistência local, limitando-se ao uso empírico das poucas opções existentes no mercado.

Nesse contexto, a busca por estratégias sustentáveis, como a utilização de nutracêuticos, ganha relevância. O bagaço de uva, um subproduto da indústria vinícola rico em compostos fenólicos e taninos, emergiu como uma alternativa promissora. A abordagem experimental, que envolveu cordeiros infectados artificialmente com *H. contortus* e ovelhas durante a lactação naturalmente infectadas, permitiu uma análise detalhada dos impactos dessa estratégia nutricional.

Em ambos os experimentos, a inclusão do bagaço de uva seco e triturado misturado à ração foi bem tolerada, sem comprometimento do consumo voluntário ou do desempenho produtivo. Conforme Amaral et al. (2019), o bagaço de uva pode substituir parcialmente volumosos em dietas de ruminantes.

No Capítulo 2, a suplementação com bagaço de uva em cordeiros artificialmente infectados com *H. contortus* resultou em redução significativa do OPG. Além disso, os animais suplementados apresentaram parasitas menores e fêmeas com menos ovos no útero em comparação ao grupo controle.

Já no Capítulo 3, a inclusão de bagaço de uva na dieta de ovelhas lactantes, naturalmente infectadas por nematoides gastrintestinais, não resultou em reduções significativas na contagem OPG ao longo do período experimental. Entretanto, uma exceção foi observada na coleta realizada no dia 14, quando o grupo suplementado apresentou uma redução significativa no OPG, especialmente durante o período de relaxamento da imunidade periparturiente. Esse período é crítico devido à imunossupressão fisiológica inerente à lactação e a suplementação alimentar pode mitigar esse efeito. Em nosso estudo, os valores de OPG mantiveram-se em níveis relativamente baixos durante todo o experimento, o que pode ser atribuído tanto à adequada quantidade de proteína bruta na dieta quanto à resistência natural da raça Santa Inês a infecções parasitárias. Rocha et al. (2011) reforçam essa observação, ao relatar aumento nas contagens de OPG mesmo em ovelhas no final da gestação e lactação suplementadas com altos níveis de proteína metabolizável, evidenciando a complexidade da resposta imunológica nesse estágio fisiológico. Os cordeiros nascidos dessas ovelhas suplementadas apresentaram maior peso ao desmame, especialmente os de partos únicos. Esse resultado sugere que a suplementação durante a lactação pode ter melhorado a qualidade nutricional do leite ou a eficiência alimentar das ovelhas, refletindo no desenvolvimento da prole.

A literatura corrobora a importância da nutrição materna, sendo que Roca Fraga et al. (2018) constataram que ovelhas submetidas a subnutrição no terço final da gestação tiveram cordeiros com redução de até 22% no peso ao nascer, enquanto a suplementação acima das exigências de mantença resultou em melhorias no desenvolvimento neonatal. Particularmente relevante para nosso estudo, Hernández Mendo et al. (2023) demonstraram que a inclusão de fontes naturais de taninos, contidos na *Guazuma ulmifolia*, na dieta de ovinos melhorou parâmetros produtivos e qualidade da carne, reforçando o potencial de compostos bioativos similares aos presentes no bagaço de uva. Embora a suplementação possa aumentar o peso ao nascer dos cordeiros, nem todas as formas de suplementação produzem diferenças significativas no peso ao nascer, sugerindo que a eficácia pode depender da estratégia nutricional específica empregada (DOVE et al., 2017).

Adicionalmente, o bagaço de uva contém compostos bioativos, como taninos condensados e flavonoides, que têm demonstrado efeitos anti-helmínticos significativos. Mena et al. (no prelo) observaram que o extrato hidroalcoólico de bagaço de uva a 500 mg/kg reduziu mais de 95% a contagem de OPG de nematoides gastrintestinais em ovinos naturalmente infectados, com efeito comparável ao obtido com o anti-helmíntico sintético levamisol. A ação anti-helmíntica é atribuída principalmente à presença de taninos condensados, que podem se ligar à cutícula de larvas e vermes adultos, comprometendo suas funções vitais e promovendo sua eliminação (ATHANASIADOU et al., 2001). Similarmente, o estudo de Sanchez et al. (2025) destacou o potencial do extrato de *Piper cubeba* em controlar infecções por *H. contortus*, sugerindo que compostos fenólicos presentes no extrato são responsáveis por sua eficácia anti-helmíntica. Esses resultados corroboram o uso de bagaço de uva como uma alternativa sustentável e eficaz no manejo integrado de parasitoses em pequenos ruminantes, oferecendo vantagens ecológicas e econômicas para a produção animal.

Os resultados dos experimentos, apresentados nos capítulos dois e três, destacaram a eficácia do bagaço de uva na redução de parâmetros parasitológicos e na melhoria do estado nutricional dos animais. Esse efeito pode ser atribuído à presença de compostos bioativos, como taninos condensados e polifenóis, que têm demonstrado propriedades anti-helmínticas e antioxidantes significativas em estudos *in vitro* e *in vivo*. Esses compostos atuam tanto na inibição do desenvolvimento dos parasitas quanto na modulação da resposta imunológica dos hospedeiros (HOSTE et al., 2012 e 2022; SOARES et al., 2018; UNIYAL et al., 2024). Embora não tenham sido observadas diferenças significativas, médias superiores de eosinófilos e imunoglobulinas específicas nos animais suplementados sugerem que um período mais prolongado de suplementação poderia tornar esses efeitos mais evidentes.

Adicionalmente ao seu efeito anti-helmíntico, o bagaço de uva tem se mostrado uma estratégia eficiente para promover sustentabilidade na pecuária. O reaproveitamento de subprodutos da vinificação, como o bagaço de uva, insere-se no contexto da economia circular, mitigando os impactos ambientais decorrentes do descarte inadequado desses resíduos e agregando valor econômico ao setor agrícola (DILEK et al., 2024; KOKKINOMAGOULOS e KANDYLIS, 2023). O estudo de Taifouris et al. (2023) demonstra que a produção de compostos de alto valor agregado, como polifenóis e óleos essenciais, apresenta maior rentabilidade em capacidades de processamento, com redução de emissões de gases de efeito estufa em comparação a processos térmicos como pirólise e combustão. Além disso, a análise de ciclo de vida aponta que a produção de ração para animais a partir do bagaço de uva apresenta menor impacto ambiental comparado a práticas tradicionais, como compostagem ou incineração, ao mesmo tempo em que gera benefícios econômicos significativos (RAMOS e FERREIRA, 2022). Tais iniciativas não apenas promovem a sustentabilidade no manejo de resíduos, mas também impulsionam a inovação tecnológica e a eficiência no uso de recursos naturais.

O uso de subprodutos agroindustriais, como o bagaço de uva, pode substituir parcialmente ingredientes convencionais da ração, reduzindo os custos com alimentação. Um estudo de Calvillo-Marín et al. (2022) demonstrou que a substituição de alfafa por subprodutos (como resíduos de cervejaria) na dieta de ovelhas em lactação reduziu os custos em até 33,8%, sem comprometer o desempenho animal. Em nosso estudo, o bagaço de uva substituiu 20% do concentrado, o que, em escala prática, reduziria os custos com ração comercial. Trabalhos futuros podem incluir análises econômicas detalhadas para quantificar essas economias. Contudo, a aplicabilidade desta estratégia está intrinsecamente vinculada a fatores logísticos: o transporte do resíduo além de regiões vinícolas tende a se tornar inviável devido ao alto volume do material e aos custos de combustível (especialmente em cenários de alta nos preços). Essa realidade restringe o uso econômico do bagaço principalmente a propriedades próximas a vinícolas, como observado no Sul do Brasil (IBGE, 2023), onde a sinergia entre produção vitivinícola e pecuária ovina é naturalmente favorecida.

Apesar dos avanços, desafios persistem devido à variabilidade na composição do bagaço de uva, influenciada por múltiplos fatores como o cultivar utilizado, o método de processamento e extração (que impacta diretamente o rendimento e qualidade dos compostos bioativos), e as condições edafoclimáticas de origem, frequentemente denominadas "efeito da safra", que desempenham papel crucial na determinação do perfil químico deste subproduto, podendo levar a variações significativas nas propriedades antioxidantes e no teor de polifenóis (ONACHE et al., 2022; LOPES et al., 2025; GUAITA et al., 2023).

Além disso, embora a dose ideal de inclusão na dieta ainda não esteja completamente estabelecida, este estudo utilizou uma proporção de aproximadamente 20% de bagaço de uva no concentrado dos animais, demonstrando resultados promissores. De forma complementar, Amaral et al. (2019) sugerem que até 300 g/kg de MS de substituição de feno por subproduto de uva é seguro, enquanto Juráček et al. (2021) relataram que a inclusão de 2% de bagaço de uva na matéria seca da dieta de ovinos resultou em melhorias significativas na digestibilidade de proteína bruta, carboidratos não fibrosos, fibra em detergente neutro e matéria orgânica, sem efeitos adversos no perfil bioquímico sanguíneo. Os autores concluíram que essa concentração é adequada para a nutrição de ovinos, destacando seu potencial como fonte de nutrientes e seu benefício na digestibilidade da proteína bruta da dieta.

Os resultados obtidos nos experimentos demonstraram que a adição do bagaço de uva à dieta de cordeiros e ovelhas pode ser considerada uma estratégia viável de suplementação alimentar, contribuindo para o controle de nematoides gastrintestinais sem comprometer o desempenho produtivo dos animais. Além disso, o bagaço de uva mostrou-se uma alternativa promissora no manejo sustentável de resíduos agroindustriais, oferecendo benefícios econômicos e ambientais. No entanto, futuros estudos são necessários para avaliar os efeitos a longo prazo do uso contínuo do bagaço de uva na dieta, incluindo sua influência na saúde ruminal, parâmetros reprodutivos e qualidade dos produtos de origem animal. Investigações adicionais também devem explorar a possibilidade de combinar o bagaço de uva com outras fontes de compostos bioativos para potencializar seus efeitos anti-helmínticos e nutricionais, bem como avaliar a viabilidade econômica da sua utilização em larga escala.

2. CONCLUSÕES GERAIS

A suplementação de cordeiros com bagaço de uva demonstrou efeitos positivos, impactando em menor tamanho e fecundidade das fêmeas de *H. contortus*, com consequente redução na contagem de OPG, além de que houve melhora nos parâmetros relacionados à resposta imune e hematológicos. Enquanto que em ovelhas lactantes apresentou potencial para otimizar o desempenho produtivo e o perfil hematológico, principalmente no início da lactação, além de sugerir um papel complementar no controle de nematoides gastrintestinais. Além disso, os cordeiros nascidos de ovelhas suplementadas apresentaram maior peso ao desmame, indicando um benefício indireto na produtividade da prole. Esses resultados destacam os benefícios econômicos e ambientais desse subproduto agroindustrial, reforçando sua viabilidade no manejo integrado de parasitas, especialmente em propriedades próximas a regiões vinícolas.

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ANEXOS

UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

fmvz - unesp
Faculdade de Medicina Veterinária e Zootecnia
Campus de Botucatu

ATESTADO

Atesto que o Projeto "AVALIAÇÃO DA ATIVIDADE ANTI-HELMÍNTICA DO BAGAÇO DE UVA EM CORDEIROS INFECTADOS ARTIFICIALMENTE COM Haemonchus contortus" **Protocolo CEUA 0542/2023** , a ser conduzido por Mateus Oliveira Mena, responsável/orientador Alessandro Francisco Talamini do Amarante, para fins de pesquisa científica/ensino - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA.

Finalidade	PESQUISA CIENTÍFICA
Vigência do projeto	16/10/2023 a 16/10/2024
Nome Comum / Espécie / Linhagem	OVINA / OVIS ARIES /
Raça	Santa Inês
Nº de animais machos	20
Nº de animais fêmeas	0
Nº de animais sexo indefinido	0
Peso médio de animais machos	16
Peso médio de animais fêmeas	0
Peso médio de animais sexo indefinido	0
Idade	0 ano(s) e 2 mes(es) e 0 dia(s).
Procedência	Própria

Projeto de Pesquisa aprovado em reunião da CEUA em 06/10/2023

JULIANY GOMES QUITZAN
Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

Faculdade de Medicina Veterinária e Zootecnia
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CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DA ATIVIDADE ANTI-HELMÍNTICA DO BAGAÇO DE UVA EM OVELHAS INFECTADAS POR NEMATOIDES GASTRINTESTINAIS DURANTE A LACTAÇÃO ", protocolada sob o CEUA nº 7450220324 (ID 000729), sob a responsabilidade de **Alessandro Francisco Talamini do Amarante** *e equipe; Mateus Oliveira Mena* - 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 Instituto de Biociências da Universidade Estadual Paulista (IBB/UNESP) na reunião de 23/05/2024.

We certify that the proposal "EVALUATION OF THE ANTIHELMINTHIC ACTIVITY OF GRAPE POMAGE IN SHEEP INFECTED BY GASTROINTESTINAL NEMATODES DURING LACTATION", utilizing 20 Ovines (20 females), protocol number CEUA 7450220324 (ID 000729), under the responsibility of **Alessandro Francisco Talamini do Amarante** *and team; Mateus Oliveira Mena* - 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 São Paulo State University/Biosciences Institute (IBB/UNESP) in the meeting of 05/23/2024.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 06/2024 a 05/2025 Área: Ciências Biológicas

Origem: Animais provenientes de outros projetos			
Espécie: Ovinos	sexo: Fêmeas	idade: 2 a 4 anos	Quantidade: 20
Linhagem: Santa Inês		Peso: 50 a 70 kg	

Botucatu, 23 de maio de 2024

Profa. Dra. Ana Carolina Inhasz Kiss
Coordenadora da Comissão de Ética no Uso de Animais
Instituto de Biociências da Universidade Estadual Paulista

Prof. Assoc. Luis Fernando Barbisan
Vice-Coordenador da Comissão de Ética no Uso de Animais
Instituto de Biociências da Universidade Estadual Paulista