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**USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR A
GLICERINA E O MELAÇO NA DIETA DE OVINOS**

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**USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR A
GLICERINA E O MELAÇO NA DIETA DE OVINOS**

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Maxwell Lima Reis

Dedico esse trabalho a você, Mãe
Rita Lima Reis (in memoriam)

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USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR A GLICERINA E O MELAÇO NA DIETA DE OVINOS

RESUMO

Este trabalho utilizou uma abordagem meta-analítica para avaliar o impacto da inclusão de glicerina e melaço em dietas para ovinos, com foco no desempenho animal, características de carcaça, parâmetros físico-químicos e perfil de ácidos graxos da carne. O Estudo 1 investigou os efeitos da glicerina bruta na dieta de ovinos, utilizando 38 artigos selecionados para a meta-análise. Os resultados mostraram que a inclusão de glicerina aumentou a digestibilidade da matéria seca, proteína bruta e fibra em detergente neutro, mas reduziu a ingestão de nitrogênio e a excreção urinária de nitrogênio. Houve também alterações na coloração e composição centesimal da carne. A análise de subgrupos revelou que o fator genético influenciou o aumento no consumo de matéria seca (CMS) e a redução de ácidos graxos saturados (AGS). O tipo de delineamento experimental afetou significativamente o consumo de matéria seca (CMS) e a digestibilidade da proteína bruta (PB). Além disso, o período de alimentação e a quantidade de concentrado contendo glicerina influenciaram os resultados, com um aumento na digestibilidade da proteína bruta e da glicemia em períodos de alimentação mais longos. Concluiu-se que a glicerina pode ser um substituto eficaz para ingredientes energéticos, melhorando a digestibilidade sem comprometer a qualidade da carne, especialmente quando ajustada para fatores como genética e delineamento experimental. O Estudo 2 avaliou o efeito do melaço na dieta de ovinos, com base em 26 artigos incluídos na meta-análise. A inclusão de melaço aumentou a digestibilidade da matéria seca, fibra em detergente ácido e neutro, propionato e butirato, mas reduziu o consumo total de matéria seca, nitrogênio uréico no sangue, pH ruminal, acetato, isovalerato e isobutirato. A análise mostrou que a resposta ao melaço pode variar conforme a idade dos animais, com uma diminuição do consumo de matéria seca total (CMS-Total) e da digestibilidade da proteína bruta em ovinos entre 6 a 8 meses de idade. Além disso, a inclusão de melaço por períodos curtos (menos de 30 dias) aumentou a digestibilidade da fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA). O melaço também aumentou o peso corporal final, ganho médio diário e rendimento de carcaça, especialmente em níveis de 100 a 200 g/kg na dieta. Esses resultados sugerem que o melaço é uma opção viável para melhorar o desempenho animal e as características de carcaça, mas a dosagem e o tempo de inclusão devem ser ajustados para maximizar os benefícios e minimizar os impactos negativos na ingestão e fermentação ruminal. Ambos os subprodutos, glicerina e melaço, demonstraram ser alternativas viáveis aos ingredientes convencionais de dieta para ovinos, com benefícios distintos. No entanto, as dosagens devem ser ajustadas cuidadosamente para equilibrar os benefícios de digestibilidade e desempenho com possíveis impactos negativos na ingestão e na fermentação ruminal. Estudos adicionais são recomendados para otimizar as dosagens e explorar mais a fundo as interações com outros componentes dietéticos e fatores de manejo.

Palavras-chaves: cordeiros, desempenho, glicerol, qualidade da carne, sacarose.

ABSTRACT

This study employed a meta-analytical approach to evaluate the impact of including glycerin and molasses in sheep diets, focusing on animal performance, carcass characteristics, physicochemical parameters, and fatty acid profile of the meat. Study 1 investigated the effects of crude glycerin in sheep diets using 38 selected articles for the meta-analysis. The results showed that the inclusion of glycerin increased the digestibility of dry matter, crude protein, and neutral detergent fiber but reduced nitrogen intake and urinary nitrogen excretion. There were also changes in meat color and centesimal composition. Subgroup analysis revealed that the genetic factor influenced the increase in dry matter intake (DMI) and the reduction in saturated fatty acids (SFA). The type of experimental design significantly affected dry matter intake (DMI) and crude protein digestibility (CPD). Additionally, the feeding period and the amount of concentrate containing glycerin influenced the results, with an increase in crude protein digestibility and glycemia observed with longer feeding periods. It was concluded that glycerin can be an effective substitute for energy ingredients, improving digestibility without compromising meat quality, especially when adjusted for factors such as genetics and experimental design. Study 2 evaluated the effect of molasses in sheep diets based on 26 articles included in the meta-analysis. The inclusion of molasses increased the digestibility of dry matter, acid detergent fiber, neutral detergent fiber, propionate, and butyrate but reduced total dry matter intake, blood urea nitrogen, rumen pH, acetate, isovalerate, and isobutyrate. The analysis showed that the response to molasses can vary according to the age of the animals, with a decrease in total dry matter intake (TMI) and crude protein digestibility in sheep aged between 6 to 8 months. Furthermore, the inclusion of molasses for short periods (less than 30 days) increased the digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF). Molasses also increased final body weight, average daily gain, and carcass yield, especially at levels of 100 to 200 g/kg in the diet. These results suggest that molasses is a viable option for improving animal performance and carcass characteristics, but the dosage and inclusion time should be adjusted to maximize benefits and minimize negative impacts on intake and rumen fermentation. Both by-products, glycerin and molasses, proved to be viable alternatives to conventional dietary ingredients for sheep, each with distinct benefits. However, dosages should be carefully adjusted to balance the benefits of digestibility and performance with potential negative impacts on intake and rumen fermentation. Further studies are recommended to optimize dosages and explore interactions with other dietary components and management factors more deeply.

Keywords: lambs, performance, glycerol, meat quality, sucrose.

1 INTRODUÇÃO

A ovinocultura de corte tem se destacado como uma atividade de relevância econômica e social no Brasil, contribuindo significativamente para a geração de emprego e renda e para a fixação das populações rurais no campo. Apesar do seu potencial promissor, a ovinocultura brasileira enfrenta desafios consideráveis que limitam sua produtividade, incluindo questões relacionadas à genética, nutrição e sanidade (Cartaxo et al., 2017). A falta de tecnologias adequadas para otimizar a produção e a produtividade contribui para a baixa competitividade da ovinocultura brasileira no mercado global.

O Brasil possui atualmente um rebanho ovino de aproximadamente 21.514.274 cabeças, distribuídas em 525.882 estabelecimentos, segundo o censo agropecuário, com a Bahia sendo o maior produtor, somando 4.660.494 cabeças (IBGE, 2022). Apesar do potencial de produção, o consumo de carne ovina no Brasil é baixo, com uma previsão de 0,519 kg per capita para 2024 (FAO, 2015). Esse baixo consumo está ligado à falta de qualificação e manejos inadequados, que afetam a qualidade da carne, resultando em um produto de menor valor comercial. As condições higiênico-sanitárias durante a produção, manipulação e comercialização são fatores críticos que influenciam a qualidade final da carne (Oliveira et al., 2023).

Nesse contexto, o uso de subprodutos da agroindústria surge como uma solução inovadora para melhorar a nutrição animal e, conseqüentemente, a produtividade da ovinocultura. A utilização de coprodutos, como a glicerina e o melaço, como ingredientes de dieta, pode proporcionar uma alternativa econômica aos ingredientes tradicionais, especialmente para pequenos e médios produtores que têm acesso a esses resíduos (Azevêdo et al., 2012). A inclusão desses ingredientes, mesmo na forma de suplementação, em sistemas intensivos de produção, pode aumentar a eficiência produtiva, pois animais que têm suas exigências nutricionais atendidas são mais resistentes a problemas sanitários e metabólicos, o que afeta diretamente a qualidade e o valor comercial do produto final (Roberto et al., 2024).

A glicerina, o principal coproduto da produção de biodiesel, representa aproximadamente 10% do volume total de biodiesel produzido (Dasari et al., 2005). Devido ao seu alto valor energético, a glicerina bruta tem sido explorada como uma fonte alternativa de energia na alimentação animal, com potencial para substituir parcialmente concentrados energéticos tradicionais, como o milho (Menten et al., 2008; Fávaro et al., 2015). O uso de glicerina em dietas para ovinos pode não apenas reduzir custos, mas

também melhorar a digestibilidade dos nutrientes, o que pode resultar em um melhor desempenho animal.

Além da glicerina, o melaço, um subproduto do processamento agroindustrial, também se destaca como um ingrediente promissor para a alimentação animal. Este coproduto pode reduzir custos e otimizar a eficiência produtiva ao enriquecer dietas convencionais, especialmente em substituição ao milho. O melaço de soja, por exemplo, é produzido a partir da extração de carboidratos do farelo de soja desengordurado e possui alto valor nutritivo (Rakita et al., 2021). Já o melaço de cana-de-açúcar, que contém sacarose e outros produtos do caldo de cana, serve como uma rica fonte de energia para ruminantes (Santana; Souza, 1984).

Embora existam resultados promissores que recomendam a inclusão de glicerina e melaço em dietas para ovinos, os estudos na literatura apresentam resultados ainda controversos. As variações nas condições experimentais, tipos de dietas e características dos animais podem levar a diferentes interpretações sobre a eficácia desses subprodutos. Nesse sentido, a meta-análise surge como uma ferramenta estatística robusta para sintetizar dados de múltiplos estudos, oferecendo uma visão mais clara e conclusiva sobre os efeitos da glicerina e do melaço na dieta de ovinos. Essa abordagem permite avaliar de maneira integrada os efeitos sobre características de carcaça, parâmetros nutricionais e eficiência alimentar (Lovatto et al., 2007; Rossi et al., 2008; Teodoro et al., 2012; Boemo et al., 2015; Claro; Mercadante; Silva, 2012; Faruk; Roos; Cisneros-Gonzalez, 2018; Sánchez-Duarte et al., 2019).

Portanto, este trabalho tem como objetivo avaliar o uso de glicerina e melaço em dietas para ovinos, utilizando uma abordagem meta-analítica para proporcionar resultados mais conclusivos sobre os impactos desses coprodutos na nutrição e desempenho animal.

2 REFERENCIAL TEÓRICO

Existe uma diferença conceitual entre subproduto e coproduto e as características que os distinguem. Basicamente, ambos são substâncias ou materiais gerados secundariamente em um processo de produção. Os coprodutos e subprodutos agroindustriais são os resíduos secundários gerados no processo de industrialização de produtos agrícolas. O que distingue coproduto de um subproduto é a existência ou não de um mercado definido para a sua comercialização. Assim, os produtos secundários de um processo agroindustrial que são demandados pelo mercado e que apresentam um valor de comercialização definido são chamados de coprodutos e aqueles que não tem potencial mercadológico ou cujo potencial não é efetivamente explorado são chamados de subprodutos (Jayathilakan et al., 2012).

Em busca de um sistema que proporcione o desempenho dos animais através de uma alimentação que tenha baixo custo e atendendo às exigências nutricionais, busca-se por alimentos alternativos, como uma alternativa para melhoria da eficiência produtiva e econômica dos sistemas de produção, os coprodutos ou resíduos de agroindústria podem ser utilizados como suplementos na alimentação animal (Silva, 2010).

2.1 Uso da glicerina na alimentação de ovinos

Dentre os vários coprodutos agroindustriais utilizados na alimentação de ruminantes, destaca-se os derivados da produção de biodiesel. A glicerina ($C_3H_8O_3$) é o principal coproduto gerado na produção do biodiesel e aproximadamente 10% do volume total de biodiesel produzido corresponde à glicerina (Dasari et al., 2005). Este coproduto resulta da formação de ésteres metílicos de ácidos graxos a partir de triglicerídeos (Chanjula; Pakdeechanuan; Wattanasit, 2015). A glicerina pode ser utilizada como ingrediente energético em rações, substituindo grãos mais caros, para aumentar a rentabilidade da atividade (Chanjula et al., 2016).

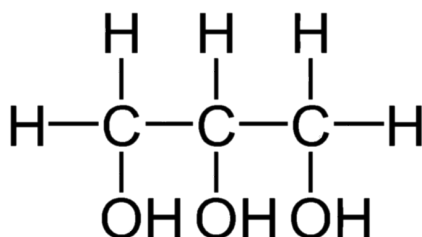


FIGURA 1- Estrutura química do glicerol

A produção de ácidos graxos de cadeia curta (AGCC) a partir da fermentação ruminal sobre os alimentos que os animais ingerem, e sua subsequente absorção, representam a maior fonte de energia para os ruminantes. As produções de acetato e propionato são as mais afetadas, pois tem relação direta da utilização do substrato pela microbiota. O propionato é utilizado, pelo animal, pela maior produção de glicogênio, constituindo a maior fonte de glicose para os ruminantes. O acetato é também o principal substrato para lipogênese (Palmquist; Mattos, 2011).

Quando ingerido pelos ruminantes, o glicerol, é convertido em propionato no rúmen (Lee et al., 2011), a maior parte é convertida em glicose para produção de energia. O propionato, por sua vez, tem um importante papel como precursor dos ácidos graxos de cadeia ímpar (Berthelot et al., 2001). Além disso, o glicerol pode reduzir a lipólise e a biohidrogenação no rúmen (Edwards et al., 2012), promovendo um maior acúmulo de ácidos graxos insaturados (UFA) na carne (Barros et al., 2015; Carvalho et al., 2015; Lage et al., 2014), resultando em um produto mais saudável.

O glicerol é absorvido pelo epitélio ruminal, metabolizado no fígado e direcionado à gliconeogênese pela ação da enzima glicerolquinase, que o converte em glicose. Parte do glicerol pode ser fermentado para propionato no rúmen, que por sua vez é convertido em oxaloacetato através do ciclo de Krebs no fígado e pode ser usado para formar glicose pela via gliconeogênica. Assim, a glicerina bruta (GC) tem uma aplicação potencial como substrato gliconeogênico para ruminantes (Krehbiel, 2008). Além de servir como fonte de energia, o glicerol também pode ter efeitos positivos na retenção de aminoácidos por inibir a atividade das enzimas fosfoenolpiruvato, carboxiquinase e glutamato desidrogenase, o que resulta na economia de aminoácidos glicogênicos, favorecendo a deposição de proteína corporal (Cerrate, et al., 2006).

Estudos realizados com glicerol já foram realizados, buscando dados sobre desempenho, parâmetros bioquímicos ruminais e caracterização de carcaças de ovinos. Em um estudo no qual foram avaliados os efeitos de níveis crescentes de glicerina bruta (0%, 6%, 12% e 18%) utilizados como substituto do milho na alimentação de cordeiros sobre as características quantitativas da carcaça, e utilizados 40 cordeiros para o tal, obteve-se resultados satisfatórios em relação à substituição proposta cujo houve efeito linear crescente para o peso corporal ao abate com a substituição do milho pela glicerina bruta. Os consumos de matéria seca e energia metabolizável, peso de corpo vazio, peso de carcaça quente e peso de carcaça fria apresentaram efeito quadrático, com níveis

máximos de glicerina bruta estimados em 10,9%, 9,8%, 10,83%, 11,78% e 11,35%, respectivamente. Também não houve efeito das dietas nas medidas morfométricas da carcaça, exceto para o perímetro torácico e o índice de compacidade da carcaça, que apresentaram efeitos quadráticos e lineares, respectivamente (Da Costa et al., 2019).

Em um outro estudo que consistiu na avaliação dos efeitos na qualidade da carne ovina da substituição do farelo de soja e da dieta de milho por farelo de amendoim e glicerina bruta, foram fornecidas dietas contendo farelo de amendoim, glicerina bruta, e farelo de amendoim mais glicerina, levando em consideração a relação volumoso:concentrado de 40:60, e ao final foram obtidos resultados somente nos perfis de ácido graxos presentes na carcaça, sem alteração físico-química (Valença et al., 2020).

2.2 Uso do melaço na nutrição de ovinos

A cana-de-açúcar é uma planta pertencente à família das gramíneas (Poaceae), gênero *Saccharum* e espécie *Saccharum officinarum*. Utilizada como matéria-prima para a produção de diversos produtos, é composta em média de 65 a 75% de água, sendo seu principal componente a sacarose (figura 2), que corresponde a cerca de 70 a 90% das substâncias sólidas solúveis (Lemos; Stradiotto, 2012). O Brasil é o maior produtor mundial de cana-de-açúcar e apresenta grandes áreas cultiváveis assim como um clima favorável, o que o torna um país promissor para exportação da cana. De acordo com a União da indústria de cana-de-açúcar (Unica, 2019), o Brasil produziu na safra de 2018/19, 620 milhões de toneladas de cana-de-açúcar, 29 milhões de toneladas de açúcar e 33 bilhões de litros de etanol.

A soja é um dos principais cultivos da agricultura mundial e brasileira, devido ao seu grande potencial de produção e ao seu valor nutritivo, o que a faz ter uma grande aplicação na nutrição humana e animal, com grande papel econômico para o país, além de constituir a matéria prima necessária para o funcionamento de vários setores (Mauad et al., 2010). A soja é uma leguminosa cujo grão contém cerca de 34% de proteínas, 31% de carboidratos, 22% de lipídeos, 5% de resíduo mineral e 8% de umidade (Gonçalves et al., 2014). Dentre os carboidratos presentes no grão, destacam-se a glicose, frutose, galactose, sacarose, estaquiose, rafinose, melibiose, ramnose e maltopentose. O melaço de soja é um xarope viscoso, de cor marrom e sabor agri-doce (Loman; Ju, 2016), gerado durante a obtenção do extrato proteico de soja. O processo de produção do extrato proteico envolve uma etapa de extração, com solução etanólica 60% (v/v), a qual

insolubiliza as proteínas e fibras da soja, que são então retiradas e desidratadas, sendo obtido a proteína concentrada de soja. A fração líquida resultante da extração retém os açúcares da soja, os quais após recuperação do etanol por evaporação, originam o melaço de soja (Siqueira et al, 2008).

O melaço destinado para alimentação animal pode ser obtido a partir da indústria da cana-de-açúcar e é utilizado em dietas para animais ruminantes como fonte de carboidratos rapidamente fermentáveis no rúmen, melhora a palatabilidade dos alimentos e reduz a poeira. Os efeitos do melaço na produção de AGCC estão relacionados ao seu nível na dieta. Níveis dietéticos mais baixos de melaço (<15% MS) não modificam o padrão de fermentação e a produção de AGCC, e o mesmo é semelhante ao observado com dietas de grãos com o desejo de produção de propionato como substrato gliconeogênico para o fígado. Em contraste, quando a inclusão do melaço excede 15%, a produção de propionato é reduzida e um incremento anormal de ácido butírico é observada (Martin e Wing, 1966; Marty e Preston, 1970; Hatch e Beeson, 1972; Olbrich e Wayman, 1972; Wiedmeier et al., 1992;).

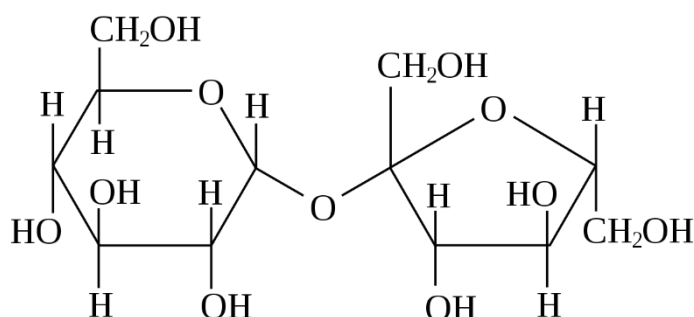


FIGURA 2- Estrutura química da sacarose

O setor sucroalcooleiro tem relevância na economia brasileira, possui um número expressivo de agroindústrias em todas as regiões do país, sendo responsável por gerar milhares de empregos diretos e indiretos. Diante disto, é considerável investigar sob o olhar do desenvolvimento sustentável como o setor impacta na sociedade, na economia e no meio ambiente, e também qual destinação que é dada aos seus resíduos e subprodutos (Silva et al., 2021).

A inovação no setor faz com que a utilização dos subprodutos da cana-de-açúcar possa minimizar os impactos ao meio ambiente, por dar uma destinação correta aos resíduos, como a utilização da vinhaça para a fertilização do solo e do bagaço da cana para a geração de energia, e pode gerar benefícios sociais, tais como empregos e

disponibilização de novos produtos para a sociedade (Neto et al., 2014).

Os trabalhos realizados com o melaço, e suas diversas origens (cana-de-açúcar, soja ou beterraba) apresentam alguns resultados significativos em relação ao desempenho e caracterização de carcaças de ovinos, e parâmetros bioquímicos ruminais. Ao se caracterizar o padrão fermentativo do glicerol, propileno glicol e melaço, incluindo a produção de ácidos graxos de cadeia curta, utilizando-se substratos de alfafa, silagem de milho, glicerol (320 e 640 μ l), propileno glicol (320 e 640 μ l) e melaço (320 μ l), foi observado o maior volume de gás produzido na maior dose de glicerol que também apresentou a taxa mais lenta de produção de gás e a maior defasagem em relação ao tempo ($P<0,05$). O propilenoglicol apresentou o menor volume de gás e foi rapidamente metabolizado com um curto intervalo de tempo e a fermentação de glicerol resultou em uma redução do acetato, um leve aumento no propionato e um incremento na percentagem de butirato. Incubações com propileno glicol também reduziu acetato e butirato, mas aumentou propionato ($P<0,05$), e o melaço apresentou características típicas de um substrato rapidamente disponível, com a taxa mais rápida de produção de gás ($P<0,05$), demonstrando uma alteração significativa na produção de ácidos graxos de cadeia curta (Ferraro et al., 2009).

Em um estudo que teve como objetivo avaliar o o efeito de inclusão dietética de milho, cevada ou melaço como fonte de energia em dietas de cama de frango processadas sobre a digestibilidade dos nutrientes, parâmetros ruminais metabólitos sanguíneos e fluxo microbiano para o duodeno em ovelhas, foram avaliadas dietas contendo cama de frango processada, com diferentes fontes de carboidratos (ou seja, milho, cevada ou melaço) na digestibilidade dos nutrientes, produção de proteína microbiana (PM), parâmetros ruminais e metabólitos sanguíneos, e as mesmas continham dieta com milho (alfafa, palha de trigo, processado CFP, grão de milho); dieta com cevada (alfafa, trigo palha, processado BL, cevada); e dieta com melaço (alfafa, palha de trigo, CFP, melaço), e os resultados obtidos demonstraram que a digestibilidade da matéria seca, proteína bruta e fibra detergente neutro e PM em ovinos alimentados com melaço foram maiores ($P<0,05$) em comparação com aqueles alimentados com dietas contendo milho ou cevada. Entretanto, ovino alimentados com melaço, a concentração de amônia ruminal foi menor do que os alimentados com outras dietas, e fontes de carboidratos nas dietas não tiveram efeito sobre a concentração de ácidos graxos voláteis (AGV) ($P>0,05$), exceto para AGV total e proporção molar de butirato que aumentou ($P<0,05$) pela alimentação do melaço.

A concentração na dieta de ovinos alimentados com melaço foi menor ($P<0,05$) do que a dieta contendo milho.

2.3 Uso da Abordagem meta-analítica na nutrição animal

A meta-análise é um recurso estatístico que conjuga respostas vinda de diferentes trabalhos primários (Santos; Cunha, 2013; Brei; Vieira; Matos, 2014; Rodrigues; Ziegelmann, 2010), permitindo avaliar suas combinações, tornando-se sendo um importante aliado na tomada de decisão.

A etimologia da meta-análise é de origem grega, onde *μετα*, quer dizer ‘depois de’ e *ανάλυση* que possui significado de “análise” (Bicudo, 2014). Os primeiros estudos conduzidos combinaram dados experimentais independentes (Cochran, 1954). Mais tarde, houve publicação de um método que serviu como modelo para essa modalidade de estudo (Mantel e Haenszel, 1959). Acredita-se que esse método fundamentou a meta-análise, e sua aplicação inicial foi aplicada nas ciências sociais, logo depois, na medicina humana, e mais à frente na agricultura (Lovatto et al., 2007).

Na agricultura, a meta-análise é potencialmente empregada, por exemplo, em experimentos de melhoramento vegetal e zoneamentos agrícolas, onde os dados coletados de mapas permitem averiguar a produtividade de culturas.

Logo, a meta-análise na agricultura tem contribuído para indicadores sociais, ambientais e sustentabilidade, relacionando suas complexidades com ênfase na viabilidade de técnicas como plantio direto, manejo do solo, agroquímicos, agroindustrialização e conservação dos recursos naturais. (Santos et al., 2020).

Na ciência animal, a técnica de meta-análise tem sido aplicada em diferentes áreas de estudos, entre elas: bovinocultura de corte (Giannotti; Packer; Mercadante, 2005); pecuária leiteira (Sánchez-Duarte et al., 2019); avicultura de corte (Boemo et al., 2015) e de postura (Faruk; Roos; Cisneros-Gonzalez, 2018); e suinocultura (Rossi et al., 2008). Além disso, meta-análises mais recentes foram conduzidas na avaliação da saúde (Taghipour et al., 2020), nutrição (Krasuska; Webb, 2018) e comportamento (Hradecká et al., 2015) de cães domésticos. Do mesmo modo, riscos de doenças pandêmicas em humanos causadas por animais silvestres na China foram estudados com uso de meta-análise (Chen et al., 2019).

As etapas da meta-análise são constituídas por quesitos operacionais, de coleta e de execução de dados, além da realização de análises e obtenção de resultados (Shelby;

Vaske, 2008). No entanto, alguns cuidados devem ser considerados no momento de aplicar a meta-análise. Por exemplo, adequar os dados à finalidade do estudo, o que pode ocasionar resultados tendenciosos (Luiz, 2002). Desse modo, algumas etapas são decisivas no momento de conduzir um estudo meta-analítico, tais como, a definição dos objetivos do estudo meta-analítico, a codificação das informações, a compilação, filtragem e investigação gráfica dos dados, análise do dispositivo experimental, e as práticas pós-análises. Os principais tipos de modelos usados na meta-análise são os de efeito fixo e de efeito aleatório.

Os modelos de efeitos fixos consideram que o efeito em evidência é igual entre os estudos. Já os efeitos aleatórios consideram que o efeito em evidência é diferente entre os estudos. Na meta-análise, combina-se estudos semelhantes, porém quando a variabilidade entre eles não é unicamente aleatória significa que estes são heterogêneos. Na presença de heterogeneidade, deve-se aplicar o modelo de efeitos aleatórios (Santos et al., 2020). A heterogeneidade é conferida pelo teste Q de Cochran (Rodrigues; Ziegelmann, 2010). Mas para quantificá-la é utilizada estatística I^2 de Higgins e Thompson (Higgins et al., 2003; Re, 2015). Os valores variam de 0 a 100%. Quando os valores estão próximos de zero, não há heterogeneidade; próximos a 25% é baixa; próximos a 50% é moderada; e próxima a 75% é alta (Higgins; Thompson, 2002; Higgins et al., 2003).

Na meta-análise, os efeitos podem ser conferidos, por exemplo, pela diferença média padronizada (DMP), conforme sugerido por Cochen (1988). Já o peso dos estudos é medido pelo inverso da variância (IV) e estimado pela DMP (Borenstein et al., 2009; Sánchez-Duarte et al., 2019). Uma etapa importante na condução de pesquisas meta-analíticas é o julgamento de vieses de publicação, designado de gráfico em Funil, feito a partir de um diagrama de dispersão. A simetria do gráfico é conferida pelo teste de Begg e Mazumdar (1994) e de Egger et al. (1997).

Uma forma de representar os resultados extraídos na meta-análise é através do *forest plot*, um gráfico que permite a visualização dos resultados. Esse gráfico mostra o intervalo de confiança e o tamanho de efeito dos estudos, esta última é descrita por um ícone comumente no formato de um quadrado, já o tamanho do quadrado é equivalente ao peso do trabalho incluído na meta-análise (Rodrigues; Ziegelmann, 2010).

Análises adicionais à meta-análise podem ser conduzidas para explicar fatores que induzem a heterogeneidade dos estudos. Entre eles, a meta-regressão que é um método estatístico utilizado em complemento da meta-análise (Sánchez-Duarte et al., 2019)

Esta Tese de Doutorado está dividida em dois artigos científicos a serem submetidos para análise na revista Journal of Animal Science, *qualis* A2.

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CAPÍTULO 1:

USE OF GLYCERIN IN DIETS FOR SHEEP: META-ANALYSIS

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ABSTRACT

The use of crude glycerin in sheep diets has gained attention as a viable alternative to replace traditional energy ingredients. Glycerin has the potential to improve nutrient digestibility and reduce feed costs. However, despite promising results, previous studies have shown varied and sometimes controversial findings regarding the effects of including glycerin in sheep diets. A meta-analysis was conducted to integrate existing data and provide a clearer and more conclusive understanding of the impact of glycerin on animal performance, physicochemical parameters, and meat quality. The meta-analysis was conducted based on 38 selected articles. The results indicated that the inclusion of glycerin increased the digestibility of dry matter, crude protein, and neutral detergent fiber, proving advantageous for the feed efficiency of sheep. However, nitrogen intake and urinary nitrogen excretion were reduced, and changes in meat color and centesimal composition were observed. Subgroup analysis revealed that factors such as animal genetics influenced the results, with an increase in dry matter intake and a reduction in saturated fatty acids. The type of experimental design used in the reviewed studies significantly affected dry matter intake and crude protein digestibility. The experimental period and the amount of concentrate containing glycerin positively influenced crude protein digestibility and blood glucose levels, while the amount of glycerin in the diet was associated with a reduction in carcass yield. It is concluded that glycerin can be included in sheep diets as a viable substitute for conventional energy ingredients, improving nutrient digestibility without causing adverse changes in meat quality.

Keywords: fatty acids, lambs, glycerol, meat quality

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1 Introduction

The global increase in demand for biofuels has led to a higher need for raw materials (such as corn, wheat, and oilseeds), which, in turn, has raised the costs of animal feed. However, the growth in biodiesel production and its byproduct, glycerin, has provided a more accessible and cost-effective energy source (Kholif, 2019) that can be included in ruminant diets. Diets with high-purity glycerin (0%, 5%, and 10%), used as a substitute for corn in the concentrate, have been tested in sheep to evaluate effects on performance, blood components, and nutrient digestibility. Results showed a decrease in dry matter intake (DMI) with higher levels of glycerin inclusion but maintained an adequate average daily gain and improved feed efficiency (Saleen and Singer, 2017). In another study, Valença et al. (2020) examined the inclusion of crude glycerin in sheep diets as a corn substitute and observed that meat quality remained unchanged, while the fatty acid profile of the meat improved.

The variation in responses to crude glycerin inclusion, particularly in lipid concentration and meat lipid profile, may be associated with various factors, such as genetics, animal age, and feeding period (Torres et al., 2021), as well as the glycerin concentration in the diets. Previous studies have reported that glycerin can have qualitative effects in ruminant diets, primarily due to increased availability of gluconeogenic compounds that act as precursors for fatty acids to be deposited in meat. This supports the hypothesis that crude glycerin reduces lipolysis, promoting an increase in the passage of unsaturated fatty acids through the rumen, which are then absorbed in the intestine and incorporated into the meat (Edwards et al., 2012; Carvalho et al., 2015; Castagnino et al., 2018).

Research has described several effects of including crude glycerin in ruminant diets. In an experiment by Brant et al. (2022), the effects of replacing ground corn with glycerin and soybean meal with cottonseed cake, either in combination or independently, were evaluated on nutrient intake, apparent digestibility, ingestive behavior, ruminal fermentation, nitrogen utilization, and blood metabolites. Lower nutrient intake was observed, along with increased digestibility of crude protein (CP) and ether extract (EE). Additionally, the interaction between energy sources altered pH and short-chain fatty acid (SCFA) concentrations, leading to an increase in butyrate and propionate. In a study by

Souza et al. (2015) using crude glycerin in diets for crossbred Santa Inês and Dorper sheep, an increase in EE digestibility was noted, influenced by the interaction between crude glycerin levels and sheep breed, as well as changes in nutrient intake and digestibility, resulting in poorer feed conversion.

Glycerin, when combined with other factors such as genetics, feeding strategies, and feeding periods, can alter fatty acid metabolism in the rumen, animal performance, and nutrient digestibility. As an alternative energy source in sheep feeding, glycerin requires further study to determine its optimal inclusion levels and the most suitable presentation forms for animal nutrition. This study aims to evaluate experimental research on glycerin inclusion using a meta-analytical approach in sheep diets.

2 Material and Methods

2.1. Data set

A comprehensive literature search was conducted using four search engines: PubMed, Science Direct, Wiley library and CAB direct. Around 5116 publications were retrieved using the following search terms: “glycerin and sheep”, “glycerin and lamb” and “glycerol and lamb”. Of the papers that were retrieved, only those that met the predetermined inclusion criteria were included in the meta-analysis. For inclusion into the meta-analysis, studies had to have the following (standardized criteria): 1) The control group does not include glycerin in the diet; 2) the treatment group includes only the inclusion of glycerin in diet and 3) study performed with total mixed ration (TMR) and/or confinement systems. A flowchart detailing the process of study identification and selection for analysis is shown in Figure 1.

2.2. Data Extraction

Based on inclusion criteria, 38 peer-reviewed publications with 98 means of treatment were rated by: first author, publication reference, country, genetic group, experimental period, experimental design, animal age (months), amount of glycerin in the diet (g/kg of DM) and amount of concentrate in the diet (g/kg of DM). The number of

replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables for control (diets with no glycerin) and treatment (diets with glycerin in diet) treatments: performance (i.e., feed intake, average daily gain; ADG), nutrient digestibility, carcass parameters (i.e., carcass weight), blood parameters, meat quality (i.e., meat color, fatty acid profile), ruminal parameters and nitrogen metabolism. The complete data set is available in Supplemental File S1.

2.3. Statistical analysis

2.3.1. Weighted mean difference and Publication bias

A meta-analysis was conducted using R Statistical Software (Metafor package, version 3.4.2; Viechtbauer, 2010). The Forest Graph (forest plot) was created using STATA software (Version 16.0; StataCorp LP, College Station, TX). The effects of glycerin inclusion in sheep diets were evaluated by examining the weighted mean difference (WMD) between glycerin treatment (diets with glycerin inclusion) and control treatment (diets with no glycerin). Treatments means were weighted by the inverse of the variance, according to the method proposed by Der-Simonian and Laird (1986) for random effects model.

Between-study variability (i.e., heterogeneity of the treatment effect) was evaluated using both the chi-square (Q) test of heterogeneity and I^2 statistics, which measures the percentage of variation due to heterogeneity (Higgins et al., 2003). Negative I^2 values were assigned as zero values. An I^2 value less than 25% indicate low heterogeneity, whereas values between 25 and 50% denote moderate heterogeneity and those above 50% denote high heterogeneity (Higgins et al., 2003).

Publication bias was evaluated using the funnel plot (Light and Pillemer, 1984) and asymmetry test (indicative of publication bias) which was carried according to the Egger regression asymmetry test, among the WMD and SE (Egger et al., 1997). Significance was declared at $P \leq 0.05$. Outliers were removed when studentized residuals outside range of -2.5 to 2.5.

2.3.2 Meta-regression and Subgroup analysis

The meta-regression analysis was conducted to identify the effects of categorical covariates and select the ones to perform the subgroup analysis. A mixed model was applied to adjust the data in the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by

$$\Theta_i = \beta + \beta_i x_{ij} + \dots \beta_{ip} x_{ip} + \mu_i$$

Where Θ_i = the true treatment effect in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_i = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, \tau^2)$. Here τ^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

To measure of between-study variance ($\text{Tau-squared} = T^2$). The moment estimator calculation of (T^2) is that used in Der-Simonian and Laird random effects meta-analysis but is less suitable when covariates are included (Thompson and Sharp, 1999). The restricted maximum likelihood estimate (REML) approach was used to estimate (T^2) because it is less likely to underestimate or produce biased estimates of variance (Thompson and Sharp, 1999; Viechtbauer, 2005).

Tests of null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010).

Meta-regression criteria were: 1) $P \leq 0.05$, for the heterogeneity test; 2) $P \geq 0.05$, for the funnel plot; 3) no observations with values for studentized residuals out of the range -2.5 to 2.5 (outliers). The WMD was evaluated by subgroup analysis when the categorical covariates (from the meta-regression analysis in Supplemental File S2) were significant at $P \leq 0.10$. The covariates were divided as following:

- Genetic type (Crossbreed, Ile de France and Santa Inês);
- Animal age (2.0-3.0 and 4.0-5.0 months);
- Experimental design (rotative = crossover, Latin Square and continuous = randomized complete block design);
- Experimental period (< 60, 60-90 and 100-130 days);
- Amount of concentrate in the diet (<150, 400-500, 600-700 and 800-900 g/kg of DM);
- Amount of crude glycerin in the diet (<30.0, 30.0-60.0, 60.0-90.0 and 100.0-200.0 g crude glycerin/kg of DM);
- Methanol in glycerin (<0.1, 38.0-48.0 and 60.0-87.0 g Methanol/kg of DM glycerin).

3 Results

The dataset comprised 38 peer-reviewed publications with 98 treatment means selected to investigate the effects of glycerin in the diet of sheep on performance, carcass and meat parameters, and meat fatty acid profile. Animals from different breeds were included in the studies: Mixed (50.00%), Santa Inês (21.42%), Arcott (6.12%), Pantaneira (6.12%), Ile de France (5.10%), Saidi (4.08%), Texel (3.06%), Merino (3.06%), and Ripollesa (2.04%). Animal age was reported as 2.0–3.0 months (38.77%), 4.0–5.0 months (13.26%), and not specified (47.96%).

Approximately 22.45% of the studies were conducted using a rotational design, while the majority (77.55%) employed a continuous design. Experimental periods varied: <60 days (9.18%), 60–90 days (47.95%), 100–130 days (12.24%), and not reported (30.61%). Diet concentrate levels were as follows: <150 g concentrate/kg DM (8.16%), 400–500 g/kg DM (12.24%), 600–700 g/kg DM (42.86%), 800–900 g/kg DM (22.45%), and unspecified in 14.28% of studies. Glycerin types included Crude Glycerin (86.73%), Semi-purified (3.06%), Refined Glycerol (6.12%), and High Purity (4.08%).

Quantities of crude glycerin in the diet were as follows: <30.0 (8.53%), 30.0–60.0 (18.29%), 60.0–90.0 (13.41%), 100.0–200.0 (47.56%), 200.0–300.0 (10.97%), and 450 g crude glycerin/kg DM in 1.21% of studies. Descriptive statistics for the full dataset on the effects of glycerin in sheep diets and crude glycerin composition are shown in

Table 1. Tables 2-4 provide an initial approach to determine whether the inclusion of glycerol affects each response parameter globally.

3.1 Dry Matter Intake, Digestibility, Nitrogen Metabolism, and Blood Parameters

Including glycerin in sheep diets increased dry matter digestibility ($P = 0.030$), crude protein ($P = 0.001$), and neutral detergent fiber (NDF; $P = 0.001$). However, it decreased nitrogen intake ($P = 0.001$) and urinary nitrogen excretion ($P < 0.0001$). Glycerin inclusion did not affect ($P > 0.05$) dry matter intake (DMI), organic matter digestibility, ether extract, fecal nitrogen excretion, nitrogen retention, blood glucose, blood urea nitrogen (BUN), or blood cholesterol (Table 2).

3.2 Performance, Carcass Parameters, and Meat Physicochemical Characteristics

The inclusion of glycerin in sheep diets had no effect ($P > 0.05$) on final body weight, average daily gain (ADG), carcass weight, subcutaneous fat thickness (SFT), loin area, mesenteric fat, final meat pH, lightness, redness, shear force, cooking loss, water-holding capacity (WHC), protein, fat, or cholesterol in the meat. However, glycerin inclusion reduced carcass yield ($P = 0.028$), yellowness ($P = 0.044$), thawing loss ($P < 0.0001$), and increased moisture ($P = 0.028$) and ash ($P = 0.050$) in the meat (Table 2).

3.3 Meat Fatty Acid Profile

The inclusion of glycerin in sheep diets reduced the concentration of lauric acid C12:0 ($P < 0.0001$), myristic acid C14:0 ($P < 0.0001$), palmitic acid C16:0 ($P < 0.0001$), palmitoleic acid C16:1 c9 ($P < 0.0001$), stearic acid C18:0 ($P < 0.0001$), vaccenic acid C18:1 t11 ($P = 0.001$), total saturated fatty acids (SFA; $P = 0.035$), and Omega-6/Omega-3 ratio ($P < 0.0001$). Glycerin inclusion increased concentrations of pentadecanoic acid C15:0 ($P < 0.0001$), heptadecanoic acid C17:0 ($P = 0.001$), oleic acid C18:1 c9 ($P < 0.0001$), linoleic acid C18:2 c9 c12 ($P < 0.0001$), conjugated linoleic acid (CLA c9 t11; $P < 0.0001$), α -linolenic acid C18:3 ω 3 ($P < 0.0001$), arachidonic acid C20:4 ($P < 0.0001$), eicosapentaenoic acid C20:5 ω -3 (EPA; $P < 0.0001$), docosahexaenoic acid C22:6 ω -3 (DHA; $P < 0.0001$), total monounsaturated fatty acids (MUFA; $P < 0.0001$), total

polyunsaturated fatty acids (PUFA; $P < 0.0001$), total Omega-3 ($P < 0.0001$), and total Omega-6 ($P < 0.0001$). Glycerin inclusion did not affect ($P > 0.05$) myristoleic acid (C14:1 n-7) or docosapentaenoic acid (DPA; C22:5 n-3) in the meat (Table 4).

3.4 Meta-Regression Analysis and Funnel Plot

As shown in Tables 2, 3, and 4, the funnel plot asymmetry test did not indicate publication bias ($P > 0.05$). The meta-regression analysis, presented in seven figures, identified genetic group and experimental period as the covariables most impacting glycerin responses in sheep diets. For response variables where covariates explained less than 50% of the heterogeneity (adjusted R^2), this suggests that unidentified factors outside our meta-analysis may have influenced the response to glycerin in sheep diets.

3.5 Subgroup Analysis

The inclusion of glycerin in Santa Inês sheep diets (Covariate = Genetic group; Figure 3) increased DMI (WMD = 0.03 kg/day; $P = 0.037$) and reduced total SFA (WMD = -3.80 g/100g; $P < 0.0001$) in the meat. Studies employing a rotational design (Figure 4) showed reduced DMI (WMD = -0.02 kg/day; $P = 0.053$), while both rotational and continuous designs increased crude protein digestibility (WMD = 13.19 g/kg DM; $P = 0.052$ and WMD = 7.89 g/kg DM; $P = 0.036$, respectively).

Independently of ages 2.0–3.0 and 4.0–5.0 months (Covariate = lamb age; Figure 5), glycerin inclusion in the diet reduced stearic acid C18:0 concentration (WMD = -1.35; $P = 0.009$ and WMD = -1.12 g/100g; $P = 0.045$, respectively) in meat. When assessing the effect of experimental period (Figure 6), glycerin inclusion in sheep diets increased crude protein digestibility (WMD = 13.62 g/kg DM; $P = 0.003$) and glycemia (WMD = 1.90 mg/dL; $P = 0.022$). Diets with 600–700 g concentrate/kg DM (Figure 7) increased DMI (WMD = 0.004 kg/day; $P = 0.056$) and crude protein digestibility (WMD = 15.54 g/kg DM; $P < 0.0001$).

In diets with 200.0–300.0 g crude glycerin/kg DM, carcass yield decreased (WMD = -1.17%; $P = 0.009$; Figure 2). Limited study numbers allowed analysis only for 100.0–200.0 g crude glycerin/kg DM on fatty acid profiles (Figure 8), showing increased concentrations of pentadecanoic acid C15:0 (WMD = 0.045 g/100g; $P < 0.0001$),

heptadecanoic acid C17:0 (WMD = 0.301 g/100g; $P = 0.005$), oleic acid C18:1 c9 (WMD = 2.125 g/100g; $P < 0.0001$), linoleic acid C18:2 c9 c12 (WMD = 0.179 g/100g; $P = 0.001$), CLA c9 t11 (WMD = 0.078 g/100g; $P < 0.0001$), α -linolenic acid C18:3 ω -3 (WMD = 0.016 g/100g; $P < 0.0001$), arachidonic acid C20:4 (WMD = 0.063 g/100g; $P < 0.0001$), and total PUFA (WMD = 0.215 g/100g; $P = 0.001$) and Total Omega-3 (WMD = 0.019 g/100g; $P = 0.004$). However, the inclusion of 100.0-200.0 gCG/kg DM in the diet reduced the concentration of lauric acid C12:0 (WMD = -0.013 g/100g; $P = 0.001$), myristic acid C14:0 (WMD = -0.420 g/100g; $P < 0.0001$), palmitic acid C16:0 (WMD = -1.027 g/100g; $P = 0.027$), palmitoleic acid (WMD = -0.048 g/100g; $P < 0.0001$), and stearic acid C18:0 (WMD = -1.076 g/100g; $P = 0.015$) in meat.

4 Discussion

Feed intake regulation in animals is a multifactorial response encompassing both the physical attributes of the diet and physiological factors arising from the animal's metabolism. Thus, when the digestibility of dietary fractions increases, it can result in high energy availability for the animal, inducing satiety through the chemostatic factor, which in turn can impact dry matter intake (DMI). Concerning the study on glycerin inclusion in diets for sheep as presented in the meta-analysis, such interference was not solely attributable to glycerin presence in the diet (DMI = 1.08 kg/day (Table 2)). However, when analyzing covariates, such as the influence of animal genetics (Santa Inês breed – WMD = 0.03 kg/day; $P = 0.037$) (Figure 3)), experimental designs adopted by researchers (rotational experimental design – WMD = -0.02 kg/day; $P = 0.053$) (Figure 6)), and the concentrate level in the diet (600–700 g concentrate/kg DM – WMD = 0.004 kg/day; $P = 0.056$) (Figure 7)), differences in DMI were observed. This reduction in DMI can also be explained by increased propionate synthesis by rumen microorganisms (Trabue et al., 2007), which stimulates greater insulin secretion (Oba and Allen, 2003). When insulin reaches the brain, it interacts with specific receptors that may reduce feed intake, or it could be due to increased energy density or diet palatability (Saleem and Singer, 2017). Another explanation may be the high fatty acid concentration (33%) in crude glycerin diets. Lipids are potent stimulators of cholecystokinin, an appetite-suppressing hormone that reduces gastric emptying (Lage et al., 2014).

The high digestibility of DM and nutrients (CP and NDF; Table 2) indicates that crude glycerin addition in sheep diets can improve nutrient absorption from the diet more effectively, as the increased metabolizable energy due to glycerin presence (3.64 Mcal/kg; Benedetti et al., 2016) compensates for reduced DMI, potentially enhancing animal performance (Saleem and Singer, 2017). Glycerin lacks NDF, and as shown in a meta-analysis on glycerin-containing diets for beef cattle, this absence diluted the effects on NDF intake and digestibility, maintaining the animals' ruminal pH stability at over 6.2, suggesting ruminal functions proceeded without significant environmental variations (stability) (Torres et al., 2021).

Despite carcass yield percentages being lower than expected (49.82%), possibly due to reduced DMI including diet concentrate, subcutaneous fat values ($P > 0.05$) showed no differences among studies analyzed in the meta-analysis with glycerin inclusion in sheep diets. This result can be attributed to reduced acetate production and increased propionate, which suppress lipogenesis around the carcass. Non-fermented glycerol in the rumen is absorbed through the intestines and rumen wall, transported to the liver, and then the enzyme glycerol kinase converts ATP to glycerol-3-phosphate and ADP, promoting gluconeogenesis (Lehninger et al., 2006) or is degraded and increases propionate production in the rumen, which consequently raises liver glucose production (González and Silva, 2006).

In sheep, meat color parameters, only meat yellowness ($P < 0.05$) showed differences, which is due to reduced beta-carotene in the diet, as corn is replaced by glycerin (Faustman and Suman, 2017).

When evaluating the genetic pattern subgroup, there was a reduction in total SFA production (WMD = -3.80 g/100g; $P < 0.0001$), as well as a reduction in stearic acid (C18:0 (WMD = -1.35; $P = 0.009$; Figure 5)). This result is beneficial for human health since reductions in these acids' intake can offer health advantages, as per Wood et al. (2003), who associated these acids' intake with an increase in low-density lipoproteins (LDL) in the blood, potentially detrimental to human health.

The experimental designs in the analyzed studies influenced intake (rotational – WMD = -0.02 kg/day, $P = 0.053$) and DM and CP digestibility (rotational and continuous

– WMD = 13.19 g/kg DM; P = 0.052 and WMD = 7.89 g/kg DM; P = 0.036, respectively), possibly due to factors under testing. These effects, when included in the statistical analysis, reveal the degree of influence in each researcher's outcomes, providing insights into the impact of error variation caused by tested variables on evaluated parameters.

Changes in fatty acid profile due to concentrate levels in glycerin-containing diets contribute to ruminal and microbiological profile shifts, reducing biohydrogenation efficiency and saturated fatty acid saturation, enabling passage to the intestine and subsequent absorption, eventually being incorporated into meat (Torres et al., 2021). Another possibility is glycerin's glycogenic potential, stimulating SCD-1 gene expression, encoding $\Delta 9$ desaturase enzyme responsible for synthesizing monounsaturated fatty acids from saturated ones, such as stearic acid (Smith et al., 2009).

5 Conclusion

This meta-analysis provides a solid foundation for decision-making in animal nutrition practices, suggesting glycerin as an effective and economically viable alternative to enhance productivity efficiency in sheep production systems. Glycerin can be included in sheep diets, improving nutrient digestibility, altering color, and enhancing the fatty acid profile, representing a suitable replacement for energetic ingredients in the diet.

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Table 1: Descriptive statistics of the complete data set for the effect of glycerin in sheep diets and chemical composition of crude glycerin in the studies

Item	Mean	Maximum	Minimum	SD	N ¹
Animal age, month	10.0	60.0	2.0	15.5	64
Experimental period, day	80	123	22	18.4	68
Control Group Diet (without glycerin in diet), g/kg of DM					
Concentrate	594.4	900.0	39.0	201.2	84
Dry matter	687.4	959.8	211.3	218.9	73
Crude protein	162.9	190.9	92.60	20.23	83
Ethereal extract	28.71	40.20	14.20	5.98	75
NDF	331.6	639.5	96.50	114.4	83
Non-fibrous carbohydrates	420.9	563.5	107.3	135.1	38
TDN	682.5	789.0	540.3	79.40	32
Starch	303.7	373.0	251.6	64.89	7
ME diet, Mcal/kg DM	5.18	11.96	2.70	3.68	18
Treatment Group Diet (with glycerin in diet), g/kg of DM					
Concentrate	594.4	900.0	39.0	201.2	84
Dry matter	692.2	947.2	228.1	215.7	73
Crude protein	161.9	189.7	86.00	22.06	83
Ethereal extract	33.37	82.50	6.30	17.22	75
NDF	315.2	621.4	97.20	104.1	83
Non-fibrous carbohydrates	410.9	528.8	82.30	131.8	38
TDN	690.7	813.0	500.2	79.97	32
Starch	148.0	285.0	27.30	85.35	7
ME diet, Mcal/kg DM	5.30	12.02	2.64	3.82	18
Crude glycerin, g/kg of DM					
Dry matter	881.5	963.7	676.1	78.91	27
Organic matter	924.9	965.0	889.0	34.14	7
Crude protein	10.06	40.00	0.600	9.81	15
Ether extract	94.57	117.8	43.50	58.72	6
Ash	58.76	111.0	20.00	33.26	13
Salts	49.93	60.00	35.70	11.96	9
Sodio	16.64	53.80	5.30	17.34	7
Methanol	22.91	87.00	0.001	30.53	22
Glycerol	695.55	895.3	362.0	201.1	25

¹Treatments mean from 38 studies are presented (Table S1 Online Data Supplement).

DM = dry matter; SD = standard deviation; NDF = neutral detergent fiber; TDN = total digestible nutrient; ME = metabolic energy.

N = Number of treatments means.

Table 2: Effects of inclusion glycerin in sheep diets on feed intake, apparent total-tract digestibility of nutrients, nitrogen metabolism and blood parameters.

Item ¹	Control ² mean (SD)	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	<i>P</i> value	<i>P</i> value	I ² (%)	<i>P</i> value
Intake, kg/d							
DMI-total	1.08 (0.31)	54	-0.007 (-0.02, 0.01)	0.319	0.002	38.90	0.575
Digestibility, g/kg of DM							
Dry matter	682.20 (82.84)	30	8.200 (0.76, 15.64)	0.030	<.0001	96.76	0.366
Organic matter	663.28 (95.24)	21	-4.283 (-11.45, 2.88)	0.241	<.0001	82.48	0.330
Crude protein	728.08 (63.43)	23	10.157 (3.76, 16.56)	0.001	<.0001	84.46	0.102
Ether extract	755.22 (153.73)	23	6.103 (-0.48, 12.69)	0.064	<.0001	90.84	0.632
NDF	494.65 (91.29)	22	10.42 (4.26, 16.57)	0.001	<.0001	70.63	0.063
Nitrogen Metabolism, g/d							
N Intake	17.73 (10.04)	16	-0.797 (-1.26, -0.33)	0.001	0.559	0.00	0.563
Urinary N excretion	7.19 (6.57)	13	-0.528 (-0.69, -0.35)	<.0001	0.442	0.36	0.812
Fecal N excretion	4.22 (2.78)	13	0.078 (-0.01, 0.17)	0.097	0.602	0.00	0.504
Retained N	6.55 (5.66)	13	-0.481 (-1.01, 0.05)	0.076	0.092	36.27	0.862
Blood parameters, mg/dl							
Glucose	68.75 (6.85)	11	0.219 (-2.07, 2.51)	0.850	0.001	69.51	0.582
Blood urea nitrogen	19.86 (9.71)	7	-1.062 (-2.19, 0.07)	0.066	0.623	0.00	0.644
Cholesterol	69.03 (13.96)	7	-0.159 (-0.49, 0.17)	0.354	0.597	0.00	0.081

¹ DMI = dry matter intake; NDF = neutral detergent fiber; DM = dry matter; N = nitrogen.

² Diets with no additives are the control treatment.

³ N represents the number of comparisons for control and glycerin treatments (complete data set is available in Supplementary File S1).

⁴ The weighted mean differences between control and glycerin treatments are presented (WMD).

⁵ I² represents the proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity.

⁶ Egger's regression asymmetry test (Funnel test).

Table 3: Effects of inclusion glycerin in sheep diets on their performance, carcass parameters and physicochemical characteristics of meat.

Item ¹	Control ² mean (SD)	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P value	P value	I ² (%)	P value
Final body weight, kg	40.50 (7.77)	50	-0.008 (-0.14, 0.12)	0.914	0.300	8.68	0.279
ADG, kg/d	0.24 (0.05)	43	-0.005 (-0.01, 0.01)	0.267	0.003	41.27	0.071
Carcass weight, kg	20.95 (6.23)	38	-0.138 (-0.33, 0.05)	0.162	0.289	10.32	0.171
Carcass dressing, %	49.82 (4.38)	37	-0.422 (-0.79, -0.04)	0.028	0.031	32.50	0.228
SFT, mm	2.56 (1.65)	29	0.021 (-0.02, 0.07)	0.405	0.735	0.00	0.156
Ribeye area, cm ²	14.43 (2.42)	32	0.195 (-0.09, 0.48)	0.185	0.958	0.00	0.957
Mesenteric fat, kg	0.68 (0.29)	7	-0.008 (-0.06, 0.05)	0.762	0.869	0.00	0.937
Physicochemical characteristics of meat							
Final pH	5.72 (0.17)	29	-0.015 (-0.04, 0.01)	0.301	<.0001	68.90	0.511
Meat colour							
Lightness, L*	36.98 (5.02)	22	-0.268 (-0.94, 0.40)	0.433	0.037	38.55	0.506
Redness, a*	15.47 (3.79)	22	-0.083 (-0.32, 0.16)	0.509	0.642	0.00	0.163
Yellowness, b*	6.25 (2.58)	21	-0.195 (-0.38, -0.01)	0.044	0.901	0.00	0.089
Shear force, kgf/cm ²	3.58 (1.22)	24	-0.009 (-0.16, 0.13)	0.898	<.0001	95.08	0.537
Thawing loss, g/kg	55.58 (21.35)	10	-3.471 (-4.04, -2.89)	<.0001	0.433	0.48	0.674
Cooking loss, g/kg	303.75 (82.88)	24	-2.044 (-10.11, 5.92)	0.615	0.114	26.71	0.327
WHC, g/kg	624.10 (204.14)	14	6.118 (-3.78, 16.01)	0.226	0.819	0.00	0.204
Chemical Composition, g/kg of DM							
Moisture	747.85 (25.97)	19	1.463 (0.15, 2.76)	0.028	0.952	0.00	0.665
Protein	199.67 (16.40)	17	0.490 (-2.96, 3.93)	0.780	<.0001	69.78	0.131
Fat	31.07 (14.27)	22	-0.423 (-1.38, 0.54)	0.391	0.889	0.00	0.216
Ash	16.22 (11.60)	15	0.261 (-0.00, 0.52)	0.050	0.593	0.00	0.235
Cholesterol, mg/100g Meat	31.78 (6.13)	6	0.128 (-0.01, 0.27)	0.089	0.801	0.00	0.696

¹ADG=average daily gain; SFT=subcutaneous fat thickness; WHC=water holding capacity. ²Diets with no additives are the control treatment. ³N represents the number of comparisons for control and glycerin treatments (complete data set is available in Supplementary File S1). ⁴The weighted mean differences between control and glycerin treatments are presented (WMD). ⁵I² represents the proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test (Funnel test).

Table 4: Effects of inclusion glycerin in sheep diets on the fatty acid (g/100g) profile of meat.

Item ¹	Control ² mean (SD)	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P value	P value	I ² (%)	P value
Lauric acid C12:0	0.14 (0.06)	14	-0.011 (-0.01, -0.01)	<.0001	0.936	0.00	0.736
Myristic acid C14:0	2.56 (0.53)	14	-0.398 (-0.49, -0.30)	<.0001	0.723	0.00	0.850
Myristoleic acid C14:1 c9	0.12 (0.08)	14	0.001 (-0.01, 0.02)	0.976	0.971	0.00	0.167
Pentadecanoic acid C15:0	0.54 (0.35)	13	0.038 (0.02, 0.05)	<.0001	0.091	36.44	0.269
Palmitic acid C16:0	23.67 (1.95)	15	-1.163 (-1.73, -0.58)	<.0001	<.0001	82.04	0.401
Palmitoleic acid C16:1 c9	1.85 (0.29)	12	-0.044 (-0.07, 0.02)	0.002	0.215	23.30	0.409
Heptadecanoic acid C17:0	1.36 (0.54)	11	0.231 (0.11, 0.35)	0.001	0.470	0.00	0.531
Stearic acid C18:0	15.99 (3.04)	14	-0.969 (-1.62, -0.31)	0.004	<.0001	73.84	0.092
Oleic acid C18:1 c9	41.17 (4.08)	14	2.167 (1.81, 2.52)	<.0001	0.525	0.00	0.905
Vaccenic acid C18:1 t11	1.55 (0.05)	9	-0.081 (-0.12, -0.03)	0.001	0.133	35.59	0.811
Linoleic acid C18:2 c9c12	4.25 (2.11)	12	0.167 (0.10, 0.23)	<.0001	0.371	7.57	0.115
CLA C18:2 cis9 trans11	0.51 (0.21)	14	0.058 (0.03, 0.07)	<.0001	0.014	51.01	0.210
α-Linolenic acid C18:3 ω-3	0.34 (0.35)	15	0.014 (0.01, 0.02)	<.0001	0.766	0.00	0.107
Arachidonic acid C20:4	0.74 (0.52)	14	0.015 (0.01, 0.03)	0.005	0.987	0.00	0.076
EPA C20:5 ω-3	0.07 (0.11)	13	0.010 (0.01, 0.01)	<.0001	0.996	0.00	0.950
DPA C22:5 ω-3	0.17 (0.06)	7	-0.008 (-0.03, 0.01)	0.452	0.783	0.00	0.095
DHA C22:6 ω-3	0.05 (0.06)	14	0.009 (0.01, 0.01)	<.0001	0.598	0.00	0.582
Total SFA	43.70 (3.82)	14	-1.248 (-2.39, -0.09)	0.035	0.001	65.47	0.244
Total MUFA	48.73 (2.53)	14	1.797 (1.19, 2.40)	<.0001	0.019	48.99	0.143
Total PUFA	6.25 (3.42)	11	0.230 (0.14, 0.31)	<.0001	0.889	0.00	0.159
Total Omega-3	0.82 (0.67)	9	0.019 (0.01, 0.03)	<.0001	0.998	0.00	0.820
Total Omega-6	5.91 (3.19)	6	0.153 (0.07, 0.22)	<.0001	0.821	0.00	0.732
Omega-6/Omega-3	17.45 (11.02)	13	-1.375 (-1.86, -0.88)	<.0001	0 0.182	25.72	0.737

¹CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid. ²Diets with no additives are the control treatment. ³N represents the number of comparisons for control and glycerin treatments (complete data set is available in Supplementary File S1). ⁴The weighted mean differences between control and glycerin treatments are presented (WMD). ⁵I² represents the

proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test (Funnel test)

Table 5: Meta-regression of the covariate effect on weighted mean differences (WMD) between glycerol and control treatments.

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹								Adjusted R ² (%)	N ⁴
	Intercept	Genetic group	EP (day)	ED	Age, months	Crude glycerin (g/kg DM)	CON (g/kg DM)	Methanol (g/kg DM of CG)		
DMI, kg/day	0.02 (0.402)	0.24 (0.039)	-0.01 (0.981)	-0.05 (0.099)	-0.01 (0.911)	0.02 (0.802)	-0.31 (0.018)	-0.01 (0.934)	20.01	54
DMd, g/kg	93.2 (0.338)	3.03 (0.906)	-34.2 (0.772)	-20.1 (0.214)	-5.31 (0.910)	-9.12 (0.862)	-38.6 (0.778)	--	0.00	30
NDFd, g/kg	-41.1 (0.487)	59.1 (0.608)	29.3 (0.505)	3.26 (0.786)	-112.0 (0.207)	-94.1 (0.352)	--	--	100	22
CPd, g/kg	143.5 (0.108)	-10.6 (0.632)	-178.9 (0.063)	35.9 (0.039)	53.5 (0.190)	79.5 (0.117)	-118.7 (0.055)	--	14.13	23
Glucose, mg/dL	4.50 (0.500)	-19.5 (0.019)	8.90 (0.054)	--	-5.30 (0.567)	6.76 (0.338)	--	--	81.66	11
ADG, kg/day	-0.01 (0.424)	-0.05 (0.488)	0.01 (0.555)	--	-0.02 (0.271)	-0.03 (0.814)	0.07 (0.410)	0.01 (0.352)	100	43
Carcass dressing, %	-5.86 (0.125)	0.62 (0.607)	2.32 (0.744)	--	0.13 (0.931)	7.67 (0.057)	3.10 (0.041)	--	100	37
pH meat	-0.04 (0.851)	0.01 (0.975)	-0.06 (0.316)	--	0.13 (0.748)	-0.54 (0.371)	-0.01 (0.842)	--	80.61	29
Lightness, L*	5.01 (0.219)	-6.11 (0.075)	-3.30 (0.103)	--	7.40 (0.046)	-11.6 (0.051)	--	--	100	22
Shear force, kgf/cm ²	-0.24 (0.894)	-0.79 (0.802)	1.13 (0.859)	--	-0.49 (0.893)	0.21 (0.908)	1.62 (0.574)	--	100	24
Protein meat	-24.9 (0.092)	-7.60 (0.782)	-16.1 (0.009)	--	42.7 (0.099)	0.60 (0.977)	--	--	100	17
Palmitic C16:0, g/100g	-1.57 (0.003)	-0.59 (0.702)	--	--	1.31 (0.505)	0.85 (0.726)	--	--	60.25	15
Stearic C18:0, g/100g	0.85 (0.330)	0.35 (0.818)	--	--	-4.02 (0.098)	2.25 (0.352)	--	--	48.43	14
CLA C18:2 c9 t11, g/100g	-0.01 (0.715)	0.10 (0.200)	--	--	0.01 (0.958)	-0.01 (0.927)	--	--	100	14
Total SFA, g/100g	0.17 (0.809)	-1.95 (0.059)	--	--	-0.51 (0.837)	2.25 (0.089)	--	--	100	14
Total MUFA, g/100g	0.73 (0.264)	3.70 (0.113)	--	--	0.27 (0.919)	-2.80 (0.103)	--	--	100	14

¹ EP = experimental period (days); ED = experimental design (rotatable vs. continuous); CON = concentrate in diet; CG = crude glycerin. ² DMI = dry matter intake; DMd = dry matter digestibility; CPd = crude protein digestibility; NDFd = neutral fiber detergent digestibility; ADG = average daily gain; CLA = conjugated linoleic acid. Adjusted R² is the proportion of the between-study variance (heterogeneity) explained by covariate. N represents the number of comparisons between glycerin and control treatments (complete data set is available in Supplementary file S1).

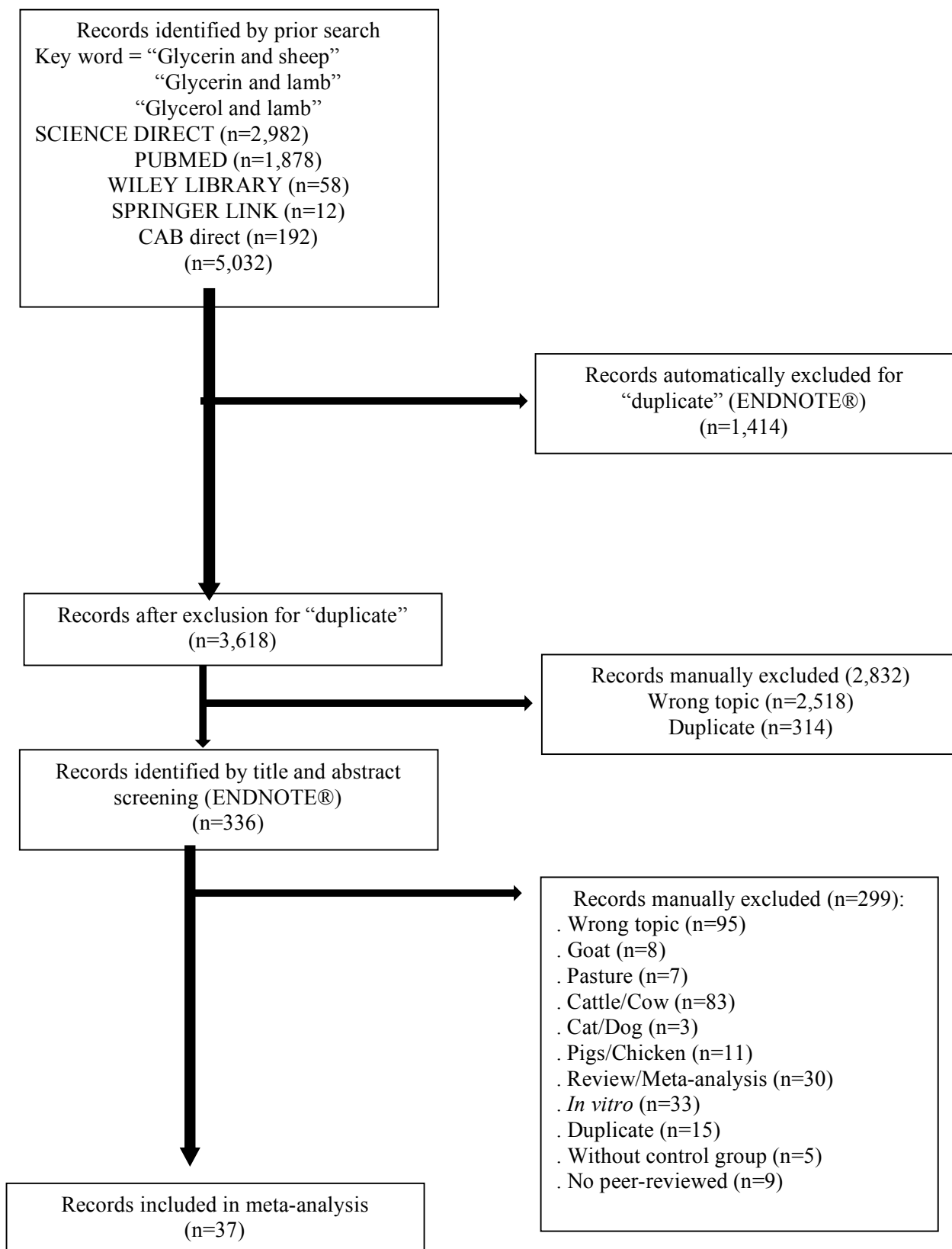


Figure 1. Flowchart showing inclusion criteria for studies used for conducting the meta-analysis on effect of inclusion glycerin in sheep diet

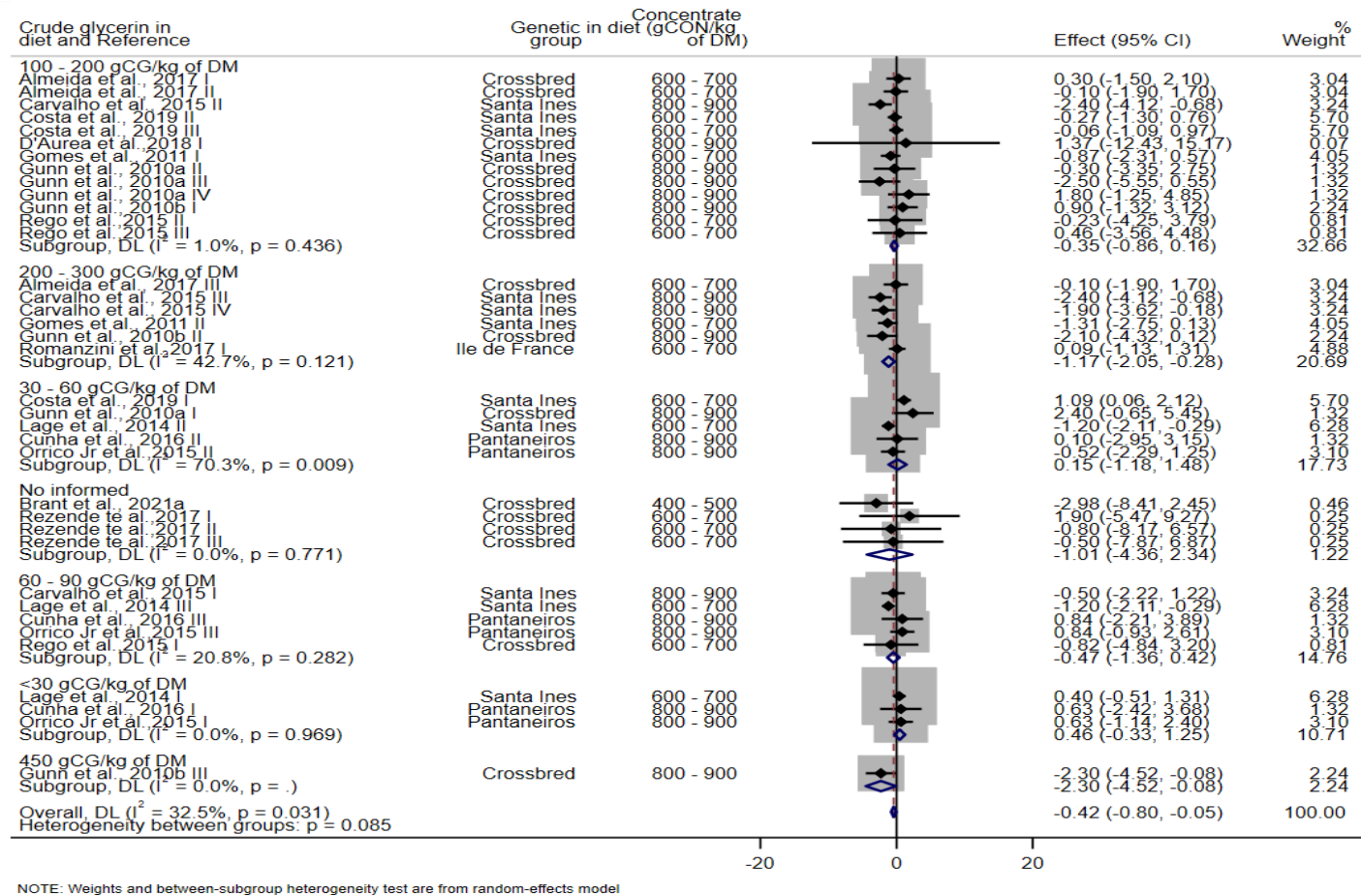


Figure 2. Forest plot (with subgroup analysis “Covariate= Crude glycerin in diet (gCG/kg of DM in diet)), showing the effect of the use of glycerin on the diet the sheep on carcass dressing (%). The x-axis shows the weighted mean difference (Effect); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an increase. Each diamond represents the mean size effect for that study, and the size of the diamond reflects the relative weighting of the study to the overall size effect estimate with larger diamonds representing greater weight. The lines connected to the diamond represents the upper and lower 95% confidence interval for the treatment effect. The dotted vertical line represents the overall size effect estimate. The diamond at the bottom represents the mean response across the studies, and the solid vertical line represents a mean difference of zero or no effect.

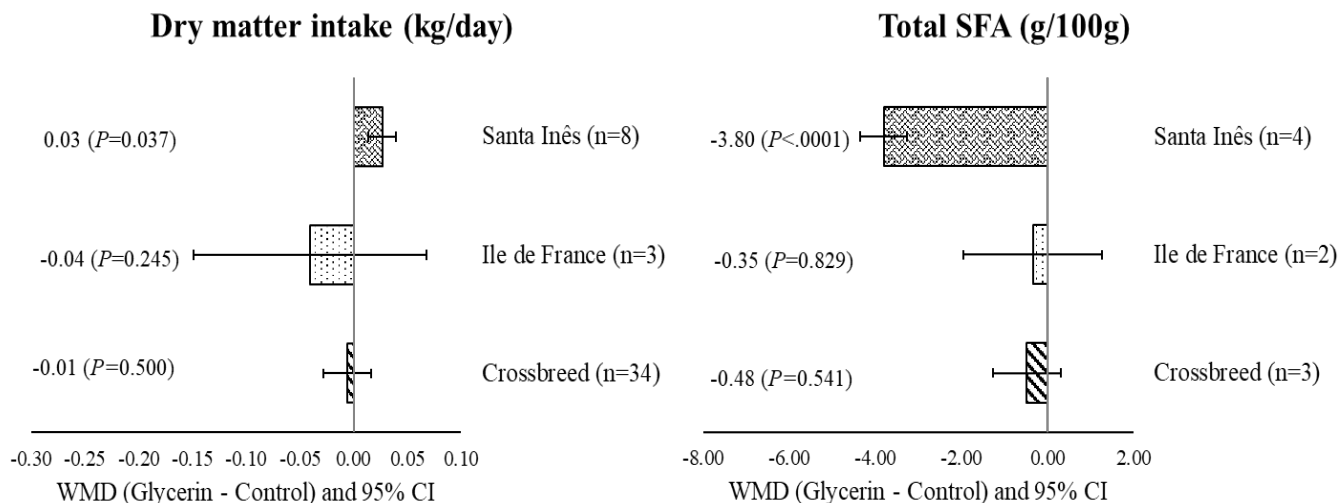


Figure 3. Subgroup analysis (subgroup = Genetic group) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between tannins and control treatments.

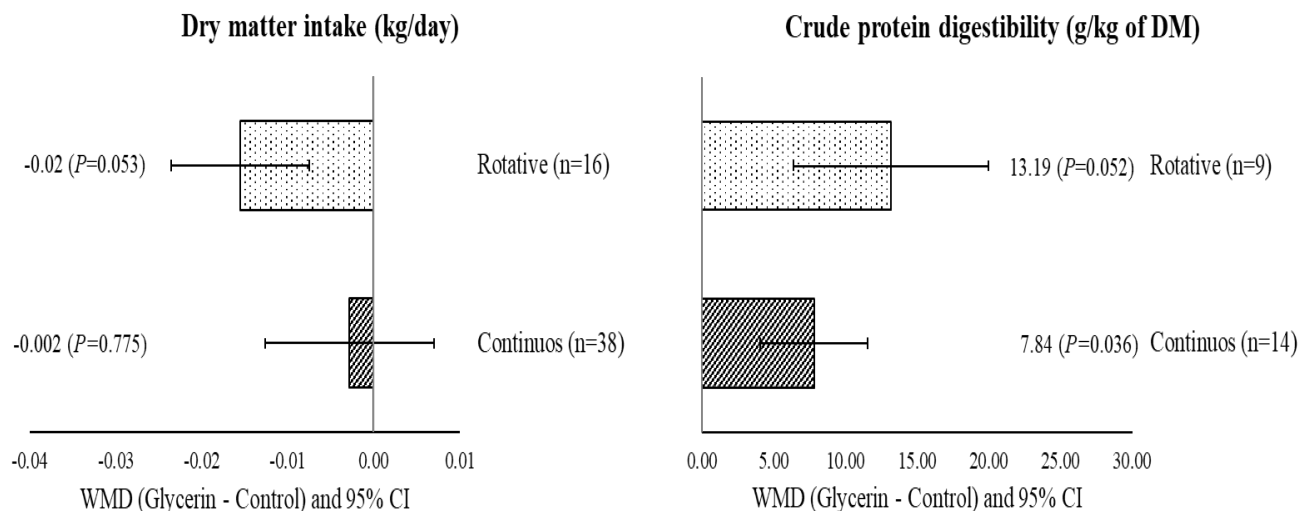


Figure 4. Subgroup analysis (subgroup = Experimental design) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between treatment and control treatments.

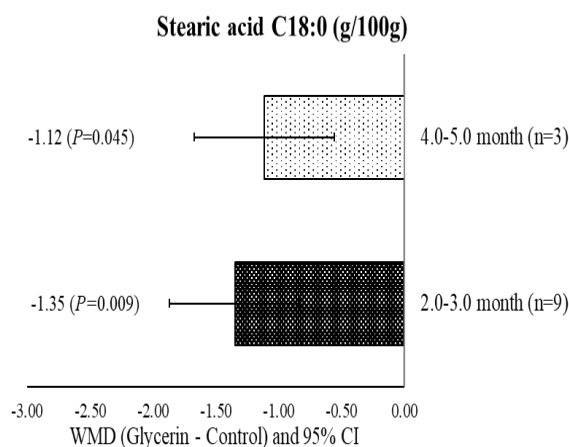


Figure 5. Subgroup analysis (subgroup = Age of lamb (month)) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between treatment and control treatments.

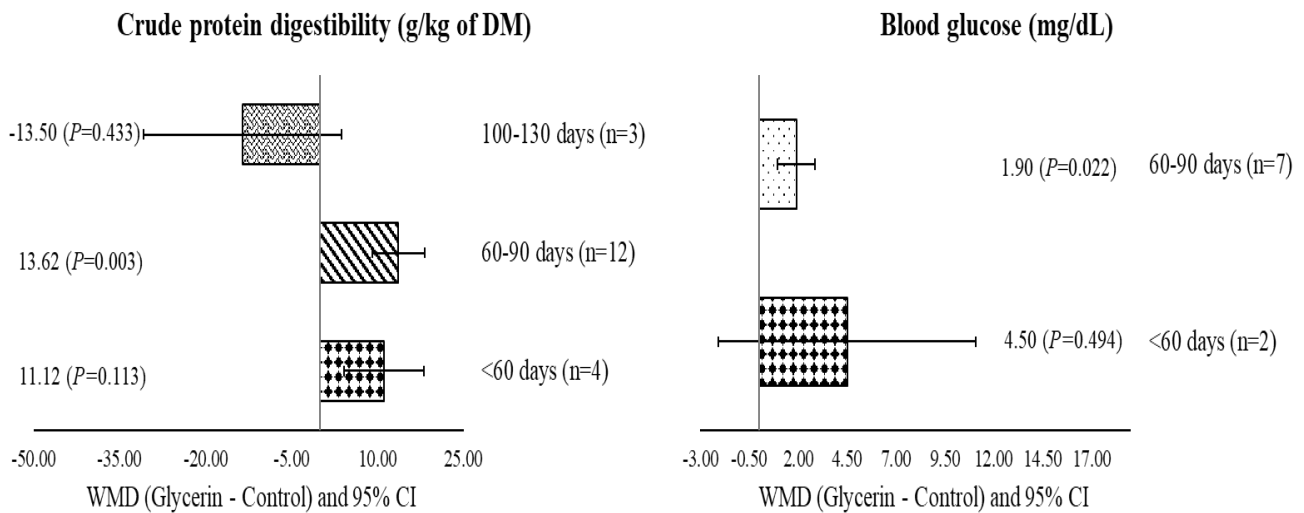


Figure 6. Subgroup analysis (subgroup = Experimental period (day)) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between treatment and control treatments.

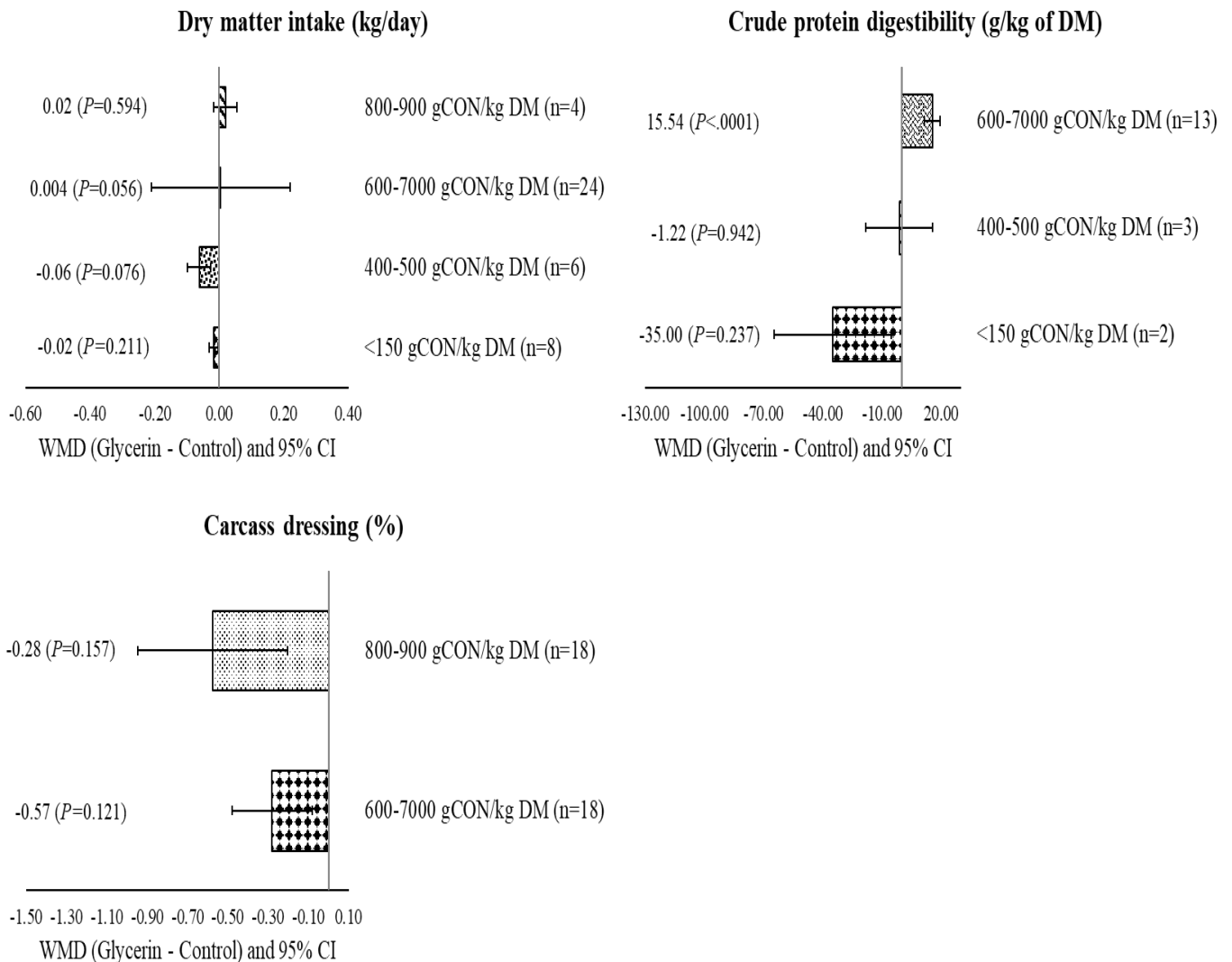


Figure 7. Subgroup analysis (subgroup = Concentrate in diet (gCON/kg of DM)) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between treatment and control treatments.

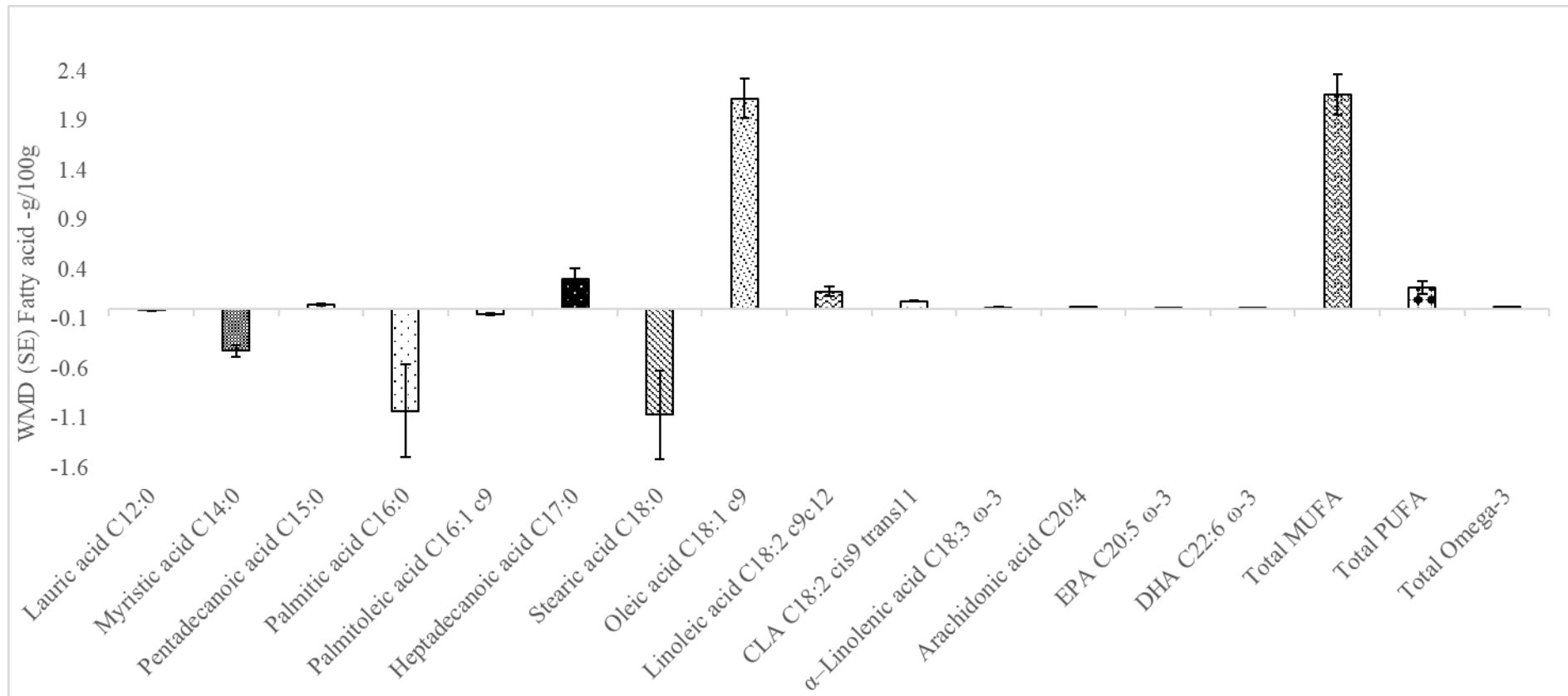


Figure 8. Subgroup analysis (subgroup = Crude glycerin in diet (g/kg of DM)) of the effect of inclusion glycerin in sheep diets, WMD = weighted mean differences between treatment and control treatments; SE = standard error. Due to the low number of samples, only the effect of the inclusion of 100.0 - 200.0 g Crude glycerin/kg of DM in the diet on the fatty acid profile in sheep meat was presented.

USE OF MOLASSES IN DIET FOR SHEEP: META-ANALYSIS

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ABSTRACT

Molasses has been considered a viable alternative for animal nutrition due to its energy value and low cost. Including molasses in sheep diets can partially replace traditional ingredients, and promote improvements in nutrient digestibility and animal performance. However, the effects of molasses inclusion in sheep diets remain a topic of debate in the scientific literature, with results varying depending on experimental conditions and methods used. To integrate existing data and provide a clearer understanding of the impacts of molasses, a comprehensive meta-analysis was conducted. The meta-analysis was based on 26 selected articles. The results indicated that the inclusion of molasses in the diet increased the digestibility of dry matter, acid detergent fiber (ADF), neutral detergent fiber (NDF), propionate, and butyrate. Molasses inclusion reduced total dry matter intake (TMI), blood urea nitrogen, rumen pH, acetate, isovalerate, and isobutyrate. There was no significant effect on forage dry matter intake, crude protein digestibility, nitrogen intake, urinary nitrogen and fecal nitrogen. Including molasses in sheep diets increased final body weight, average daily gain, carcass weight, and carcass yield. Molasses was found to be particularly effective in improving the digestibility of dry matter, crude protein, NDF, and ADF, especially at inclusion levels between 100 and 200 g/kg in the diet. However, in animals aged between 6 to 8 months, there was a decrease in TMI and crude protein digestibility. It is concluded that molasses can be included in sheep diets, providing improvements in animal performance and carcass parameters, such as final weight and carcass yield.

Keywords: carcass, lambs, performance, sucrose.

1. Introduction

Molasses is an intermediate byproduct of sugar and bioethanol production and refining derived from sugarcane, soybean, and beet. Rich in sugars, molasses was initially added to animal feed to enhance the palatability and acceptability of fibrous feedstuffs. Due to its composition, it serves as an energy source for ruminants (Andriguetto et al., 2002; Martel et al., 2011). Regardless of its origin, molasses is considered an energy feed due to its high content of readily fermentable sugars, and it is also rich in bioavailable minerals. Its low cost makes molasses a popular partial substitute for cereals (it has approximately 2/3 the energy value of corn) in feed formulations for various livestock species. In terms of some physicochemical properties and total energy value, beet and

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cane molasses exhibit similar characteristics. Both are high-density liquids (1.3) and have an acidic pH (5 to 5.8) (Mordenti et al., 2021).

Research has demonstrated that adding molasses in urea mineral blocks to ruminant diets results in improved animal performance and certain blood parameters (SungChinTial et al., 2023). Lestari et al. (2016) observed that supplementing diets with molasses in urea mineral blocks increased the availability of energy, non-protein nitrogen, minerals, vitamins, and other nutrients for ruminants. Additionally, replacing corn and barley grains with molasses (up to 100 g/kg DM) led to linear increases in organic matter (OM) digestibility and ash-free neutral detergent fiber, as well as improved animal performance (Zahedi-Moghadam, Azarfar, and Azizi, 2023).

Studies using soybean molasses as an alternative energy source have been conducted to assess the use of this byproduct in sheep diets. Pereira Júnior et al. (2024) evaluated performance, blood parameters, carcass yield, and meat quality, finding that soybean molasses increased fat deposition, reduced lipid oxidation, and altered energy metabolism, making it a viable option in ruminant nutrition. Rodrigues et al. (2020) also reported that including soybean molasses in sheep diets increased meat tenderness and intramuscular fat content.

Given the need to use cost-effective and organic ingredients, it is essential to obtain data on the use of molasses in ruminant diets. The practice of including this ingredient has been common for many years, both in production and scientific research. Therefore, this study aims to evaluate experimental studies on the inclusion of molasses in sheep diets using a meta-analytical approach.

2 Material and Methods

2.1. Data set

A comprehensive literature search was conducted using four search engines: PubMed, Science Direct, Wiley library, Springer link and CAB direct. Around 8,375 publications were retrieved using the following search terms: “molasses and sheep” and “molasses and lamb”. Of the papers that were retrieved, only those that met the predetermined inclusion criteria were included in the meta-analysis. For inclusion into the meta-analysis, studies had to have the following (standardized criteria): 1) The control group does not include molasses in the diet; 2) the treatment group includes molasses in diet and 3) study performed with total mixed ration (TMR) and/or confinement systems. A flowchart detailing the process of study identification and selection for analysis is shown in Figure 1.

2.2. Data Extraction

Based on inclusion criteria, 26 peer-reviewed publications with 73 means of treatment were rated by: first author, publication reference, country, genetic group, experimental period, experimental design, animal age (months), forage type, molasses type, amount of molasses in the diet (g/kg of DM) and amount of concentrate in the diet (g/kg of DM). The number of replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables for control (diets with no additives) and molasses (diets with molasses) treatments: performance (i.e., feed intake, average daily gain; ADG), nutrient digestibility, carcass parameters (i.e., carcass weight), blood parameters, meat quality (i.e., meat color, fatty acid profile), ruminal parameters and nitrogen metabolism. The complete data set is available in Supplemental File S1.

2.3. Statistical analysis

2.3.1. Weighted mean difference and Publication bias

A meta-analysis was conducted using R Statistical Software (Metafor package, version 3.4.2; Viechtbauer, 2010). The Forest Graph (forest plot) was created using STATA software (Version 16.0; StataCorp LP, College Station, TX). The effects of inclusion molasses in sheep diets were evaluated by examining the weighted mean difference (WMD) between tannins treatment (diets with molasses) and control treatment (diets with no molasses). Treatments means were weighted by the inverse of the variance, according to the method proposed by Der-Simonian and Laird (1986) for random effects model.

Between-study variability (i.e., heterogeneity of the treatment effect) was evaluated using both the chi-square (Q) test of heterogeneity and I^2 statistics, which measures the percentage of variation due to heterogeneity (Higgins et al., 2003). Negative I^2 values were assigned as zero values. An I^2 value less than 25% indicate low heterogeneity, whereas values between 25 and 50% denote moderate heterogeneity and those above 50% denote high heterogeneity (Higgins et al., 2003).

Publication bias was evaluated using the funnel plot (Light and Pillemer, 1984) and asymmetry test (indicative of publication bias) which was carried according to the Egger regression asymmetry test, among the WMD and SE (Egger et al., 1997). Significance was declared at $P \leq 0.05$. Outliers were removed when studentized residuals outside range of -2.5 to 2.5.

2.3.2 Meta-regression and Subgroup analysis

The meta-regression analysis was conducted to identify the effects of categorical covariates and select the ones to perform the subgroup analysis. A mixed model was applied to adjust the data in the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by:

$$\Theta_i = \beta + \beta_1 x_{ij} + \dots + \beta_p x_{ip} + \mu_i$$

Where Θ_i = the true treatment effect in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_j = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, \tau^2)$. Here τ^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

To measure of between-study variance ($\text{Tau-squared} = \tau^2$). The moment estimator calculation of (τ^2) is that used in Der-Simonian and Laird random effects meta-analysis but is less suitable when covariates are included (Thompson and Sharp, 1999). The restricted maximum likelihood estimate (REML) approach was used to estimate (τ^2) because it is less likely to underestimate or produce biased estimates of variance (Thompson and Sharp, 1999; Viechtbauer, 2005).

Tests of null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010).

Meta-regression criteria were: 1) $P \leq 0.05$, for the heterogeneity test; 2) $P \geq 0.05$, for the funnel plot; 3) no observations with values for studentized residuals out of the range -2.5 to 2.5 (outliers). The WMD was evaluated by subgroup analysis when the categorical covariates were significant at $P \leq 0.10$.

The covariates were divided as following: Animal age (< 4.0, 6.0-8.0, 16.0-18.0, 24.0 and 39.0-65.0 months); Experimental design (rotative = crossover, Latin Square and continuous = randomized complete block design); Experimental period (< 30, 30-60, 60-90 and 100-110 days); Amount of concentrate in the diet (<200, 200-500 and 500-750 g/kg of DM); Molasses type (Liquid molasses [cane], Soybean molasses and Urea-molasses block (UMB)), Forage type (straw, hay, silage and straw + hy) and Amount of molasses in the diet (50.0-100.0, 100.0-200.0 and 200.300-.0 g molasses /kg of DM).

3 Results

The dataset comprised 26 peer-reviewed publications with 73 treatment means selected to investigate the effects of molasses inclusion in sheep diets on performance, nitrogen metabolism, carcass traits, and meat parameters. Figure 1 presents the study selection criteria and a description of the complete dataset for molasses effects in sheep diets. Tables 1–3 represent the initial approach to determine whether molasses inclusion in the diet globally affects each response parameter.

3.1 Dry Matter Intake, Nutrient Intake, Digestibility, Nitrogen Metabolism, Blood, and Rumen Parameters

The inclusion of molasses in the diet increased dry matter digestibility ($P = 0.003$), acid detergent fiber (ADF; $P = 0.005$), neutral detergent fiber (NDF; $P = 0.002$), propionate ($P < 0.0001$), and butyrate ($P = 0.002$). However, molasses inclusion reduced total dry matter intake (DMI-total; $P = 0.040$; Table 1), blood urea nitrogen (BUN; $P = 0.001$), rumen pH ($P = 0.005$), acetate ($P = 0.002$), isovalerate ($P = 0.001$), and isobutyrate ($P = 0.005$; Table 3). Conversely, there was no effect ($P > 0.05$) of molasses inclusion on forage dry matter intake (DMI-forage), crude protein digestibility, total protein, albumin, glucose, creatinine, cholesterol (Table 1), ammonia, valerate, nitrogen intake, urinary nitrogen, fecal nitrogen, and retained nitrogen (Table 3).

3.2 Performance, Carcass Traits, and Blood Parameters

Molasses inclusion in sheep diets increased final body weight ($P = 0.001$), average daily gain (ADG; $P < 0.0001$), carcass weight ($P = 0.001$), and carcass yield ($P < 0.0001$). However, molasses inclusion affected meat lightness (L^* ; $P = 0.023$). There was no effect ($P > 0.05$) on feed efficiency, subcutaneous fat thickness, loin eye area, pH, red intensity (a^*), yellow intensity (b^*), cooking loss, moisture, protein, or meat minerals (Table 2).

3.3 Meta-Regression Analysis and Funnel Plot Asymmetry

As seen in Tables 1, 2, and 3, publication bias for funnel plot asymmetry was not evident ($P > 0.05$). The meta-regression analysis is presented in Table 4, with age and experimental period as primary covariates impacting the response of molasses inclusion in sheep diets.

3.4 Subgroup Analysis

Inclusion of molasses in the diets of animals aged 6–8 months resulted in decreased DMI-total (WMD = -10.64 g/day; $P < 0.0001$) and crude protein digestibility (WMD = -26.45 g/kg DM; $P = 0.054$). Conversely, molasses inclusion in animals aged 24 months increased DMI-total (WMD = 203.75 g/day; $P = 0.001$), crude protein

digestibility (ADG = 28.91 g/kg DM; $P < 0.0001$), and ADF digestibility (DMI = 22.48 g/kg DM; $P = 0.015$). The experimental design (Figure 3) used in studies affected the responses, where continuous design studies showed increased ADF digestibility (WMD = 19.05 g/kg DM; $P = 0.005$).

The experimental period or molasses intake period (<30 days) increased NDF and ADF digestibility (WMD = 25.26; $P = 0.010$ and WMD = 25.00 g/kg DM; $P = 0.001$, respectively). A molasses consumption period of 60–90 days increased ADF digestibility (WMD = 45.91 g/kg DM; $P = 0.014$) and ruminal propionate (WMD = 4.53 mol/100 mol; $P = 0.014$).

The type of forage used in studies affected the response to molasses inclusion (Figure 5). Studies that included molasses with hay and straw mixtures as forage showed an increase in ADF digestibility (WMD = 25.99 g/kg DM; $P = 0.001$), while diets using straw or silage as forage presented increased carcass weight (WMD = 2.66 kg; $P = 0.001$ and WMD = 0.78 kg; $P = 0.002$, respectively). Molasses inclusion in the 50–100 g/kg range showed a decrease in DMI-total (ADG = -9.75 g/day; $P = 0.001$) but increased ADF digestibility (WMD = 9.63 g/kg; $P = 0.001$; Figure 6). Inclusion of 200–300 g molasses/kg DM increased both ADF and NDF digestibility (WMD = 42.33; $P = 0.021$ and WMD = 47.20 g/kg DM; $P < 0.0001$, respectively) and carcass weight (ADG = 1.39 kg; $P = 0.018$).

4 Discussion

Molasses inclusion in sheep diets enhanced dry matter digestibility ($P = 0.003$), ADF ($P = 0.005$), and NDF ($P = 0.002$). This effect may be attributed to the soluble carbohydrates added to the diet (Pereira Junior et al., 2018; Arruda et al., 2019), allowing for better nutrient absorption. Soybean molasses, with high solubility, is described by Hailemariam et al. (2021) as a dietary component that bypasses the rumen more easily, reducing microbial fermentation and converting non-protein nitrogen into ammonia, then into urea in the liver, as indicated by blood urea. Fimbres-Durazo et al. (2013) observed a linear reduction in blood urea nitrogen when molasses was added as an energy source in lamb diets.

In our meta-analysis, blood urea nitrogen (BUN) decreased ($P = 0.001$), and molasses types showed no differences among studies in the meta-analysis database, suggesting that soluble carbohydrates generally influence ruminal nitrogen metabolism (Pereira Júnior et al., 2024).

Molasses inclusion increased final body weight ($P = 0.001$), ADG ($P < 0.0001$),

carcass weight ($P = 0.001$), and carcass yield ($P < 0.0001$) in sheep, likely due to improved digestibility, energy intake, and rumen propionate production, which supports fat synthesis (Mushi et al., 2009; Jacques et al., 2011; Ladeira et al., 2018). However, Torres et al. (2023) found reductions in ADG, carcass weight, and subcutaneous fat in cattle fed molasses, suggesting species-specific differences in metabolism and diet utilization.

ADF digestibility ($WMD = 25.99$ g/kg DM; $P = 0.001$; Figure 5) improved when straw was present in the diet, particularly with 200–300 g molasses/kg DM (Figure 6). This may be due to ruminal microbial efficiency in degrading complex carbohydrates, provided by an additional soluble carbohydrate energy source. Certain fungi species can break down lignified tissues, facilitating fiber degradation and making cellulose more available to rumen microbes (Akin, 1988; Akin and Benner, 1988; Theodorou et al., 1996; Lee et al., 2000).

5 Conclusion

Molasses can be included in sheep diets to improve animal performance and carcass parameters, such as final weight and carcass yield. Additionally, it enhances the utilization of low-quality forages (straw) by promoting the degradation of complex carbohydrates bound to lignin. It is essential to collect data on the impact of molasses types and compositions in diets, as the current meta-analysis included primarily sugarcane molasses, leaving the effects of other types less clear for the evaluated parameters.

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Table 1: Effects of inclusion molasses in sheep diets on feed intake, apparent total-tract digestibility of nutrients and blood parameters

Item ¹	Control ² mean (SD)	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	<i>P</i> value	<i>P</i> value	I ² (%)	<i>P</i> value
Intake, g/d							
DMI-total	885.12 (335.89)	31	-5.956 (-11.64, -0.27)	0.040	0.003	45.87	0.205
DMI-forage	610.05 (297.78)	16	13.437 (-15.36, 42.23)	0.306	<.0001	84.09	0.082
Digestibility, g/kg of DM							
Dry matter	601.56 (112.95)	36	14.012 (4.67, 23.34)	0.003	<.0001	74.70	0.093
Crude protein	677.91 (106.02)	23	8.043 (-6.87, 22.96)	0.291	<.0001	78.64	0.521
ADF	454.13 (102.04)	23	19.298 (5.99, 32.60)	0.005	<.0001	75.90	0.375
NDF	526.78 (95.55)	15	21.203 (7.87, 34.53)	0.002	<.0001	75.03	0.098
Blood parameters, mg/dl							
Total protein, g/dl	7.92 (0.18)	7	-0.065 (-0.49, 0.36)	0.765	0.227	26.42	0.284
Albumin, g/dl	3.80 (0.50)	5	-0.020 (-0.16, 0.12)	0.779	0.802	0.00	0.448
Blood urea nitrogen	13.53 (17.42)	6	-0.349 (-0.54, -0.15)	0.001	0.248	24.72	0.947
Glucose	62.82 (11.05)	8	-1.240 (-2.85, 0.37)	0.136	0.727	0.00	0.525
Creatinine	0.99 (0.05)	5	0.008 (-0.03, 0.06)	0.721	0.382	4.31	0.836
Cholesterol	75.12 (9.67)	5	-2.508 (-5.13, 0.12)	0.067	0.963	0.00	0.613

¹DMI = dry matter intake; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber. ²Diets with no additives are the control treatment. ³N represents the number of comparisons for control and molasses treatments (complete data set is available in Supplementary File S1). ⁴The weighted mean differences between control and molasses treatments are presented (WMD). ⁵I² represents the proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test (Funnel test).

Table 2: Effects of inclusion molasses in sheep diets on their performance, carcass parameters and physicochemical characteristics of meat

Item ¹	Control ² mean (SD)	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P value	P value	I ² (%)	P value
Final body weight, kg	31.08 (7.06)	21	1.295 (0.32, 2.27)	0.001	<.0001	63.38	0.194
ADG, g/d	77.63 (113.55)	25	31.668 (17.90, 45.43)	<.0001	<.0001	96.25	0.095
Feed efficiency, kg/kg	0.103 (0.09)	12	0.003 (-0.01, 0.01)	0.676	<.0001	73.63	0.255
Carcass weight, kg	12.76 (3.81)	14	1.223 (0.52, 1.92)	0.001	<.0001	74.35	0.256
Carcass dressing, %	44.27 (4.49)	15	0.962 (0.53, 1.39)	<.0001	0.466	0.00	0.786
SFT, mm	3.33 (0.50)	7	-0.268 (-0.67, 0.13)	0.192	0.127	39.64	0.868
Ribeye area, cm ²	11.53 (1.41)	9	-0.174 (-0.66, 0.30)	0.476	0.048	48.65	0.379
Physicochemical characteristics of meat							
Final pH	5.78 (0.31)	7	0.006 (-0.03, 0.05)	0.798	0.894	0.00	0.708
Meat colour							
Lightness, L*	31.50 (0.78)	6	-0.522 (-0.07, -0.07)	0.023	0.503	0.00	0.207
Redness, a*	11.69 (1.32)	6	0.074 (-0.62, 0.76)	0.835	<.0001	84.23	0.893
Yellowness, b*	4.41 (2.79)	7	-0.138 (-0.38, 0.10)	0.262	0.013	62.70	0.144
Cooking loss, g/kg	263.48 (12.39)	6	-8.229 (-17.34, 0.88)	0.077	0.118	43.01	0.351
Chemical Composition, g/kg of DM							
Moisture	711.60 (7.11)	7	-0.167 (-8.60, 8.26)	0.969	<.0001	85.86	0.286
Protein	205.34 (4.70)	7	0.157 (-2.73, 3.05)	0.915	0.003	69.34	0.612
Ash	14.28 (3.10)	7	0.080 (-0.35, 0.51)	0.717	0.127	39.58	0.469

¹ADG = average daily gain; SFT = subcutaneous fat thickness. ²Diets with no additives are the control treatment. ³N represents the number of comparisons for control and molasses treatments (complete data set is available in Supplementary File S1). ⁴The weighted mean differences between control and molasses treatments are presented (WMD). ⁵I² represents the proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test (Funnel test).

Table 3: Effects of inclusion molasses in sheep diets on rumen parameters and nitrogen metabolism

Item ¹	Control ² mean (SD)	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P value	P value	I ² (%)	P value
pH	6.53 (0.58)	15	-0.059 (-0.10, -0.01)	0.005	0.430	1.83	0.503
N-NH ₃ , mg/dL	15.65 (6.56)	9	-0.360 (-1.86, 1.15)	0.639	0.003	64.83	0.430
SCFA, mol/100mol							
Acetate	67.54 (12.74)	18	-1.882 (-3.09, -0.66)	0.002	<.0001	68.33	0.140
Propionate	19.47 (5.43)	19	1.857 (0.92, 2.78)	<.0001	<.0001	76.91	0.564
Butyrate	7.30 (2.25)	17	1.009 (0.36, 1.65)	0.002	<.0001	83.60	0.076
Isovalerate	0.79 (0.54)	10	-0.071 (-0.11, -0.03)	0.001	0.450	0.00	0.342
Valerate	1.09 (0.35)	10	0.073 (-0.02, 0.16)	0.114	0.447	0.00	0.169
Isobutyrate	0.38 (0.23)	9	-0.083 (-0.14, -0.02)	0.005	0.006	62.35	0.061
Nitrogen metabolism, g/day							
N Intake	18.29 (9.99)	9	0.052 (-0.04, 0.15)	0.284	0.172	30.83	0.118
Urinary N excretion	5.07 (4.94)	7	-0.088 (-0.28, 0.11)	0.384	0.252	23.20	0.067
Fecal N excretion	7.83 (2.65)	9	0.022 (-0.05, 0.09)	0.587	0.525	0.00	0.249
Retained N	3.43 (2.29)	13	0.266 (-0.33, 0.89)	0.377	0.831	0.00	0.629

¹ SCFA = short- chain fatty acid. ²Diets with no additives are the control treatment. ³N represents the number of comparisons for control and molasses treatments (complete data set is available in Supplementary File S1). ⁴The weighted mean differences between control and molasses treatments are presented (WMD). ⁵I² represents the proportion of total variation of effect size estimates that is due to heterogeneity. P value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test (Funnel test).

Table 4: Meta-regression of the covariate effect on weighted mean differences (WMD) between molasses and control treatments

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹								Adjusted R ² (%)	N ⁴
	Intercept	Age, months	ED	EP (day)	Molasses type	Molasses (g/kg DM)	Concentrate (g/kg DM)	Forage type		
DMI-total, g/day	164.1 (0.281)	-174.7 (0.090)	-98.1 (0.282)	81.7 (0.561)	--	-19.1 (0.098)	63.8 (0.687)	-60.1 (0.643)	86.75	31
DMI-forage, g/day	14.6 (0.863)	90.2 (0.153)	-38.1 (0.497)	60.5 (0.151)	--	--	--	--	13.54	16
DMd, g/kg	28.9 (0.909)	48.1 (0.532)	32.8 (0.774)	-83.1 (0.621)	-3.0 (0.852)	-48.5 (0.717)	-11.6 (0.912)	9.9 (0.904)	95.97	36
CPd, g/kg of DM	96.6 (0.158)	-42.1 (0.088)	-2.4 (0.960)	146.3 (0.010)	-72.5 (0.026)	-2.9 (0.857)	15.4 (0.665)	--	75.67	23
ADF, g/kg of DM	-5.4 (0.976)	-6.8 (0.001)	164.7 (0.058)	-143.9 (0.002)	-90.0 (0.242)	-10.5 (0.449)	-56.3 (0.479)	72.2 (0.001)	97.78	23
NDF, g/kg of DM	107.7 (0.015)	53.8 (0.023)	-10.4 (0.526)	-93.2 (0.003)	--	-34.0 (0.001)	-60.9 (0.151)	--	100	15
BWF, kg	-8.8 (0.235)	-0.8 (0.751)	--	9.6 (0.078)	--	0.9 (0.823)	3.5 (0.418)	7.2 (0.154)	0.00	21
ADG, g/day	-69.2 (0.268)	53.5 (0.366)	--	27.2 (0.495)	79.9 (0.159)	7.41 (0.842)	46.7 (0.451)	71.4 (0.118)	1.10	25
Feed efficiency, kg/kg	0.02 (0.842)	-0.1 (0.066)	--	0.2 (0.227)	--	-0.1 (0.952)	--	0.1 (0.325)	58.42	12
Carcass weight, kg	-0.1 (1.000)	-0.2 (0.844)	--	0.5 (0.715)	--	0.9 (0.658)	--	4.5 (0.018)	0.00	14
Ribeyer area, cm ²	-0.4 (0.675)	-0.5 (0.768)	--	0.3 (0.829)	--	0.4 (0.878)	--	2.3 (0.226)	0.00	9
N-NH ₃ , mg/dl	-3.7 (0.361)	-7.9 (0.055)	10.6 (0.024)	-10.1 (0.015)	--	1.4 (0.495)	--	--	54.01	9
Acetate, mol/100mol	-15.1 (0.668)	5.7 (0.796)	7.9 (0.715)	1.4 (0.921)	0.5 (0.959)	-0.9 (0.815)	--	--	99.67	18
Propionate, mol/100mol	-10.9 (0.375)	6.9 (0.297)	4.8 (0.432)	10.6 (0.086)	--	1.3 (0.583)	--	--	100	19
Butyrate, mol/100mol	2.0 (0.511)	1.1 (0.586)	-1.1 (0.515)	1.2 (0.414)	--	-2.3 (0.068)	--	--	77.90	17

¹ EP = experimental period (days); ED = experimental design. ² DM = dry matter; DMI = dry matter intake; DMd = dry matter digestibility; CPd = crude protein digestibility; NDFd = neutral fiber detergent digestibility; ADFd = acid fiber detergent digestibility; BWF = body weight final; ADG = average daily gain. Adjusted R² is the proportion of the between-study variance (heterogeneity) explained by covariate. N represents the number of comparisons between molasses and control treatments (complete data set is available in Supplementary file S1).

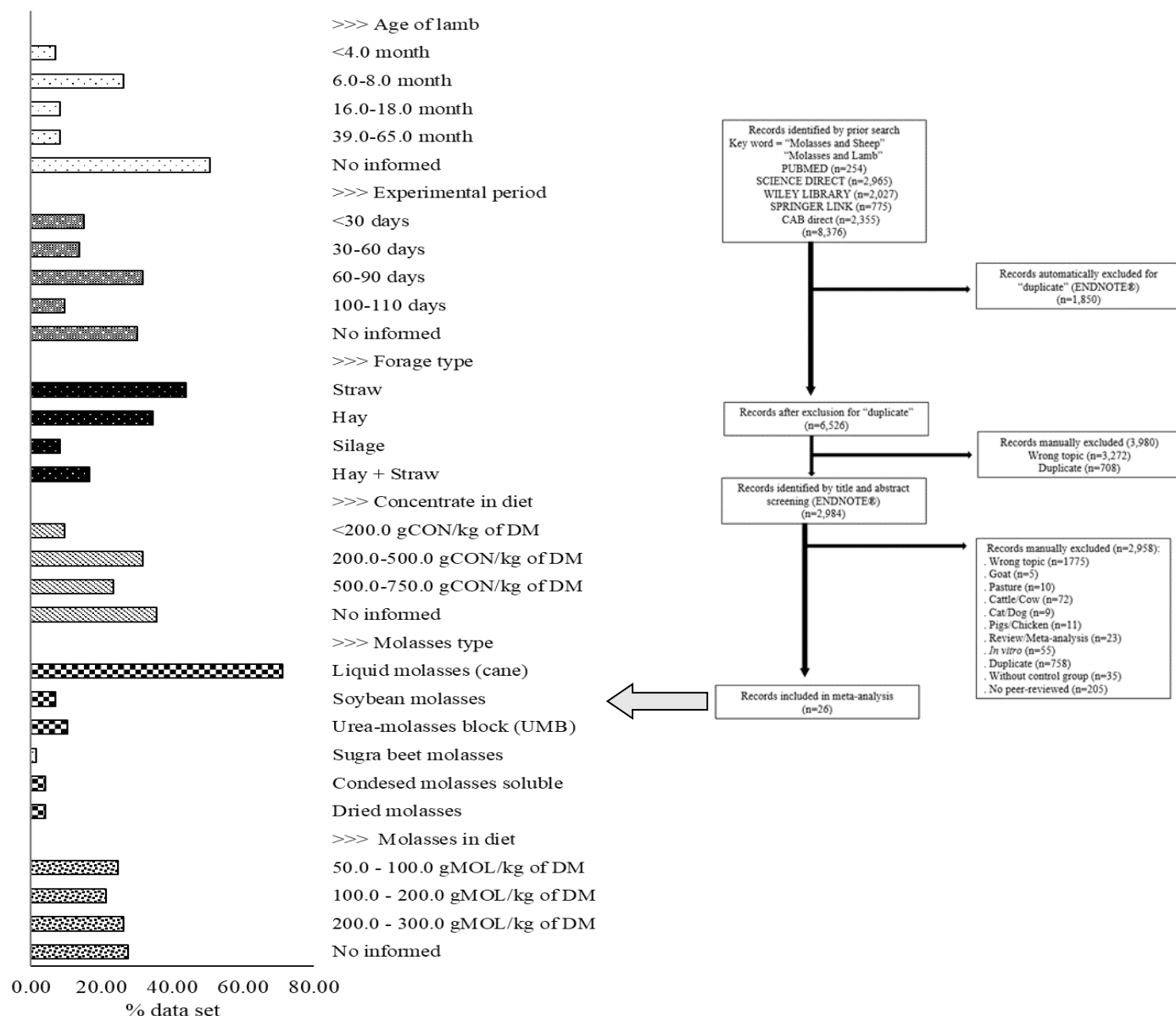


Figure 1. Flowchart showing inclusion criteria for studies and descriptive of the complete data set used for conducting the meta-analysis on effect of tannin in sheep diet

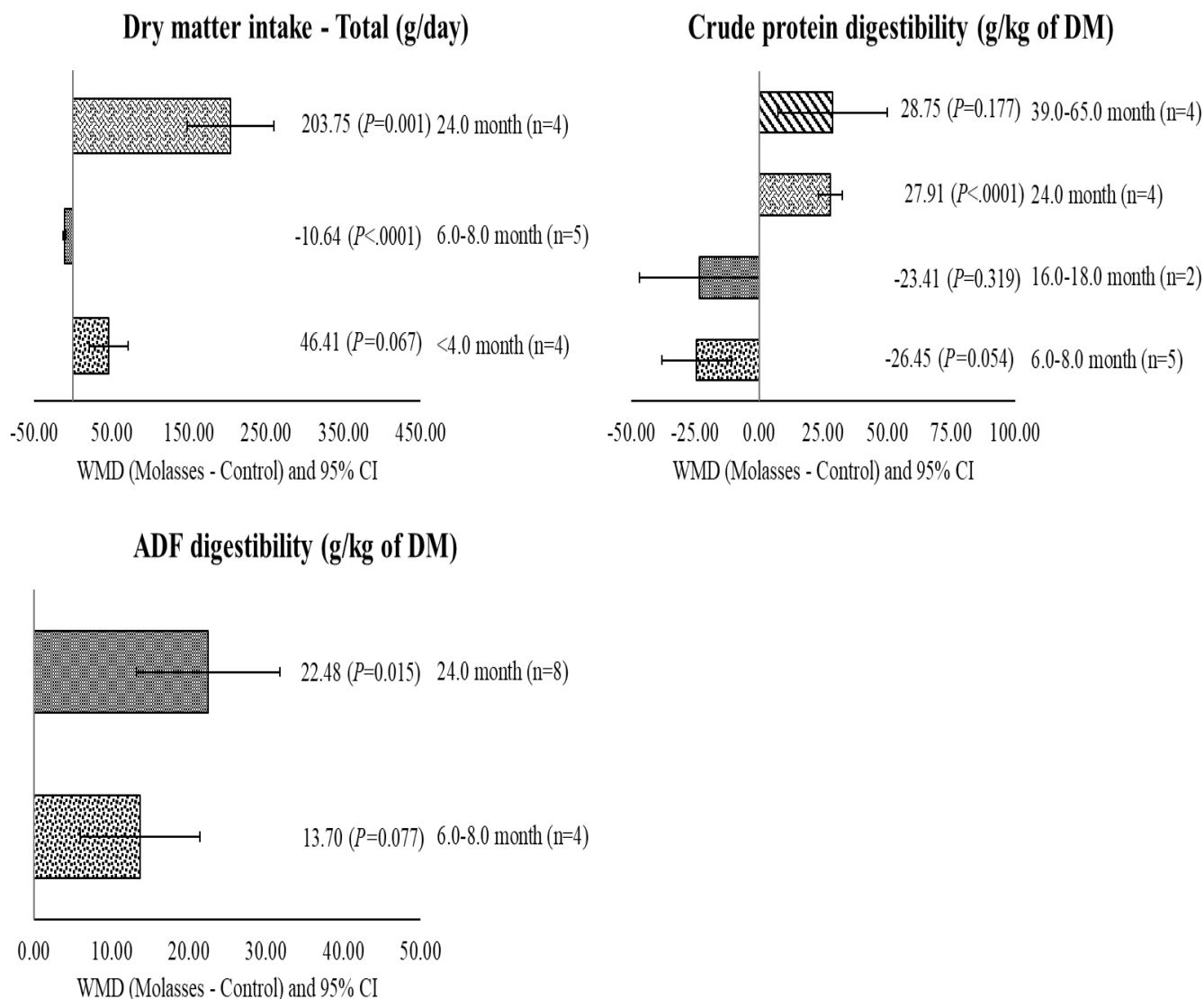


Figure 2. Subgroup analysis (subgroup = Age of lamb (month)) of the effect of inclusion molasses in sheep diets, WMD = weighted mean differences between molasses and control treatments.

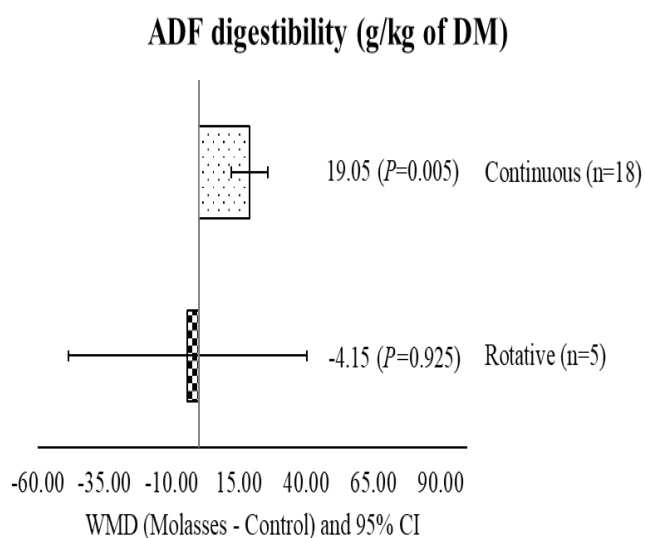


Figure 3. Subgroup analysis (subgroup = Experimental design) of the effect of inclusion molasses in sheep diets, WMD = weighted mean differences between molasses and control treatments.

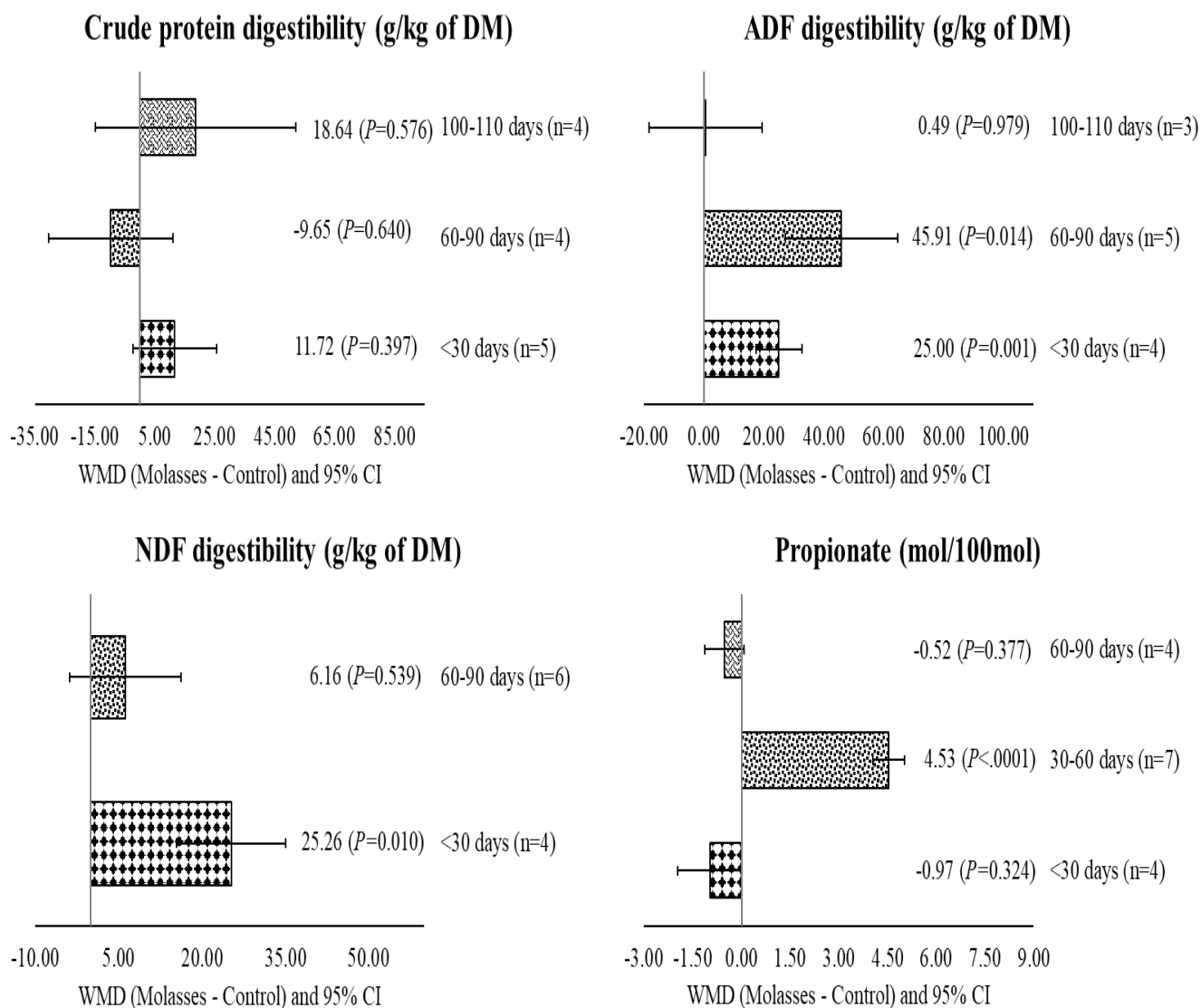


Figure 4. Subgroup analysis (subgroup =Experimental period (day)) of the effect of inclusion molasses in sheep diets, WMD = weighted mean differences between molasses and control treatments.

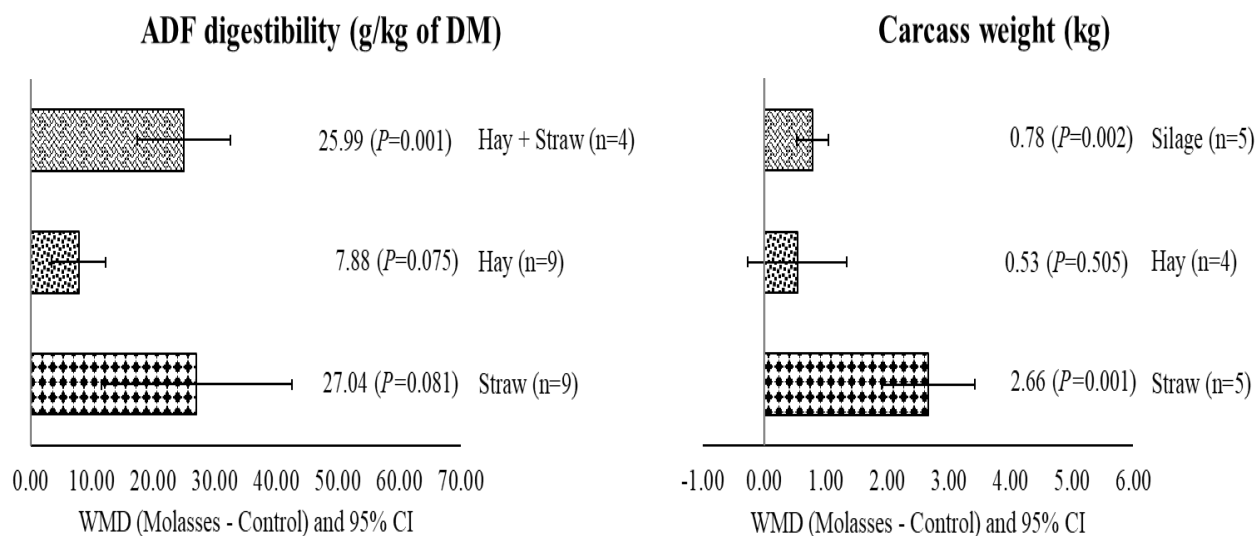


Figure 5. Subgroup analysis (subgroup =Forage type) of the effect of inclusion molasses in sheep diets,

WMD = weighted mean differences between molasses and control treatments.

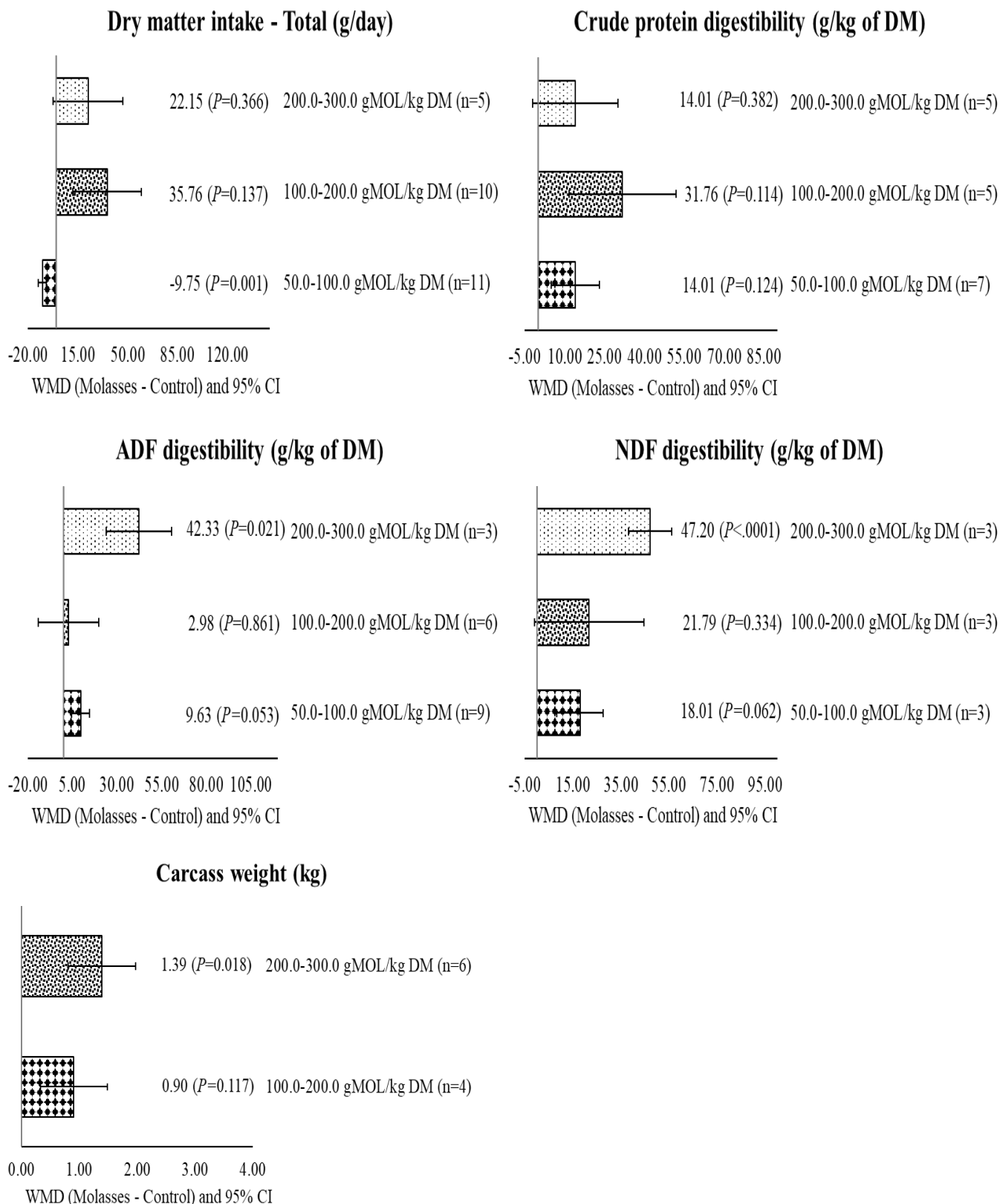


Figure 6. Subgroup analysis (subgroup = molasses (MOL) in diet (g/kg of DM)) of the effect of tannins supplementation to sheep diets, WMD = weighted mean differences between treatment and control treatments.