UNIVERSIDAD AUTÓNOMA CHAPINGO

DEPARTAMENTO DE ENSEÑANZA, INVESTIGACIÓN

Y SERVICIO EN ZOOTECNIA

POSGRADO EN PRODUCCIÓN ANIMAL

PRODUCTIVIDAD, EMISIONES DE METANO, ESTADO ANTIOXIDANTE Y CALIDAD DE CARNE DE RUMIANTES SUPLEMENTADOS CON TANINOS: METAANÁLISIS

TESIS

Que como requisito parcial
para obtener el grado de:

MAESTRO EN CIENCIAS EN INNOVACIÓN GANADERA

Presenta:

JOSÉ FELIPE ORZUNA ORZUNA

Bajo la supervisión de: **Alejandro Lara Bueno, Dr.**

APROBADA

Chapingo, Estado de México, enero de 2022

PRODUCTIVIDAD, EMISIONES DE METANO, ESTADO ANTIOXIDANTE Y CALIDAD DE CARNE DE RUMIANTES SUPLEMENTADOS CON TANINOS: METAANÁLISIS

Tesis realizada por **JOSÉ FELIPE ORZUNA ORZUNA**, bajo la supervisión del Comité Asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

MAESTRO EN CIENCIAS EN INNOVACIÓN GANADERA

DIRECTOR:

Dr. Alejandro Lara Bueno

ASESOR:

Ph.D. Germán David Mendoza Martínez

ASESOR:

Dr. Luis Alberto Miranda Romero

CONTENIDO

LISTA DE CUADROS

LISTA DE FIGURAS

DEDICATORIAS

A mi padre por ser el pilar fundamental en todo lo que soy, en toda mi educación, tanto académica, como de la vida, por su apoyo incondicional a través del tiempo. Muchas gracias por haberme enseñado que con trabajo y esfuerzo es posible cumplir las metas. Todo este trabajo ha sido posible gracias a ti papá.

A mi novia Griselda por apoyarme y motivarme para seguir trabajando para alcanzar mis metas. Tu ayuda ha sido fundamental en este proceso. Muchas gracias, amor.

AGRADECIMIENTOS

A mi Alma Mater, la Universidad Autónoma Chapingo y especialmente al Posgrado en Producción Animal por brindarme la oportunidad de desarrollarme profesionalmente en esta etapa de mi vida.

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por el financiamiento otorgado para el desarrollo de mis estudios de posgrado.

Al Dr. Alejandro Lara Bueno, por toda su ayuda, paciencia y tiempo que dedicó en la realización de esta tesis. Gracias por compartir su conocimiento y experiencia, por creer siempre en mi capacidad, por corregirme, aconsejarme y motivarme cuando fue necesario.

Al Ph.D. Germán David Mendoza Martínez y al Dr. Luis Alberto Miranda Romero por sus consejos y apoyo brindado a través de este estudio y la realización de este manuscrito.

A la empresa Nuproxa México S. de R. L. de C. V., por la donación del aditivo herbal utilizado para la realización del trabajo experimental

DATOS BIOGRÁFICOS

Datos personales

Nombre	José Felipe Orzuna Orzuna
Fecha de nacimiento	26 de mayo de 1996
Lugar de nacimiento	Chiautla de Tapia, Puebla.
CURP	OUOF960526HPLRRL06
Profesión	Ingeniero Agrónomo Especialista en Zootecnia
Cédula Profesional	12171874

Desarrollo académico

Maestría en Ciencias (2020-2021)	DEIS en Zootecnia Posgrado en Producción Animal Universidad Autónoma Chapingo
Licenciatura (2015-2019)	DEIS en Zootecnia Universidad Autónoma Chapingo

RESUMEN GENERAL

PRODUCTIVIDAD, EMISIONES DE METANO, ESTADO ANTIOXIDANTE Y CALIDAD DE CARNE DE RUMIANTES SUPLEMENTADOS CON TANINOS: METAANÁLISIS[1]

Los antibióticos se han utilizado como promotores del crecimiento en rumiantes. Sin embargo, la aparición de bacterias resistentes a estos fármacos ha conducido a la búsqueda de productos alternativos con efectos similares a los antibióticos, pero de origen natural. En un primer estudio, se evaluaron los efectos de la suplementación dietética con taninos (TANs) en el comportamiento productivo, la fermentación ruminal y las emisiones de metano (CH_4) en bovinos para carne usando metaanálisis (MA). La suplementación con TANs no afectó el comportamiento productivo ($p>0.05$); sin embargo, mejoró la concentración ruminal de propionato y butirato, y redujo la concentración ruminal de nitrógeno amoniacal y las emisiones de CH_4 ($p<0.01$). En un segundo estudio se evaluaron los efectos de la suplementación dietética de una mezcla poliherbal (MP) en el comportamiento productivo, las características de la canal y la calidad de la carne de corderos. No hubo efectos ($p>0.05$) de MP en comportamiento productivo, características de canal, color y composición química de la carne. La fuerza de corte disminuyó, mientras que pH y pérdida por goteo de la carne aumentaron linealmente ($p<0.05$) cuando se incrementó la dosis de MP. En un tercer estudio se evaluaron los efectos de la suplementación dietética con TANs en el comportamiento productivo, calidad de la carne y estado antioxidante del suero sanguíneo de ovinos usando MA. La suplementación con TANs no afectó el consumo de MS ni la calidad de la carne ($p>0.05$); sin embargo, aumentó la ganancia de peso, la capacidad antioxidante total y redujo la conversión alimenticia ($p<0.05$). En conclusión, es posible utilizar TANs para reducir emisiones de CH_4 en bovinos y mejorar el comportamiento productivo y estado antioxidante en ovinos. Además, la MP podría utilizarse para mejorar la terneza de la carne de ovinos.

Palabras clave: polifenoles, taninos, mezcla poliherbal, ovinos, bovinos.

[1] Tesis de Maestría en Ciencias en Innovación Ganadera, Posgrado en Producción Animal, Universidad Autónoma Chapingo
Autor: José Felipe Orzuna Orzuna
Director: Alejandro Lara Bueno

GENERAL ABSTRACT

PRODUCTIVITY, METHANE EMISSIONS, NTIOXIDANT STATUS AND MEAT QUALITY OF RUMINANTS SUPPLEMENTED WITH TANNINS: META-ANALYSIS [2]

Antibiotics have been commonly used as growth promoters in ruminants. However, the emergence of bacteria resistant to these drugs has led to the search for alternative products with similar effects to antibiotics, but of natural origin. In a first study, the effects of dietary tannin supplementation (TANs) on productive performance, ruminal fermentation, and methane (CH_4) emissions in beef cattle were evaluated using meta-analysis (MA). Supplementation with TANs did not affect growth performance ($p>0.05$); however, it improved the ruminal concentration of propionate and butyrate, and reduced the ruminal concentration of ammonia nitrogen and the emissions of CH_4 ($p<0.01$). In a second study, the effects of dietary supplementation of a polyherbal mixture (PM) on productive performance, carcass characteristics and quality of lamb meat were evaluated. There were no effects ($p>0.05$) of MP on productive behavior, carcass characteristics, color, and chemical composition of the meat. The shear force decreased, while the pH and drip loss of the meat increased linearly ($p<0.05$) when the dose of PM was increased. In a third study, the effects of dietary supplementation with TANs on productive performance, meat quality and antioxidant status of sheep blood serum were evaluated using MA. Supplementation with TANs did not affect DM intake or meat quality ($p>0.05$); however, it increased weight gain, total antioxidant capacity, and reduced feed conversion ($p<0.05$). In conclusion, it is possible to use TANs to reduce CH_4 emissions in cattle and improve productive performance and antioxidant status in sheep. Furthermore, PM could be used to improve the tenderness of sheep meat

Keywords: polyphenols, tannins, polyherbal mixture, sheep, cattle.

[2] Master of Science Thesis in Livestock Innovation, Graduate Program in Animal Production, Universidad Autónoma Chapingo
Author: José Felipe Orzuna Orzuna
Advisor: Alejandro Lara Bueno

1. INTRODUCCIÓN GENERAL

Durante varias décadas los antibióticos se han utilizado como promotores del crecimiento en animales (Huang, Liu, Zhao, Hu, & Wang, 2018). Sin embargo, el uso inapropiado de estos productos da como resultado la acumulación de residuos tóxicos en la carne, los cuáles pueden afectar la salud del consumidor (Wang et al., 2017). Además, la aparición de cepas bacterianas con resistencia a los efectos de los antibióticos (Callaway, Lillehoj, Chuanchen, & Gay, 2021), y la prohibición de estos compuestos en algunos países (Valenzuela-Grijalva, Pinelli-Saavedra, Muhlia-Almazan, Domínguez-Díaz, & González-Ríos, 2017) han conducido a la industria y a los investigadores a la búsqueda de productos alternativos con efectos similares a los antibióticos, pero de origen natural. Entre ellos, los aditivos fitogénicos (AFGs) han recibido especial atención y son de los productos más estudiados en la última década (Huang et al., 2018).

De acuerdo con Windisch (2008), los AFGs son compuestos derivados de plantas que pueden incorporarse en la dieta para ayudar a mejorar la productividad del ganado a través de mejoras en las propiedades del alimento, la promoción de mayor rendimiento productivo y la mejora de la calidad de los productos alimenticios derivados del ganado. Los AFGs pueden clasificarse con base en su origen botánico (hierbas, especias, etc.), composición (taninos, saponinas, etc.), procesamiento (extractos, aceites esenciales y compuestos aislados) y modo de acción (Valenzuela-Grijalva et al., 2017). Entre los AFGs, los taninos (TANs) han recibido especial atención y son de los compuestos bioactivos más estudiados, particularmente en rumiantes (Huang et al., 2018). Los TANs son un grupo de compuestos polifenólicos presentes en una amplia variedad de plantas, los cuales se pueden dividir en taninos hidrolizables y taninos condensados con base en su estructura química (Naumann, Tedeschi, Zeller, & Huntley, 2017; Serra, Salvatori,

& Pastorelli, 2021). Los TANs pueden tener efectos positivos en los animales, tales como antioxidantes, antimicrobianos, antiparasitarios, inmunomoduladores y antiinflamatorios (Huang et al., 2018; Naumann et al., 2017). Se han realizado diversos estudios para evaluar los efectos de la suplementación dietética con extractos de TANs y plantas ricas en TANs sobre el comportamiento productivo (Fernandes et al., 2021; Pathak, Dutta, Pattanaik, Chaturvedi, & Sharma, 2017), las emisiones de metano entérico (Fagundes et al., 2020; Suybeng, Charmley, Gardiner, Malau-Aduli, & Malau-Aduli, 2020), la calidad de la carne (Dentinho et al., 2020; Valenti et al., 2019), y el estado antioxidante del suero sanguíneo (Liu, Li, Lv, Zhao, & Xiong, 2016; Wang, Giller, Hillmann, Marquardt, & Schwarm, 2019) de ovinos y bovinos. Sin embargo, los resultados observados son aún inconsistentes, probablemente como consecuencia de la variabilidad en las condiciones de alimentación, la edad de los animales, el tipo de producto utilizado, la dosis y la fuente de TANs empleadas en los diferentes estudios (Huang et al., 2018). Por lo anterior, identificar los factores que contribuyen a esta variabilidad es un aspecto clave en el desarrollo de productos conteniendo TANs que puedan ser utilizados para reducir las emisiones de metano, mejorar el comportamiento productivo, la calidad de la carne, y el estado antioxidante de ovinos y bovinos.

Por otro lado, la suplementación dietética con mezclas poliherbales (MP) ha mostrado efectos positivos en el comportamiento productivo y las características de calidad de la carne y la canal de ovinos y bovinos durante la engorda final (Lee et al., 2015; Razo et al., 2020; Orzuna-Orzuna et al., 2021). Asimismo, en terneros se ha reportado que el uso de MP puede aumentar el recuento de anticuerpos contra *Clostridium spp*. y reducir la incidencia de enfermedades (Sánchez et al., 2021), además de mejorar el crecimiento y el estado de salud durante el período prerumiante hasta el destete, a través de cambios en la expresión génica (Díaz-Galván et al., 2021). Las mezclas herbales

se diferencian de los aceites esenciales y extractos en que pueden contener varios compuestos bioactivos responsables de efectos biológicos positivos (Sánchez et al., 2021). Sin embargo, los efectos de los compuestos bioactivos en los sistemas biológicos pueden depender de la eficiencia de su absorción y transformación metabólica extensa (Čobanová et al., 2020).

Hipótesis

La suplementación con taninos y mezclas poliherbales como aditivos fitogénicos mejoran el comportamiento productivo, el estado antioxidante del suero sanguíneo, y reducen las emisiones de metano entérico de ovinos y bovinos, sin afectar la calidad de la carne de estos animales.

Objetivo general

- Evaluar el efecto de distintos fitogénicos en el comportamiento productivo, las emisiones de metano, la calidad de la carne y el estado antioxidante del suero sanguíneo de rumiantes.

Objetivos particulares

- Evaluar el efecto de la suplementación dietética de taninos en el comportamiento productivo, la ingesta y digestibilidad de nutrientes, los parámetros ruminales y las emisiones de metano entérico de bovinos para carne, a través de un metaanálisis.

- Determinar los efectos de dosis crecientes de una mezcla poliherbal conteniendo saponinas, flavonoides y polisacáridos, en el comportamiento productivo, características de la canal y calidad de la carne de corderos alimentados con dietas altas en concentrado.

- ❖ Evaluar el efecto de la suplementación dietética con taninos en el comportamiento productivo, las características de la canal, la calidad y estabilidad oxidativa de la carne, y el estado antioxidante del plasma sanguíneo de ovinos, través de un metaanálisis.

Estructura de la tesis

El Capítulo 2 es un metaanálisis que sintetiza de forma cuantitativa la información publicada de 2010 hasta 2020 sobre los efectos de la suplementación dietética de taninos en el comportamiento productivo, la fermentación ruminal, la digestibilidad del alimento ingerido y las emisiones de metano entérico en bovinos productores de carne.

En el Capítulo 3 se presenta el resultado de un trabajo experimental en el que se evaluaron los efectos de diferentes dosis de una mezcla herbal conteniendo saponinas, flavonoides y polisacáridos en el comportamiento productivo, las características de la canal y la calidad de la carne de corderos durante la engorda final.

Con base en los resultados de los capítulos 2 y 3, se observó que las variables en las que más impacto tienen los fitogénicos, son las variables de comportamiento productivo y calidad de la carne. Por lo tanto, en el Capítulo 4 se presenta un metaanálisis que sintetiza de forma cuantitativa la información publicada entre enero de 2010 y junio de 2021 sobre los efectos de la suplementación dietética de taninos en el comportamiento productivo, la calidad de la carne y el estado antioxidante del suero sanguíneo de ovinos.

Literatura citada

Callaway, T. R., Lillehoj, H., Chuanchuen, R., & Gay, C. G. (2021). Alternatives to Antibiotics: A Symposium on the Challenges and Solutions for Animal Health and Production. Antibiotics, 10(8), 471, doi:10.3390/antibiotics10050471

Čobanová, K., Váradyová, Z., Grešáková, Ľ., Kucková, K., Mravčáková, D., & Várady, M. (2020). Does Herbal and/or Zinc Dietary Supplementation Improve the Antioxidant and Mineral Status of Lambs with Parasite Infection? Antioxidants, 9, 1172, doi:10.3390/antiox9121172

Dentinho, M. T. P., Paulos, K., Francisco, A., Belo, A. T., Jerónimo, E., Almeida, J., Bessa, R. J. B., & Santos-Silva, J. (2020). Effect of soybean meal treatment with Cistus ladanifer condensed tannins in growth performance, carcass and meat quality of lambs. Livestock Science, 236, 104021, doi:10.1016/j.livsci.2020.104021

Díaz, G. C., Méndez, O. E. T., Martínez, G. D., Gloria, T. A., Hernández, G. P. A., Espinosa, A. E., Palacios, M. M., Lara, B. A., Mendoza, M. G. D., & Velázquez, C. L. A. (2021). Influence of a polyherbal mixture in dairy calves: growth performance and gene expression. Frontiers in Veterinary Science, 7, 623710, doi:10.3389/fvets.2020.623710

Fagundes, G. M., Benetel, G., Welter, K. C., Melo, F. A., Muir, J. P., Carriero, M. M., Souza, R. L. M., Meo-Filho, P., Frighetto, R. T. S., Berndt, A., & Bueno, I. C. S. (2020). Tannin as a natural rumen modifier to control methanogenesis in beef cattle in tropical systems: Friend or foe to biogas energy production? Research in Veterinary Science, 132, 88–96, doi:10.1016/j.rvsc.2020.05.010

Fernandes, J., Pereira, F. J., Menezes, D., Caldas, A. C., Cavalcante, I., Oliveira, J., Silva, J. J., Cézar, M., & Bezerra, L. (2021). Carcass and meat quality in lambs receiving natural tannins from Mimosa tenuiflora hay. Small Ruminant Research, 198, 106362, doi:10.1016/j.smallrumres.2021.106362

Huang, Q., Liu, X., Zhao, G., Hu, T., & Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition, 4(2), 137-150, doi:10.1016/j.aninu.2017.09.004

Lee, S. J., Kim, D. H., Guan le, L., Ahn, S. K., Cho, K. W., & Lee, S. S. (2015). Effect of medicinal plant by-products supplementation to total mixed ration on growth performance, carcass characteristics and economic

efficacy in the late fattening period of Hanwoo steers. Asian-Australasian Journal of Animal Sciences, 28(12), 1729–1735, doi:10.5713/ajas.15.0290

Liu, H., Li, K., Lv, M., Zhao, J., & Xiong, B. (2016). Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs. Meat Science, 116, 236–242, doi:10.1016/j.meatsci.2016.02.024

Naumann, H. D., Tedeschi, L. O., Zeller, W. E., & Huntley, N. F. (2017). The role of condensed tannins in ruminant animal production: advances, limitations and future directions. Revista Brasileira de Zootecnia, 46, 929-949, doi:10.1590/s1806-92902017001200009

Orzuna-Orzuna, J. F., Dorantes-Iturbide, G., Lara-Bueno, A., Mendoza- Martínez, G. D., Miranda-Romero, L.A., & Hernández-García, P. A. (2021). Growth Performance, Carcass Characteristics, and Blood Metabolites of Lambs Supplemented with a Poly-herbal Mixture. Animals, 11(4), 955, doi:10.3390/ani11040955

Pathak, A. K., Dutta, N., Pattanaik, A. K., Chaturvedi, V. B., & Sharma, K. (2017). Effect of condensed tannins from Ficus infectoria and Psidium guajava leaf meal mixture on nutrient metabolism, methane emission and performance of lambs. Asian-Australasian Journal of Animal Sciences, 30(12), 1702–1710, doi:10.5713/ajas.17.0060

Razo, O. P. B., Mendoza, M. G. D., Vázquez, S. G., Osorio, T. A. I., González, S. J. F., Hernández, G. P. A., Torre, H. M. E., & Espinosa, A. E. (2020). Polyherbal feed additive for lambs: Effects on performance, blood biochemistry and biometry. Journal of Applied Animal Research, 48, 419–424, doi:10.1080/09712119.2020.1814786

Sánchez, N., Lee-Rangel, H. A., Martínez-Cortés, I., Mendoza, G. D., Hernández, P. A., Espinoza, E., Vazque, V. A., Flores, R. R., Roque- Jimenez, A., Campillo-Navarro, M., & Relling, A. E. (2021). A polyherbal phytogenic additive improved growth performance, health, and immune response in dairy calves. Food and Agricultural Immunology, 32(1), 482- 498, doi:10.1080/09540105.2021.1967296

Serra, V., Salvatori, G., & Pastorelli, G. (2021). Dietary Polyphenol Supplementation in Food Producing Animals: Effects on the Quality of Derived Products. Animals, 11, 401, doi:10.3390/ani11020401

Suybeng, B., Charmley, E., Gardiner, C. P., Malau-Aduli, B. S., & Malau-Aduli, A. E. O. (2020). Supplementing northern Australian beef cattle with Desmanthus tropical legume reduces in-vivo methane emissions. Animals, 10(11), 2097, doi:10.3390/ani10112097

Valenti, B., Natalello, A., Vasta, V., Campidonico, L., Roscini, V., Mattioli, S., Pauselli, M., Priolo, A., Lanza, M., & Luciano, G. (2019). Effect of different dietary tannin extracts on lamb growth performances and meat oxidative stability: Comparison between mimosa, chestnut and tara. Animal, 13(2), 435–443, doi:10.1017/S1751731118001556

Valenzuela-Grijalva, N.V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Domínguez-Díaz, D., & González-Ríos, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. Journal of Animal Science and Technology, 59, 8, doi:10.1186/s40781-017-0133-9

Wang, H., Ren, L., Yu, X., Hu, J., Chen, Y., He, G., & Jiang, Q. (2017). Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. Food Control, 80, 217–225, doi:10.1016/j.foodcont.2017.04.034

Wang, S., Giller, K., Hillmann, E., Marquardt, S., & Schwarm, A. (2019). Effect of supplementation of pelleted hazel (Corylus avellana) leaves on blood antioxidant activity, cellular immune response and heart beat parameters in sheep. Journal of Animal Science, 97(11), 4496-4502, doi:10.1093/jas/skz288

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86(14), 140-148, doi:10.2527/jas.2007-0459

This chapter was published in *Sustainability*

2. EFFECTS OF DIETARY TANNINS' SUPPLEMENTATION ON GROWTH PERFORMANCE, RUMEN FERMENTATION, AND ENTERIC METHANE EMISSIONS IN BEEF CATTLE: A META-ANALYSIS

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara- Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1] and Pedro Abel Hernández-García [3]

[1]Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo 56230, México; jforzuna@gmail.com (J.F.O.-O.); griseldi0993@gmail.com (G.D.-I.); albertomiranda@correo.chapingo.mx (L.A.M.-R.)

[2]Departamento de Producción Agrícola y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, México City 04960, México; gmendoza@correo.xoc.uam.mx

[3]Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca 56900, México; pedro_abel@yahoo.com

Corresponding author.

E-mail address: alarab_11@hotmail.com (A. Lara-Bueno).

sustainability

MDPI

Review

Effects of Dietary Tannins' Supplementation on Growth Performance, Rumen Fermentation, and Enteric Methane Emissions in Beef Cattle: A Meta-Analysis

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1] and Pedro Abel Hernández-García [3]

[1] Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo 56230, Mexico; jforzuna@gmail.com (J.F.O.-O.); griseldi0993@gmail.com (G.D.-I.); albertomiranda@correo.chapingo.mx (L.A.M.-R.)
[2] Departamento de Producción Agrícola y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, Mexico City 04960, Mexico; gmendoza@correo.xoc.uam.mx
[3] Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de Mexico, Amecameca 56900, Mexico; pedro_abel@yahoo.com
* Correspondence: alarab_11@hotmail.com

Abstract: The environmental sustainability of beef production is a significant concern within the food production system. Tannins (TANs) can be used to minimize the environmental impact of ruminant production because they can improve ruminal fermentation and ruminants' lifetime performances and mitigate methane (CH_4) emissions. The objective of this study was to evaluate the effects of dietary supplementation with TANs as sustainable natural alternative to reduce the environmental impact on growth performance, rumen fermentation, enteric CH_4 emissions, and nitrogen (N) use efficiency of beef cattle through a meta-analysis. A comprehensive search of studies published in scientific journals that investigated the effects of TANs' supplementation on the variables of interest was performed using the Scopus, Web of Science, and PubMed databases. The data analyzed were extracted from 32 peer-reviewed publications. The effects of TANs were assessed using random-effects statistical models to examine the standardized mean difference (SMD) between TANs' treatments and control (non-TANs). The heterogeneity was explored by meta-regression and subgroup analysis was performed for the covariates that were significant. TANs' supplementation did not affect weight gain, feed consumption, feed efficiency, or N use efficiency ($p > 0.05$). However, it reduced the concentration of ammonia nitrogen in rumen (SMD = −0.508, $p < 0.001$), CH_4 emissions per day (SMD = −0.474, $p < 0.01$) and per unit dry matter intake (SMD = −0.408, $p < 0.01$), urinary N excretion (SMD = −0.338, $p < 0.05$), and dry matter digestibility (SMD = −0.589, $p < 0.001$). Ruminal propionate (SMD = 0.250) and butyrate (SMD = 0.198) concentrations and fecal N excretion (SMD = 0.860) improved in response to TANs' supplementation ($p < 0.05$). In conclusion, it is possible to use TANs as a CH_4 mitigation strategy without affecting cattle growth rate. In addition, the shift from urinary to fecal N may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N. Therefore, the addition of tannins in the diet of beef cattle could be used as a sustainable natural alternative to reduce the environmental impact of beef production.

Keywords: feed efficiency; bioactive compounds; climate change; meta-regression; sustainability

check for updates

Citation: Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Hernández-García, P.A. Effects of Dietary Tannins' Supplementation on Growth Performance, Rumen Fermentation, and Enteric Methane Emissions in Beef Cattle: A Meta-Analysis. *Sustainability* **2021**, *13*, 7410. https://doi.org/10.3390/su13137410

Academic Editor: Giuseppe Todde

Received: 15 May 2021
Accepted: 28 June 2021
Published: 2 July 2021

1. Introduction

Minimizing enteric methane (CH_4) emissions from ruminant production while improving feed conversion efficiency and growth rate is a goal for sustainable livestock production [1]. In addition, the nitrogen (N) excreted by ruminants is the main source of nitrous oxide (N_2O) emissions in livestock systems [2] and can contribute to air and water pollution [3]. Therefore, strategies based on changing the composition and concentration of

urinary compounds by diet manipulation could be considered potential options to mitigate urine N_2O emissions and consequently improve sustainability in ruminant production [4]. Among these strategies, dietary tannins' (TANs') supplementation has received special attention, particularly in ruminants [5]. TANs are a group of polyphenolic compounds that are present in a wide variety of plants and can have positive effects in animals, such as antimicrobial, antiparasitic, antioxidant, anti-inflammatory, and immunomodulatory [5]. According to Naumann et al. [6], TANs are generally classified based on their chemical structure into two groups: condensed tannins (CTs) and hydrolysable tannins (HTs). CTs consist of flavan-3-ol subunits linked together to form oligomers and polymers, whereas HTs are esters of gallic or ellagic acid linked to a polyol core [6].

In ruminants, previous studies [7–9] have shown that dietary supplementation with TANs improves the utilization efficiency of ingested feed. In addition, TANs have been successfully used to reduce enteric CH_4 production, urinary N excretion, and N_2O emissions [7,10] and to increase the duodenal flux of microbial protein and amino acids [11]. TANs-rich plants and TANs' extracts have also shown positive impact on rumen microbial activity [12], ruminal fermentation rate [10], antioxidant status, and health of ruminants [13,14]. However, TANs can also reduce the digestion of protein in the rumen and the entire gastrointestinal tract [15]. Therefore, the intake of TANs in combination with a medium-poor quality diet (e.g., insufficient crude protein in the diet) may not generate nutritional benefits and is detrimental to performance ([6,8,15]. For example, some studies have reported negative effects of dietary supplementation with TANs on digestibility, productive performance, and ruminal fermentation [2,16], while other studies have not observed significant effects on digestibility, productive performance, CH_4 emissions, and urinary and fecal nitrogen excretion in response to TAN supplementation [7,11,16].

Particularly, in beef cattle, several studies have been conducted to evaluate the effect of dietary supplementation with TANs on the growth performance [17,18], nutrient intake and digestibility [19,20], ruminal parameters [21,22], enteric CH_4 emissions [23,24], and urinary and fecal N excretion [17,21]. However, the results observed to date have been non-conclusive because their effects vary widely, even within the same plant species [14]. The variations in the chemical and botanical origin of TANs, processing methods, feeding conditions, physiological state of animals, and supplementation levels used are factors that could contribute to the variability of the effects observed in animals supplemented with TANs [5,14,25]. Therefore, identifying and controlling this variability is a key aspect in the development of TANs-containing products that can be used as feed additives to improve the sustainability of beef production.

Although some classical reviews [5,6,14,25] previously suggested that dietary supplementation with TANs can improve productivity and decrease enteric CH_4 production in ruminants, these studies did not use a meta-analytical approach and none focused only on beef cattle. Meta-analysis (MA) is a statistical tool that allows combining and synthesizing data published in different studies in a quantitative way [26–28]. In addition, MA can be used to explore sources of heterogeneity, which provides additional information on factors contributing to the variability of the observed results [29], and it also helps to identify potential areas for further research [26]. MA has been frequently used in clinical and biomedical research, but its implementation in animal science-related research is still limited [30]. The objective of this meta-analysis was to evaluate the effect of dietary supplementation with tannins as sustainable natural alternative to reduce the environmental impact on the growth performance, nutrient intake and digestibility, ruminal parameters, enteric CH_4 emissions, and nitrogen use efficiency of beef cattle. In addition, we examined the heterogeneity of the responses by meta-regression analysis to identify factors contributing to the variability observed in the response variables.

2. Materials and Methods

2.1. Literature Search and Study Selection

A comprehensive literature search in the scientific databases of Web of Science, Scopus, and PubMed was carried out to identify studies that investigated the effect of TANs' supplementation on growth performance, nutrient intake and digestibility, ruminal fermentation, and enteric CH_4 emissions in beef cattle. In all databases, the keywords "tannin, chestnut, quebracho, leucaena, birdsfoot, lotus, sainfoin, onobrychis, sulla, hedysarum, proanthocyanidin, growth, digestibility, fermentation, methane, bull, steer and cattle" were used, among which were TANs and the most common TANs-containing plants [31]. A total of 613 scientific publications published between 2010 and 2020 were identified. These publications went through a two-step selection process, as previously described by Herremans et al. [31]. First, a selection was performed using titles and abstracts excluding in vitro and simulation studies, reviews, and articles that did not measure the variables of interest. Subsequently, to be considered, studies had to meet several inclusion criteria previously reported by other authors [31,32]: (1) studies on adult (male, weaned or older) and confined beef cattle; (2) data on growth performance, nutrient intake and digestibility, ruminal fermentation, urinary and fecal excretion, or in vivo CH_4 emissions (measured with respirometry chambers, the sulfur hexafluoride "SF6" tracer technique, or the Green-Feed system (C-Lock Inc., Rapid City, SD, USA)); (3) similarity between control and experimental groups, except for the presence of TANs; (4) quantification or possible determination of dietary TANs' doses; (5) peer-reviewed journal articles written in English; (6) experimental design employed (rotating or continuous); (7) least squares means of the control and experimental groups with variability measures (standard error or standard deviation); and (8) sample size used.

2.2. Data Extraction

Based on the selection criteria, only 32 articles were included in the database for the final analysis. The response variables extracted for the meta-analysis included daily weight gain, feed efficiency (determined as weight gain/feed intake (G:F), kg/kg), final body weight, intake and digestibility of dry matter (DM) and nutrients (organic matter, crude protein, ether extract, neutral detergent fiber digestibility, and acid detergent fiber digestibility), ruminal parameters (ruminal concentration of propionate, butyrate, acetate, total volatile fatty acids, ammonia nitrogen, and protozoa), in vivo CH_4 emissions (per day and per unit of dry matter intake), and urinary and fecal N excretion. Moreover, when available, additional data were collected, such as characteristics of the published study (author, year of publication), amount of forage in the diet (g/kg DM), source of chemical or botanical origin of TANs, experimental design used (rotational or continuous), period of TANs' supplementation (days), chemical composition of diet, number of replicates, type of TANs (CTs, HTs, or mixture of both), method of TANs' inclusion (extract or naturally present in the diet), and amount of TANs in the diet (g/kg DM). The references of the articles included in the data set are listed in Table A1 in Appendix A. Averages, standard deviation (SD), and number of repetitions for each treatment were extracted from these articles. When the articles presented the SD of each experimental group, these values were used directly in the meta-analysis. In cases where the SD was not reported, it was calculated by multiplying the standard error means (SEM) by the square root of the sample size, using the equation $SD = SEM \times \sqrt{n}$, as previously reported by Higgins and Thomas [33], where n = number of replicates.

2.3. Calculations and Statistical Analysis

Regarding the data involved in the meta-analysis and meta-regression, these were analyzed using the Open Meta-analyst for Ecology and Evolution software [34] and the statistical software R (version 3.6.3) using the "metafor" package [35]. The response variables were analyzed through the standardized mean difference (SMD), also called effect size (ES), in which the difference between the means of the experimental and control groups

was standardized using the SD of the groups with and without TANs [36]. The SMDs were calculated using the methods previously described by DerSimonian and Laird [37] for random effects models. The SMD is a more robust estimation of the ES when there is heterogeneity in the data set [38]. On the other hand, using the SAS statistical program [39], the chemical composition variables of the diets and the response parameters extracted were analyzed with the MEANS procedure to obtain descriptive statistics values. Differences in the composition of the diets of the control and TANs-supplemented treatments were evaluated by the MIXED procedure, using the studies as random effect and Tukey's test to detect differences between treatments, as previously reported by Torres et al. [40].

2.4. Heterogeneity

Measurement of heterogeneity was performed using chi-square test (Q) and the I^2 (percentage of variation) statistic [41]. Due to the relatively low power of the Q test to detect heterogeneity among a small number of treatment comparisons, an α level of 0.10 was used [38,42]. I^2 values range from 0 to 100%. Values close to 25% indicate low heterogeneity, close to 50% indicate moderate heterogeneity, and close to 75% indicate high heterogeneity among studies [27,29]. Likewise, I^2 values greater than 50% indicate significant heterogeneity [32].

2.5. Publication Bias

According to Littell et al. [43], the visual inspection of funnel plots generally used to assess publication bias is subjective and must be balanced with additional analyses. Accordingly, three methods were used to assess evidence of publication bias: (1) the funnel plot [44], (2) Egger's regression asymmetry test [45], and (3) Begg's adjusted rank correlation [46]. A bias was considered to be present when the funnel plot showed asymmetry or when at least one of the statistical methods (Egger's test or Begg's test) was significant ($p < 0.10$). The tests to assess publication bias are inappropriate when significant heterogeneity (Q) is detected with an $\alpha \leq 0.10$ and when the variable to be assessed is not reported in at least 10 studies because it may lead to false-positive claims [47]. Consequently, funnel plots, Egger's test, and Begg's test were only performed for variables that met the aforementioned criteria. In cases where statistical evidence of publication bias was found, the trim-and-fill method of Duval and Tweedie was used to estimate the number of possible missing observations [48].

2.6. Meta-Regression

The sources of heterogeneity of parameters that showed an I^2 greater than 50% [27] or Q with an α level of $\leq$0.10 [42] were evaluated by a meta-regression analysis. The meta-regression analysis was only performed for response variables that were reported in at least 10 studies [43]. Meta-regression was estimated using the DerSimonian and Laird method of moments, which is well established for estimating the variance between studies [27]. In the meta-regression, continuous and categorical variables were used. The continuous variables were TANs' doses (g/kg DM), difference of NDF content in the diets (g/kg DM), and duration of the experimental phase (days). The categorical variables were type of TANs (CTs, HTs, or mixture of both), source of botanical or chemical origin of the TANs, method by which the TANs were supplied (extract or as part of some dietary ingredient), animal's age ($\leq$12 and >12 months old), and the experimental design used (rotational or continuous). When categorical co-variables were significant at an α level of $\leq$0.05, SMD was assessed by subgroup analysis. Likewise, when the meta-regression was significant ($p \leq 0.05$) for continuous co-variables, these were evaluated by subgroup analysis dividing the co-variables as follows: level of TANs' supplementation in the diet ($\leq$12 and >12 g/kg DM) and experimental period ($\leq$90 and >90 days).

3. Results

3.1. Study Attributes and Excluded Studies

The online search using three databases of scientific publications from January 2010 to December 2020 returned a total of 613 publications (Figure S1). After exclusion of duplicate papers and selection of titles and abstracts, 46 full-text articles were evaluated. Of these, 32 articles met the inclusion criteria (Table A1) and were used to obtain quantitative data for meta-analysis.

The descriptive statistics and means test for diet composition are presented in Table 1. Except for NDF content, no significant differences were observed between the control and the TANs' treatment for the rest of the nutrient components of the diet ($p > 0.05$). This indicates that it is possible to exclude the effects of the chemical composition of the diets on the response of the animals to TANs' supplementation for the data set.

Table 1. Descriptive statistics of the complete data set for the effect of tannins' supplementation to beef cattle diets.

Parameter	NC	Mean		Median		Minimum		Maximum		SD	
Dietary Features		Control	Tannin	Control	Tannin	Control	Tannin	Control	Tannin	Control	Tannin
Forage g/kg DM	105	506.9	509.1	498.0	425.0	50.0	30.0	1000	1000	358.9	372.5
DM, g/kg	80	647.8	645.7	700.0	702.5	256.0	256.0	927.0	928.0	211.7	210.5
OM, g/kg DM	50	927.5	928.3	936.0	936.4	835.1	835.8	953.0	953.0	30.24	30.10
CP, g/kg DM	105	124.4	129.0	132.5	134.5	30.10	30.10	204.0	205.0	40.60	36.46
EE, g/kg DM	61	38.31	39.52	32.10	35.50	17.50	17.50	61.0	61.0	13.98	13.68
NDF, g/kg DM	97	430.5 [a]	423.7 [b]	409.0	404.4	163.0	163.0	763.5	770.0	177.2	172.8
ADF, g/kg DM	73	259.1	259.7	226.5	224.5	82.10	82.10	468.5	487.0	117.7	120.2
Starch, g/kg DM	31	364.8	362.9	415.8	422.6	48.0	23.0	575.0	575.0	180.6	183.3
Ca, g/kg DM	41	6.18	6.33	5.55	6.15	5.30	5.30	7.50	7.50	0.836	0.792
P, g/kg DM	41	4.11	4.10	4.20	4.10	3.60	3.60	4.50	4.50	0.319	0.313
Tannin, g/kg DM	105	-	14.61	-	12.10	-	0.46	-	60	-	12.29
Duration, days	99	93		90		28		180		33.38	
Extracted response parameters											
FBW, kg	31	457.5	458.2	443.5	437	189.5	204.3	621	616	122.1	122.6
DMI, kg/d	73	8.357	8.136	8.20	7.84	3.80	3.60	12.60	12.76	2.267	2.456
OMI, kg/d	46	6.837	6.820	6.540	6.690	1.185	1.155	12.440	12.480	2.321	2.391
CPI, kg/d	26	0.828	0.957	0.705	0.990	0.194	0.167	2.090	2.200	0.513	0.494
EEI, kg/d	8	0.232	0.232	0.170	0.180	0.160	0.150	0.410	0.390	0.096	0.098
NDFI, kg/d	38	3.679	3.524	3.760	3.740	1.810	1.900	4.630	4.730	0.959	0.835
ADFI, kg/d	17	2.521	2.453	2.850	2.500	1.260	1.280	3.500	3.620	0.674	0.664
ADG, kg/d	37	1.258	1.273	1.370	1.320	0.018	0.120	2.080	2.140	0.589	0.545
FE, kg/kg	22	0.153	0.150	0.163	0.159	0.092	0.092	0.206	0.198	0.037	0.033
DMD, g/kg DM	49	622.0	594.3	628.0	601.9	411.9	428.5	810.5	797.7	77.57	95.08
OMD, g/kg DM	59	660.1	632.0	660.0	646.3	451.7	442.0	820.0	810.0	84.48	100.4
CPD, g/kg DM	43	571.6	541.2	679.0	635.0	276.2	79.57	767.6	770.9	183.9	226.1
EED, g/kg DM	23	689.4	679.4	713.0	699.0	447.0	435.0	857.3	891.0	112.7	116.7
NDFD, g/kg DM	47	561.4	534.9	576.0	518.1	385.0	405.0	771.0	776.9	90.11	80.54
ADFD, g/kg DM	24	494.1	415.4	532.0	413.6	403.0	219.1	549.1	561.0	54.72	88.33
Ruminal pH	57	6.637	6.621	6.700	6.680	5.810	5.890	7.190	7.430	0.337	0.356
NH3-N, mg/dL	57	11.25	10.59	10.63	8.16	2.48	1.73	30.40	36.50	6.338	7.673
Total VFA, mM	54	84.72	86.49	74.01	78.42	35.80	32.72	158	141	29.30	28.81
Acetate, % molar	54	60.39	60.67	67.80	66.09	31.42	38.41	74.10	74.40	11.54	10.41
Propionate, % molar	54	19.39	19.85	18.74	18.47	6.58	9.25	36.80	38.0	6.987	6.505
Butyrate, % molar	54	11.94	12.38	10.33	11.70	6.10	5.30	19.40	19.77	3.799	3.866
Protozoa, log10/mL	26	5.508	5.306	5.480	5.595	1.310	0.930	11.90	10.60	3.540	3.161
CH4, L/d	26	150.6	135.7	128.8	107.0	44.16	29.20	331.7	302.4	80.86	88.44
CH4, L/DMI	28	19.93	18.76	20.10	14.78	5.60	5.43	31.22	51.80	9.35	12.38

Table 1. *Cont.*

Parameter Dietary Features	NC	Mean		Median		Minimum		Maximum		SD	
		Control	Tannin	Control	Tannin	Control	Tannin	Control	Tannin	Control	Tannin
UNE, g/d	35	56.64	54.95	54.80	46.0	4.30	9.0	168.0	167.0	47.88	44.48
FNE, g/d	31	57.10	66.73	49.88	62.0	16.20	19.50	126.0	146.0	32.64	38.04
NUE, %	22	25.76	20.75	25.34	16.45	16.89	6.20	39.15	39.0	7.43	11.62

NC: number of comparisons; SD: standard deviation; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; P: phosphorus; ADG: average daily gain; FE: feed efficiency; FBW: final body weight; DMI: DM intake; OMI: OM intake; CPI: CP intake; NDFI: NDF intake; ADFI: ADF intake; EEI: EE intake; DMD: DM digestibility; OMI: OM digestibility; CPD: CP digestibility; NDFD: NDF digestibility; ADFD: ADF digestibility; EED: EE digestibility; NH_3-N: nitrogen ammonia; VFA: volatile fatty acids; CH_4: methane; FE: determined as weight gain/feed intake (G:F), kg/kg; UNE: urinary nitrogen excretion; FNE: fecal nitrogen excretion; NUE: nitrogen use efficiency; [a], [b]: in the same row (only applies to dietary features), means followed by different letters differ significantly by the Tukey's test ($p < 0.05$).

The studies included in this meta-analysis were conducted in 10 different countries (Table A1). The experimental doses of TANs ranged from 0.46 to 60 g/kg DM, while the duration of the experimental periods varied from 28 to 180 days (Table 1). The TANs used were divided into CTs, HTs, and mixture of both. Of the treatments, 53.3% used CTs, 12.4% used HTs, and 34.3% used mixtures of CTs and HTs. On the other hand, 77% of the treatments used TANs' extracts in the diets, while 23% used parts of plants, forages, or subproducts that contained TANs in natural form (Table A1). Regarding TANs' sources, most of the treatments (34.3%) used TANs from quebracho tree (*Schinopsis spp.*), 19% used TANs from *Acacia mearnsii*, and 14.3% used TANs from pistachio tree (*Pistacia vera*). On the other hand, 32.4% of the treatments supplied TANs from chestnut tree (*Castanea sativa*), *Leucaena leucocephala*, tannic acid, and mixtures of these or other sources (Table A1).

3.2. Growth Performance and Nutrient Intake

In general, no significant effects of TANs' inclusion in beef cattle diets were found ($p > 0.05$) for final body weight (FBW), dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI), ether extract intake (EEI), neutral detergent fiber intake (NDFI), acid detergent fiber intake (ADFI), average daily gain (ADG), or feed efficiency (FE; Table 2). However, there was tendency in reduction of FE ($p = 0.06$).

Table 2. Growth performance and nutrient intake of beef cattle supplemented with tannins.

Variable	N	NC	SMD	SE	95% CI		*p*-Value	Heterogeneity		
					Lower	Upper		Q	*p*-Value	I2 (%)
Final bodyweight	11	31	−0.041	0.102	−0.241	0.158	0.68	38.642	0.13	22.36
Dry matter intake	25	73	−0.010	0.078	−0.163	0.144	0.90	102.879	<0.05	30.01
Organic matter intake	16	46	0.062	0.086	−0.106	0.230	0.47	22.526	0.99	0
Crude protein intake	9	26	0.321	0.171	−0.014	0.657	0.06	46.693	<0.05	46.46
Ether extract intake	3	8	−0.026	0.241	−0.499	0.447	0.91	6.723	0.45	0
Neutral detergent fiber intake	14	38	−0.167	0.096	−0.355	0.022	0.08	20.042	0.99	0
Acid detergent fiber intake	6	17	−0.189	0.135	−0.453	0.075	0.16	4.241	0.99	0
Average daily gain	13	37	0.059	0.083	−0.104	0.222	0.47	35.49	0.49	0
Feed efficiency	7	22	−0.287	0.150	−0.581	0.007	0.06	43.045	<0.05	51.21

N: number of studies; NC: number of comparisons; SMD: standardized mean difference; CI: confidence interval of SMD; SE: standard error; Q: chi-squared statistic and associated significance level (*p*-value); I^2: percentage of variation.

3.3. Digestibility, Ruminal Parameters, and Methane Emissions

There were no significant effects of TANs' inclusion in beef cattle diets ($p > 0.05$) for ether extract digestibility (EED), ruminal pH, ruminal concentration of total volatile fatty acids (VFA), acetate and protozoa, or for nitrogen use efficiency (NUE; Table 3). However, we observed a negative impact ($p < 0.05$) of TANs' inclusion in the diets on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), and acid detergent fiber digestibility (ADFD). On the other hand, rumen propionate, butyrate concentration, and fecal nitrogen excretion

(FNE) increased ($p < 0.05$) in response to TANs' supplementation. We observed a positive impact (reduction) of TANs' inclusion ($p < 0.05$) in the diets for ruminal ammonia nitrogen concentration (NH_3-N), urinary nitrogen excretion (UNE), and for enteric CH_4 emissions per day (MED) and per unit of dry matter intake (MEDMI; Table 3).

Table 3. Nutrient digestibility, rumen parameters, and enteric methane emissions of beef cattle supplemented with tannins.

Parameter	N	NC	SMD	SE	95% CI		*p*-Value	Heterogeneity		
					Lower	Upper		Q	*p*-Value	I2 (%)
Dry matter digestibility	17	49	−0.589	0.124	−0.833	−0.346	<0.001	97.833	<0.001	50.94
Organic matter digestibility	21	59	−0.612	0.108	−0.825	−0.400	<0.001	108.599	<0.001	46.59
Crude protein digestibility	15	43	−0.903	0.210	−1.315	−0.492	<0.001	173.687	<0.001	75.82
Ether extract digestibility	8	23	−0.328	0.215	−0.750	0.094	0.12	61.615	<0.001	64.29
NDFD	18	47	−0.370	0.150	−0.644	−0.076	0.01	127.334	<0.001	63.87
ADFD	9	24	−0.716	0.151	−1.012	−0.419	<0.001	37.107	<0.05	38.02
Ruminal pH	20	57	−0.171	0.099	−0.364	0.022	0.08	98.287	<0.001	43.02
Ruminal NH3-N	20	57	−0.508	0.128	−0.759	−0.258	<0.001	148.223	<0.001	62.22
Total VFA	19	54	0.021	0.124	−0.223	0.265	0.86	139.359	<0.001	61.97
Acetate	19	54	0.041	0.115	−0.184	0.267	0.72	120.090	<0.001	55.87
Propionate	19	54	0.250	0.107	0.040	0.460	0.02	103.404	<0.001	48.74
Butyrate	19	54	0.198	0.079	0.042	0.354	0.01	61.204	0.20	13.40
Protozoa	8	26	−0.745	0.397	−1.523	0.033	0.06	235.732	<0.001	89.39
Methane emissions/day	9	26	−0.474	0.155	−0.178	−0.171	0.002	50.007	<0.05	48.01
Methane emissions/unit of DMI	10	28	−0.408	0.155	−0.712	−0.105	0.008	56.848	<0.001	52.50
Urinary nitrogen excretion	12	35	−0.338	0.149	−0.630	−0.046	0.023	83.931	<0.001	59.49
Fecal nitrogen excretion	11	31	0.860	0.138	0.589	1.131	<0.001	48.304	0.018	37.89
Nitrogen use efficiency	8	22	−0.273	0.262	−0.786	0.239	0.296	75.726	<0.001	72.27

N: number of studies; NC: number of comparisons; SMD: standardized mean difference; CI: confidence interval of SMD; SE: standard error; Q: chi-squared statistic and associated significance level (*p*-value); I^2: percentage of variation; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; NH_3-N: ammonia nitrogen; VFA: volatile fatty acids; DMI: dry matter intake.

3.4. Analysis of Publication Bias

The tests to assess publication bias are inappropriate when there is significant heterogeneity (Q) ($p \leq 0.10$) and when the variable to be assessed is not reported in at least 10 studies [47]. Therefore, this analysis was only performed for ADG, FBW, OMI, NDFI, and ruminal butyrate concentration. The visual inspection of the funnel plots showed presence of publication bias for all variables analyzed (Figures S2a, S3a, S4a, S5a and S6a). Egger's test showed publication bias for ADG, FBW, OMI, and NDFI ($p < 0.05$), but did not detect publication bias for butyrate ($p = 0.87$). On the other hand, Begg's test only detected publication bias for ADG and OMI ($p < 0.05$), while FBW, NDFI, and butyrate were not significant ($p > 0.10$). The trim-and-fill method indicated that the number of missing observations for ADG and FBW were seven and nine, respectively, both on the left side of the funnel plot (Figures S2b and S3b), whereas, for OMI, NDFI, and butyrate, the missing observations were 14, 7, and 13, respectively, all on the right side of the funnel plot (Figures S4b, S5b and S6b).

3.5. Meta-Regression

Significant heterogeneity (Q) was observed for DMI, FE ($p < 0.05$; Table 2), DMD, OMD, CPD, EED, NDFD, ADFD, ruminal pH, ruminal NH_3-N concentration, total VFA, acetate, propionate and protozoa, MED, and MEDMI, as well as for UNE and FNE ($p < 0.001$; Table 3). Although significant heterogeneity existed, it is not advisable to use meta-regression when there are fewer than 10 studies that reported the response variable of interest [43]. Consequently, this analysis was only performed for the variables DMI, DMD,

OMD, CPD, NDFD, ruminal pH, ruminal concentration of NH_3-N, total VFA, acetate and propionate, MEDMI, and UNE as well as for the FNE.

Except for age, there was no significant relationship ($p > 0.05$) between DMI and the moderators used (level of supplementation, period of supplementation, type of TANs, method of TANs' supply, source of botanical or chemical origin of TANs, NDF content in the diet, and experimental design). The dose of TANs supplied in the diets explained 63.4, 69.1, 25.8, 33.4, 17.2, and 31.7% of the observed heterogeneity for DMD, OMD, NDFD, ruminal acetate and propionate concentration, and FNE, respectively ($p < 0.05$). The period of TANs' supplementation only had a significant relationship ($p < 0.05$) with the MEDMI, explaining only 21.95% of the observed heterogeneity. The type of TANs explained ($p < 0.05$) only 7.25, 7.16, 19.5, 6.7, and 17.4% of the observed heterogeneity in CPD, NDFD, NH_3-N, total VFA, and UNE, respectively. A significant relationship ($p < 0.05$) was observed between CPD and MEDMI with the method of inclusion of TANs in the diet (extract or naturally present in plant parts), where the inclusion method explained 14.5, 23.2, 70.3, and 84.85% of the observed heterogeneity in CPD, MEDMI, UNE, and FNE, respectively. The source of botanical or chemical origin of TANs explained ($p < 0.05$) 48.7, 13.3, 83.7, 17.3, 18, 61, 82.3, and 100% of the heterogeneity observed in CPD, NDFD, NH_3-N, total VFA, propionate, MEDMI, UNE, and FNE, respectively. A significant relationship ($p < 0.05$) was observed between CPD and MEDMI with the NDF content of the diets, where variation in NDF content explained 16 and 48.8% of the heterogeneity observed in CPD and MEDMI, respectively. The experimental design used (rotating or continuous) explained ($p < 0.05$) 19.8, 42, 29.7, 48.2, and 33.1% of the observed heterogeneity for DMD, OMD, NDFD, ruminal pH, and MEDMI, respectively. The age ($\leq$12 and >12 months old) explained ($p < 0.05$) 31.4, 100, 49.7, 55.1, and 79.7% of the heterogeneity observed in DMI, CPD, ruminal pH, NH_3-N, and total VFA, respectively.

3.6. Subgroup Analysis

Regarding the type of TANs, supplementation with HTs and mixture of CTs with HTs decreased CPD ($p < 0.001$), while there was no change in CPD in animals supplemented with CTs ($p > 0.05$; Figure 1). NDFD decreased (SMD = −0.633; $p < 0.001$) in beef cattle supplemented with CTs, but there was no change in NDFD with supplementation of HTs and mixture of CTs with HTs ($p > 0.05$; Figure S7). Ruminal NH_3-N concentration decreased ($p < 0.001$) with supplementation of HTs (SMD = −0.980) and mixture of CTs with HTs (SMD = −0.582). However, NH_3-N was not affected in animals supplemented with CTs ($p > 0.05$; Figure S8). The ruminal concentration of total VFA increased in study animals using CTs (SMD = 0.253; $p = 0.04$) but decreased when using HTs (SMD = −0.491; $p = 0.03$). No significant changes in ruminal concentration of total VFA were observed in study animals using mixtures of CTs and HTs ($p > 0.05$; Figure 2). UNE decreased with supplementation of HTs and mixture of CTs (SMD = −0.445; $p = 0.03$ with HTs (SMD = −0.900; $p < 0.001$). However, UNE was not affected in animals supplemented with CTs (SMD = −0.338; $p > 0.05$).

With respect to the source of botanical or chemical origin of the TANs, Figure 3 shows that, except for plant mixtures, all TANs' sources modified CPD ($p < 0.05$; Figure 3).

Figure 4 shows that NDFD decreased ($p < 0.05$) only when TANs came from *Acacia mearnsii* and quebracho. Ruminal NH3-N concentration was not affected by TANs when they came from a mixture of plants ($p > 0.05$). However, it increased when the TANs came from *Leucaena leucocephala* (SMD = 76.47; $p < 0.001$) and decreased in studies using *Acacia mearnsii*, quebracho, chestnut, pistachio, and tannic acid as a source of TANs ($p < 0.05$; Figure S9).

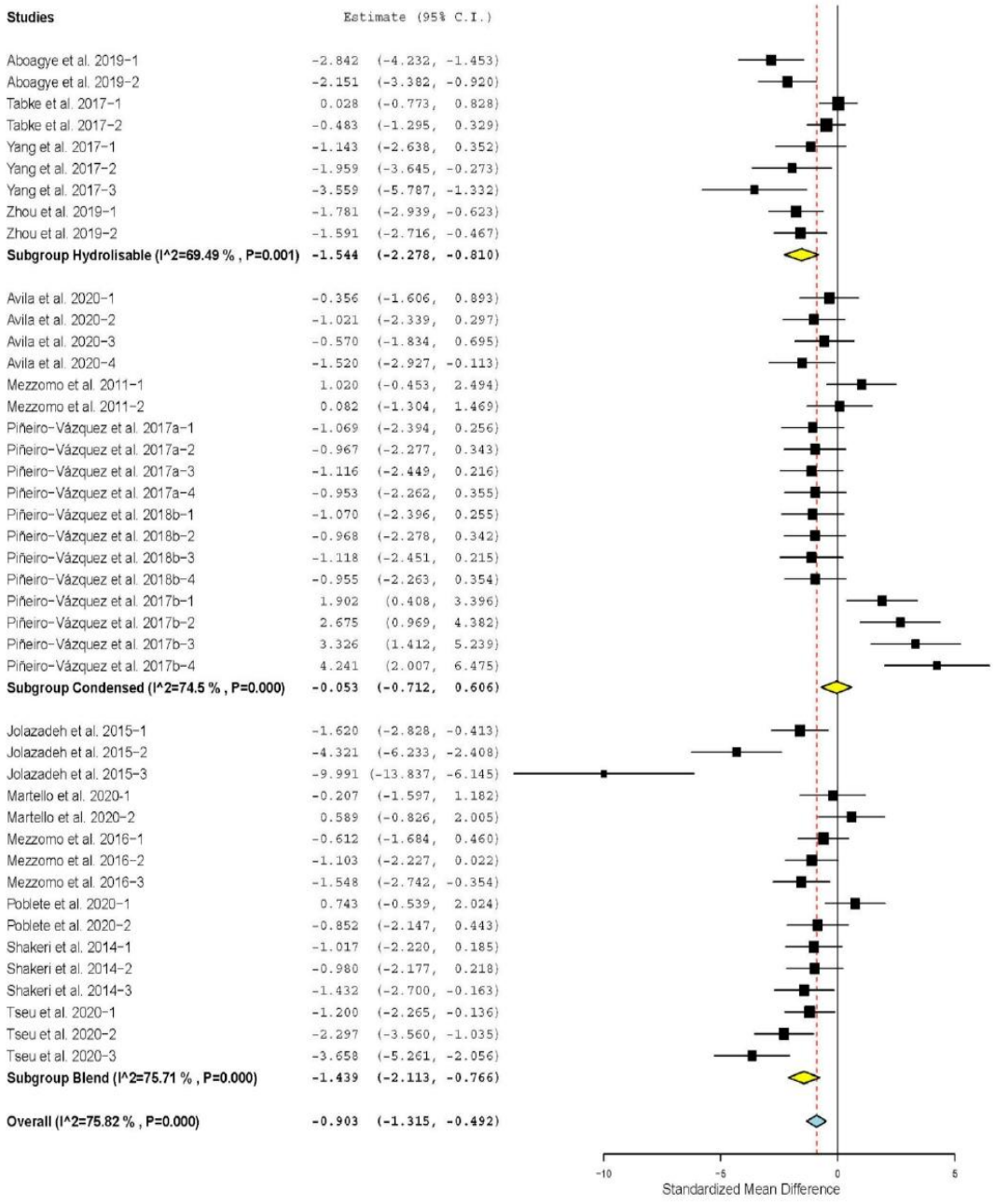

Figure 1. Forest plot of the effect size or standardized mean difference and 95% confidence interval of tannin type on crude protein digestibility (CPD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of total CPD, while points to the right of the line indicate increase in total CPD concentration.

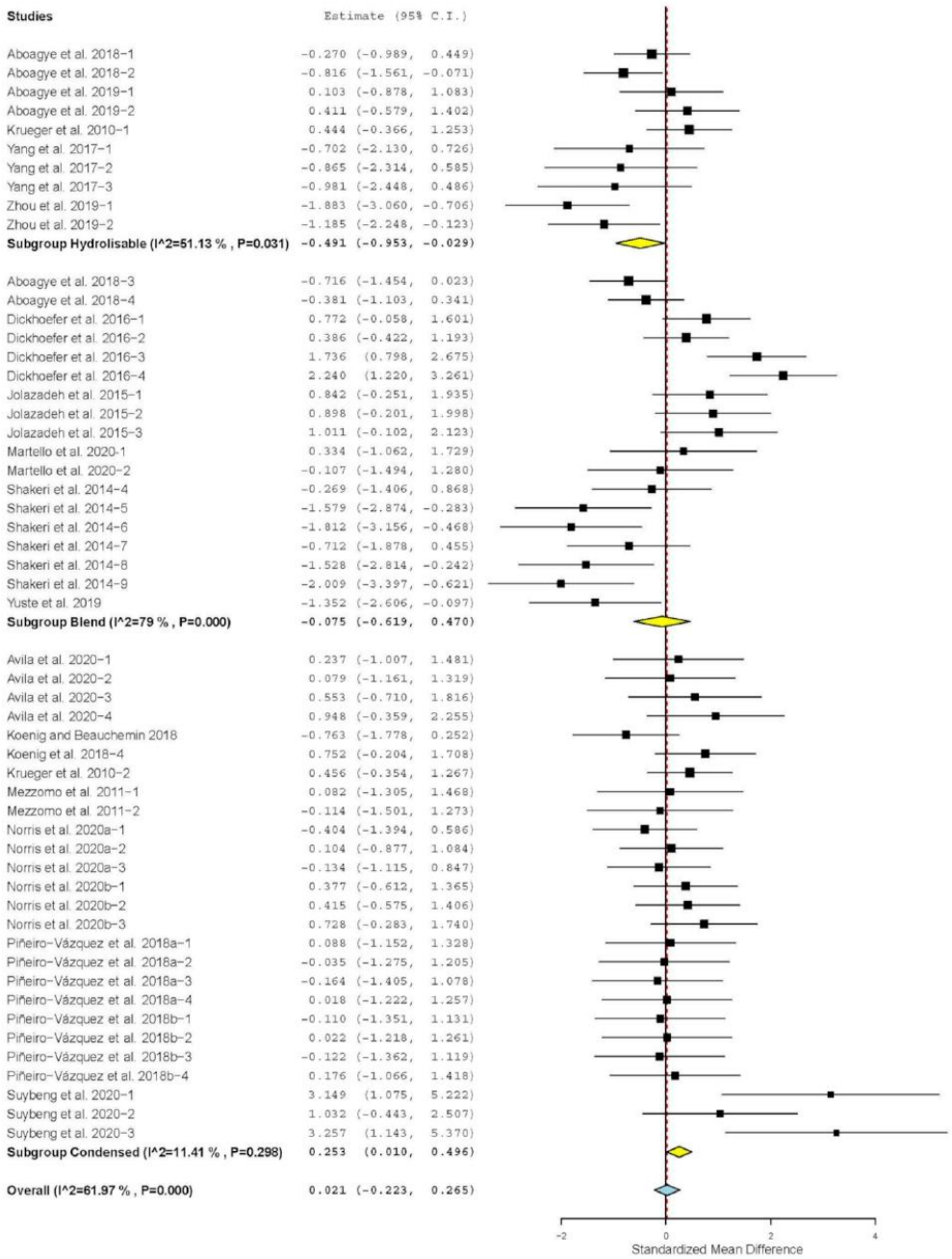

Figure 2. Forest plot of effect size or standardized mean difference and 95% confidence interval of tannin type on ruminal concentration of total volatile fatty acids (VFA) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of total VFA, while points to the right of the line indicate increase in total VFA concentration.

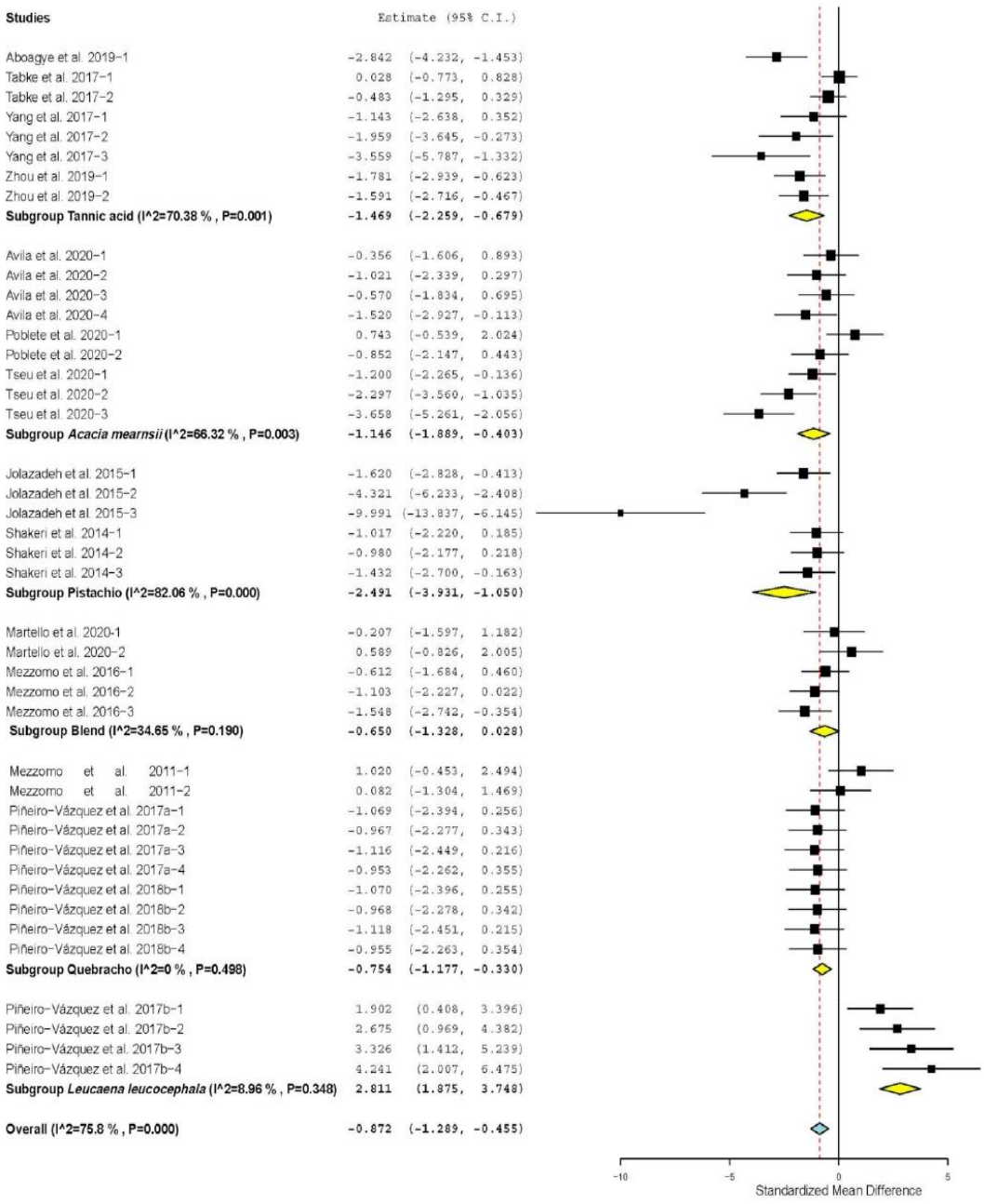

Figure 3. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on crude protein digestibility (CPD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduced CPD, while points to the right of the line indicate increased CPD.

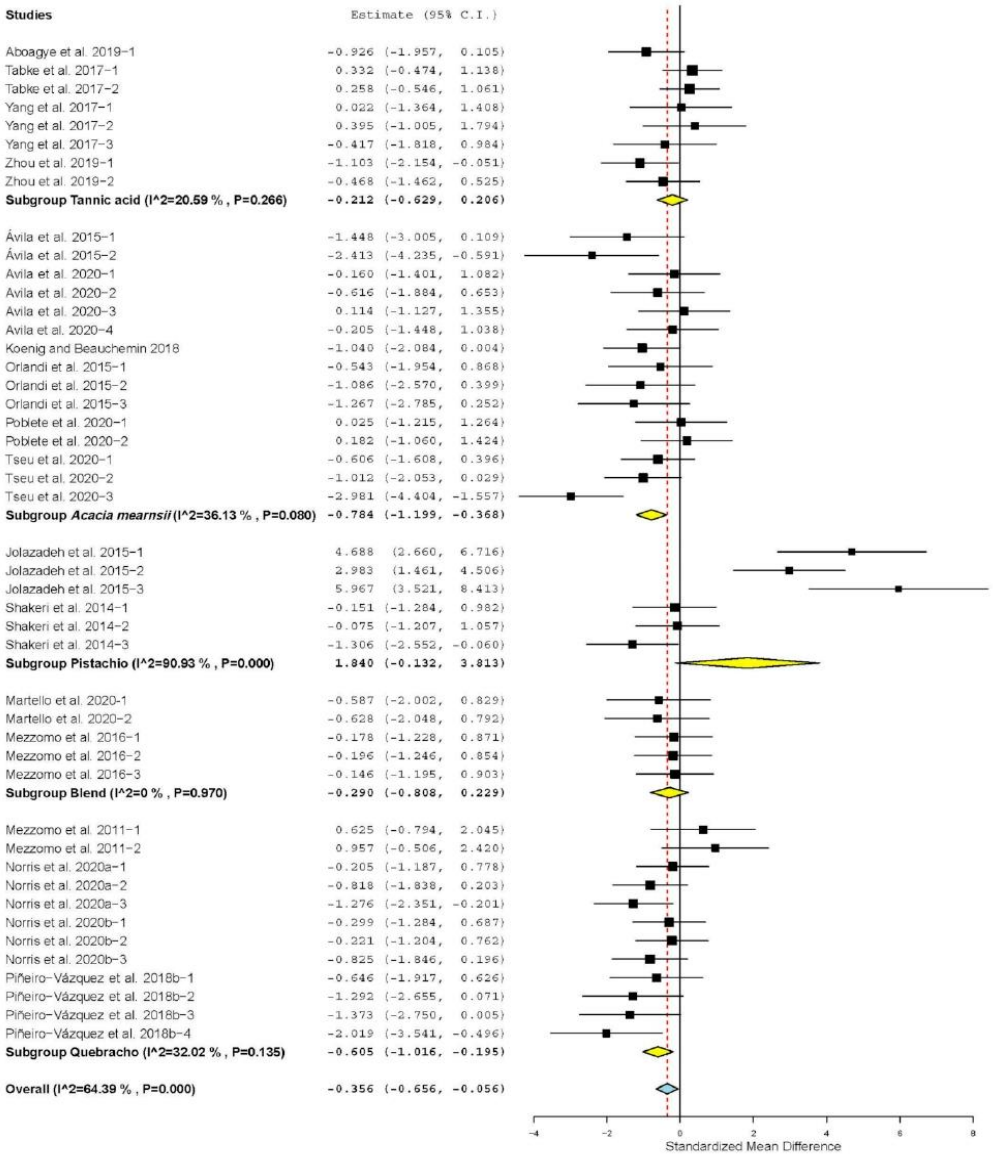

Figure 4. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on neutral detergent fiber digestibility (NDFD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduced NDFD, while points to the right of the line indicate increased NDFD.

Supplementation with TANs decreased the concentration of total VFA in ruminal liquid when tannic acid was the source of chemical origin of the TANs (SMD = −0886; $p = 0.004$). On the other hand, the ruminal concentration of total VFA increased (SMD = 0.431; $p = 0.018$) when quebracho was used as the source of TANs (Figure 5).

Dietary supplementation with TANs increased ruminal propionate concentration only when TANs were obtained from quebracho tree and pistachio ($p < 0.001$), while ruminal

propionate concentration was reduced (SMD = −1.104; $p = 0.046$) when TANs were from plant mixtures (Figure 6).

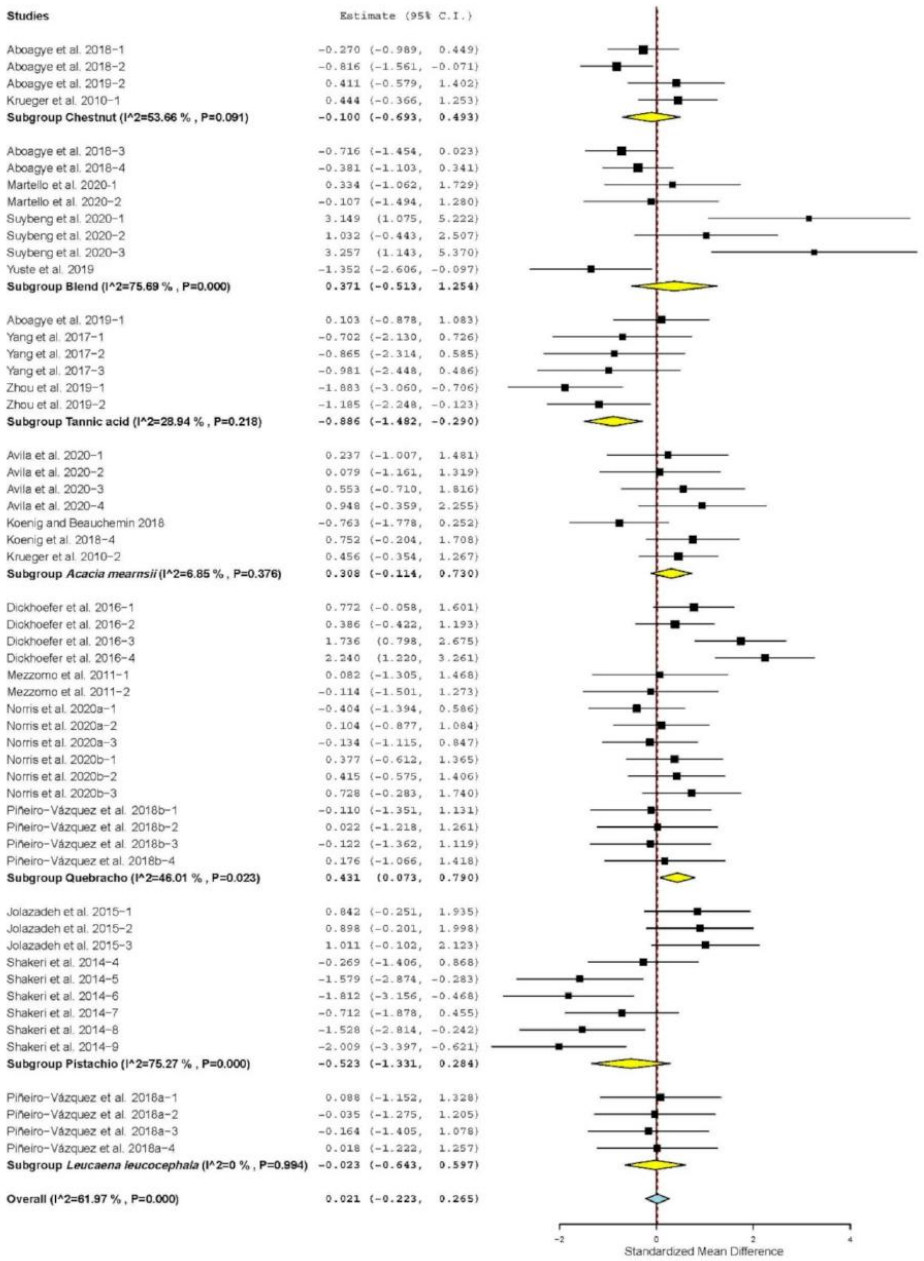

Figure 5. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on ruminal concentration of total volatile fatty acids (VFA) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in ruminal concentration of total VFA, while points to the right of the line indicate increase in total VFA.

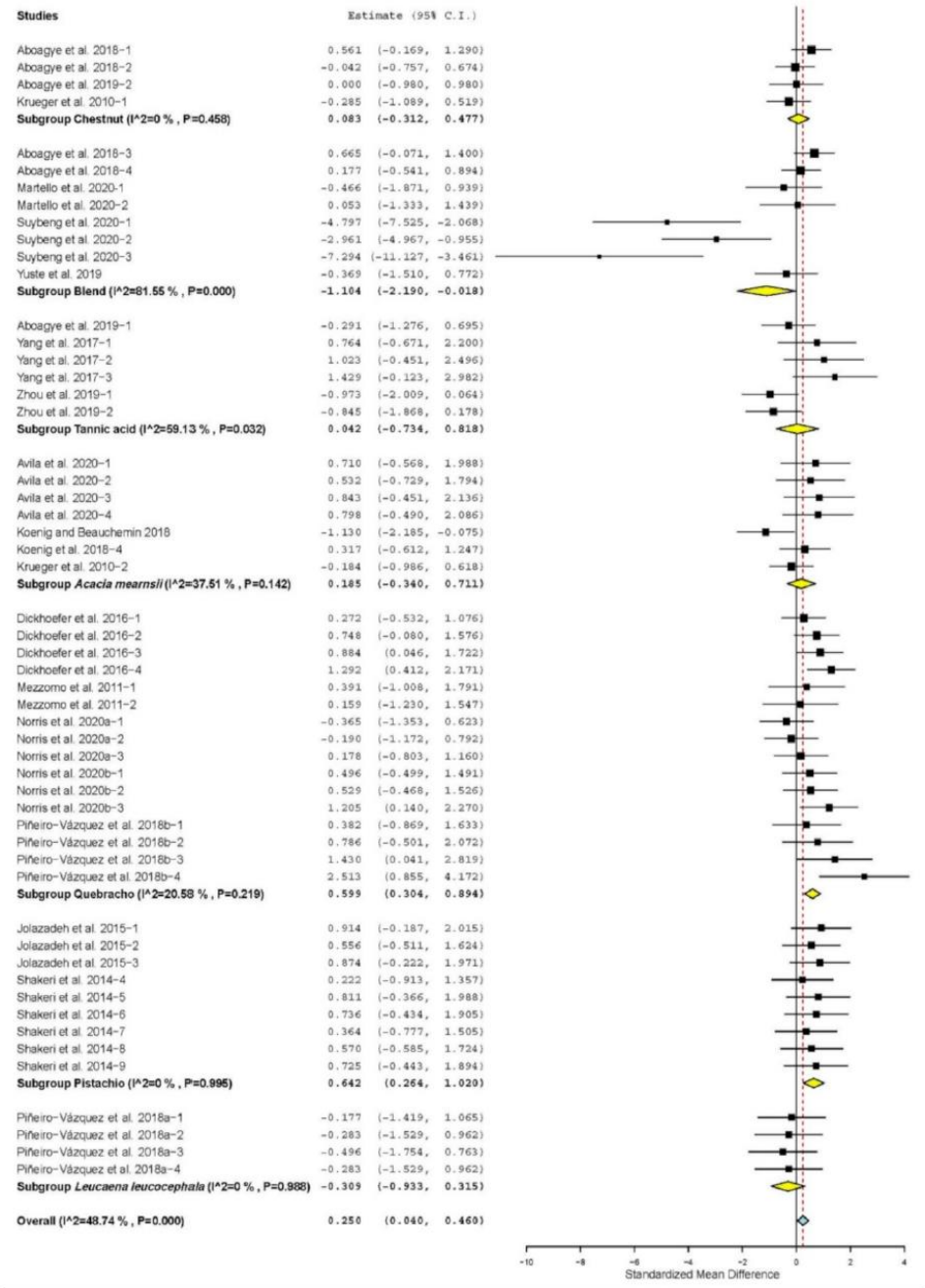

Figure 6. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on ruminal propionate concentration in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of ruminal propionate, while points to the right of the line indicate increase of propionate.

Dietary supplementation with TANs significantly reduced MEDMI only in animals from studies using tannic acid and *Leucaena leucocephala* as a source of TANs ($p < 0.001$; Figure 7).

Figure 8 shows that UNE decreased ($p < 0.05$) only when TANs came from chestnut, *Acacia mearnsii*, quebracho, and *Leucaena leucocephala* ($p < 0.05$). However, when TANs were supplied as a part of the diet ingredients, FNE was not affected (SMD = −0.368; $p > 0.05$). However, UNE was not affected by TANs when they came from a mixture of plants ($p > 0.05$). On the other hand, FNE was not affected by TANs when they came from a mixture of plants and *Leucaena leucocephala* ($p > 0.05$). However, it increased ($p < 0.001$) when the TANs came from *Acacia mearnsii* and quebracho (Figure 9).

With respect to the method by which TANs were included in the diets, CPD decreased when TANs were added to the diets in the form of extracts (SMD = −1.199; p <0.001). However, when TANs were contained in the ingredients of the diets, CPD was not affected ($p = 0.179$; Figure S10). MEDMI decreased significantly when TANs were supplied as part of the diet ingredients (SMD = −0.982; $p < 0.001$); however, when TANs were added to the diets in the form of extracts, MEDMI was not affected ($p > 0.05$; Figure 10). UNE decreased when TANs were added to the diets in the form of extracts (SMD = −0.558; $p < 0.001$). However, UNE increased when TANs were contained in the ingredients of the diets (SMD = 2.078; $p < 0.001$). On the other hand, UFE increased significantly when TANs were added to the diets in the form of extracts (SMD = $p < 0.001$); however, when TANs were supplied as a part of the diet ingredients, FNE was not affected (SMD = −0.368; $p > 0.05$).

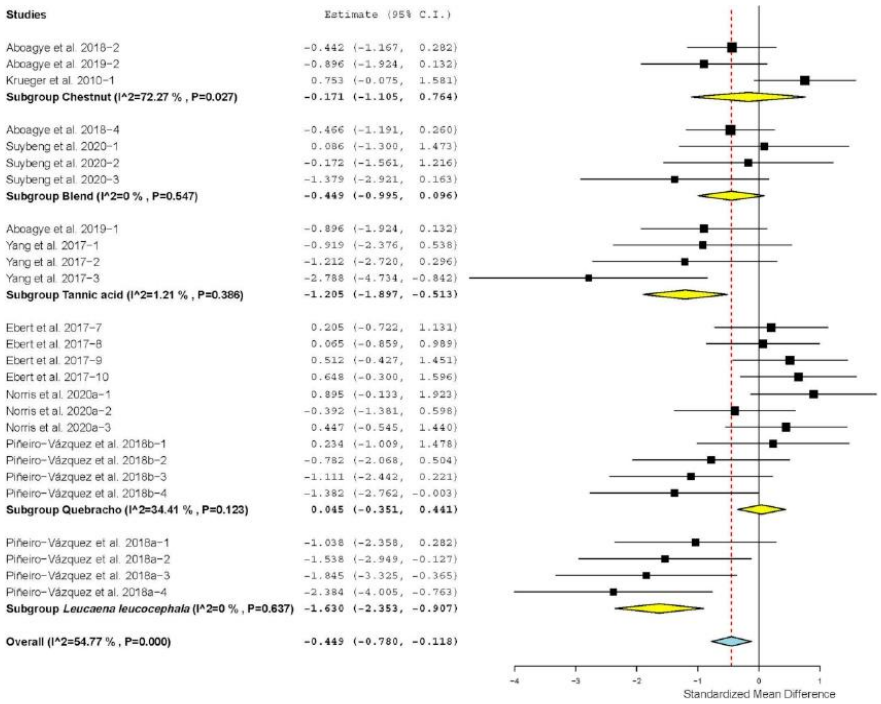

Figure 7. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical or chemical origin of tannin on enteric methane emissions per unit dry matter intake (MEDMI) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in MEDMI, while points to the right of the line indicate increase in MEDMI.

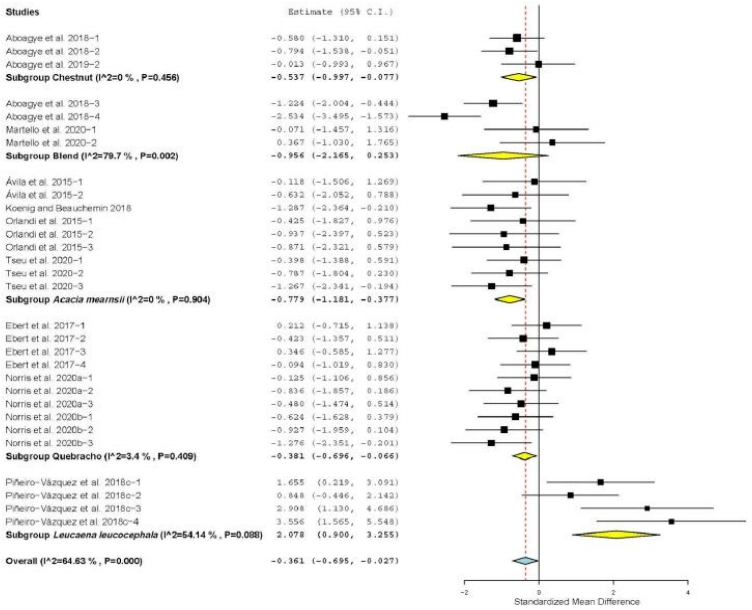

Figure 8. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical or chemical origin of tannin on urine nitrogen excretion (UNE) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in UNE, while points to the right of the line indicate increase in UNE.

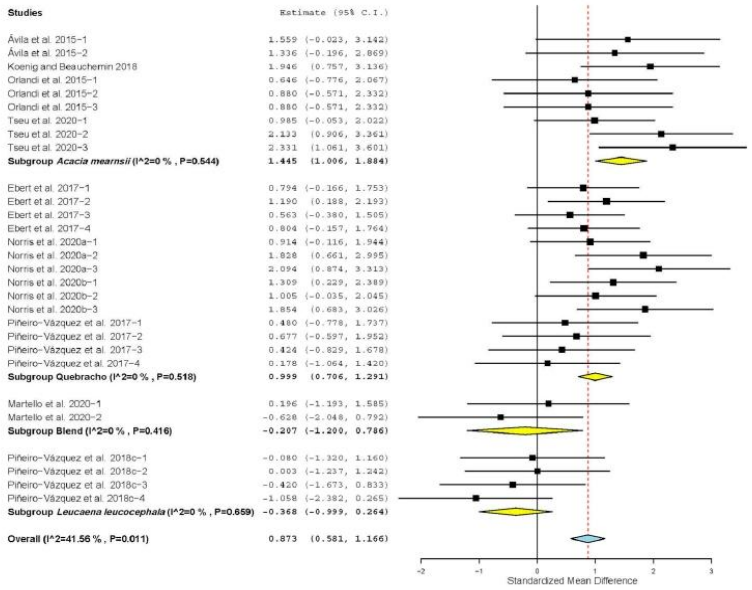

Figure 9. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical or chemical origin of tannin on fecal nitrogen excretion (FNE) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in FNE, while points to the right of the line indicate increase in FNE.

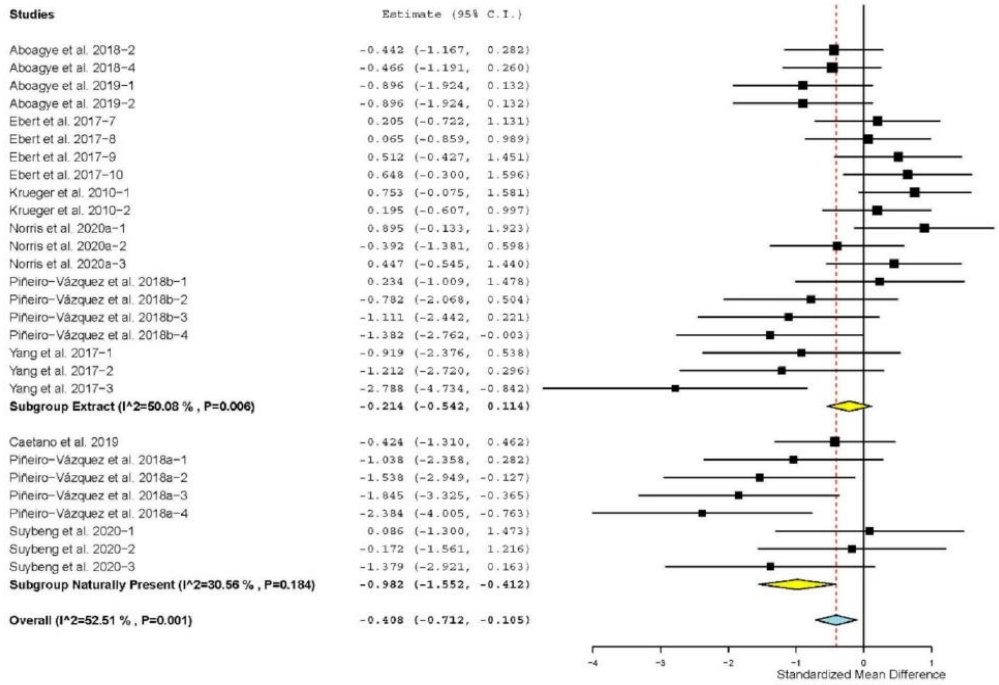

Figure 10. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the tannin inclusion method on enteric methane emissions per unit dry matter intake (MEDMI) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in MEDMI, while points to the right of the line indicate increase in MEDMI.

Regardless of TANs' supplementation, animals from studies that used rotational experimental designs (i.e., Latin squares and crossover designs) had lower DMD (SMD = −0.765; $p < 0.001$), while no differences were observed regarding DMD in animals from studies that used continuous experimental designs (i.e., completely randomized and randomized blocks designs; $p > 0.05$). OMD decreased in animals from studies that used rotating experimental designs (SMD = −0.856; $p < 0.001$), while no difference was observed in DMD in animals from studies that used continuous experimental designs ($p > 0.05$). Studies that used rotating experimental designs had lower NDFD (SMD = −0.704; $p < 0.001$); however, NDFD was not affected in animals from studies that used continuous experimental designs ($p > 0.05$). Ruminal pH was not affected by the type of experimental design used ($p > 0.05$). MEDMI decreased in animals from studies that used rotating experimental designs (SMD = −0.836; $p < 0.001$), while no differences were observed with respect to MEDMI in animals from studies that used continuous experimental designs ($p > 0.05$).

Regarding the level of TANs' supplementation, animals in studies using doses greater than 12 g/kg DM showed lower DMD (SMD = −0.917; $p < 0.001$), while no differences were observed in DMD in animals in studies using doses lower than 12 g/kg DM ($p > 0.05$). OMD was lower in animals supplemented with doses of TANs higher than 12 g/kg DM (SMD = −0.976; $p < 0.001$), but doses lower than 12 g did not change OMD ($p > 0.05$). Studies using TANs' doses higher than 12 g/kg DM had lower NDFD (SMD = −0.775; $p < 0.001$); however, NDFD was not affected when TANs' doses lower than 12 g/kg DM were used ($p > 0.05$). The concentration of acetate in the ruminal fluid increased in animals from studies that used TANs' doses lower than 12 g/kg DM (SMD = 0.387; $p = 0.038$), while there was no effect when more than 12 g TANs were used ($p > 0.05$). Animals in studies that

used TANs' doses higher than 12 g/kg DM showed higher rumen propionate concentration (SMD = 0.319; $p = 0.010$), whereas TANs' doses lower than 12 g/kg DM did not change rumen propionate concentration ($p > 0.05$). FNE increased significantly regardless of the dose of TANs used; however, the effect was greater (SMD = 1.119; $p < 0.001$) when doses greater than 12 g/kg DM were used compared to doses of less than 12 g/kg DM (SMD = 0.482; $p < 0.01$).

Regarding the period of supplementation with TANs, it was observed that the MEDMI decreased in the animals of studies that used experimental periods ranging from 90 to 180 days (SMD = −0.793; $p = 0.002$). However, when the supplementation period was shorter (less than 90 days), MEDMI was not affected ($p > 0.05$).

Regarding the age, animals younger than 12 months old showed lower DMI (SMD = −1.249; $p < 0.05$), while no differences were observed in DMI for animals older than 12 months (SMD = 0.104; $p > 0.05$). CPD was lower in animals younger than 12 months old (SMD = −1.090; $p < 0.001$), but animals older than 12 months old did not change (SMD = 0.201; $p > 0.05$). Ruminal pH was lower in animals older than 12 months old (SMD = −0.767; $p < 0.05$), while no differences were observed in ruminal pH for animals younger than 12 months old (SMD = 0.154; $p > 0.05$). Ruminal concentration of NH_3-N decreased in animals younger than 12 months old (SMD = −0.745; $p < 0.05$), while there was no effect in animals older than 12 months old (SMD = −0.030; $p > 0.05$). The ruminal concentration of total VFA increased in animals older than 12 months old (SMD = 0.753; $p = 0.01$) but decreased in animals younger than 12 months old (SMD = −1.245; $p < 0.001$).

4. Discussion

The environmental sustainability of beef production is a significant concern within the food production system [49]. Current literature suggests that TANs can be supplemented to improve the sustainability of both dairy and beef cattle by reducing CH_4 emissions and enhancing animal performance [1,25]. In ruminants, some studies suggest that dietary supplementation with TANs increases duodenal amino acid flux [11], reduces enteric CH_4 production [7,10], and improves the rumen microbial activity [50]. Consequently, it was expected that beef cattle supplemented with TANs in the diet would have higher growth rate. However, the present meta-analysis showed that ADG and FBW were not affected by dietary supplementation with TANs. A positive relationship exists between improved productivity and both environmental and economic sustainability [49]. This suggests that TANs do not affect growth rate or environmental or economic sustainability in beef cattle. Nevertheless, these results should be interpreted carefully considering that both variables were subject to publication bias. Similar to our results, a meta-analysis conducted by Méndez-Ortiz et al. [51] showed that CTs' intake did not affect significantly the weight gain of growing lambs.

There is considerable interest in improved feed efficiency as a means of augmenting the economic and environmental sustainability of beef production systems [52]. It has been reported that dietary inclusion of TANs reduces ruminal protein degradation, resulting in higher efficiency of nitrogen utilization [5,25]. On the other hand, enteric CH_4 emissions represent losses of 2–12% of energy intake in ruminants [53]. In the present meta-analysis, the values observed for ruminal NH_3-N concentration and CH_4 emissions indicated a reduction in ruminal protein degradation and enteric CH_4 emissions. This could be associated with higher efficiency of protein utilization and energy consumed. However, these effects did not modify the feed efficiency. This suggests that TANs do not affect either environmental or economic sustainability in beef cattle.

Some review articles have hypothesized that the presence of TANs in the diet may negatively affect feed intake in ruminants due to their astringent nature [5,54]. However, in the present meta-analysis, no changes in DM or nutrient intake were observed in response to dietary supplementation with TANs. Such absence probably occurred because the average dose of TANs used was 14.6 g/kg DM and the negative effects of TANs on the intake seem to occur with doses higher than 50 g/kg DM [6]. Similar to our results, two

previously conducted meta-analyses reported that dietary supplementation with TANs at average concentrations of 46.3 and 9.5 g/kg DM did not affect significantly the feed intake of growing lambs and dairy cows in production, respectively [31,51]. These results together suggest that TANs can be used in beef cattle and other ruminants during their different productive stages without negative effects on feed intake.

With respect to total tract digestibility, dietary supplementation with TANs reduced the digestibility of DM and the dietary nutrients. Similar to our results, a meta-analysis conducted by Herremans et al. [31] reported that dietary supplementation with TANs at average doses of 9.5 g/kg DM reduced the digestibility of DM and dietary nutrients in dairy cows. However, in their study they observed that it does not affect the milk production and its composition. The rumen microbial activity and the endogenous digestive enzyme activity can be affected when large amounts of TANs are present in the diet [5], resulting in lower nutrient digestibility [6]. Additionally, the reduction and/or elimination of rumen protozoa leads to lower NDFD and ADFD [55]. In the present meta-analysis, the rumen protozoa were not significantly affected by dietary supplementation with TANs, although the population was reduced by 3.7 % ($p = 0.06$). This would partially explain the lower NDFD and ADFD observed in TANs-supplemented animals. In addition, it has been reported that TANs can have negative effects on fibrolytic bacteria in the rumen [56], which would also partly explain the lower NDFD and ADFD observed. On the other hand, the lower CPD observed in the response of dietary supplementation with TANs could be explained due to an excessive ruminal protection of TANs on the protein in the diets [5].

The type of TANs used only explained about 7% of the observed heterogeneity in nutrient digestibility, while the TANs' dose explained between 25 and 69%. An analysis of subgroups revealed that DMD, OMD, and NDFD were affected only when the used dose exceeded 12 g/kg DM, but doses lower than 12 g/kg DM had no significant impact. These results confirm the hypothesis of Aboagye and Beauchemin [25], who suggested that the impact of TANs in ruminants depends on the dose of TANs in the diet rather than the type of TANs used.

Regarding the TANs' source, it explained between 13 and 48% of the heterogeneity observed for CPD and NDFD. Although most of the TANs' sources used by the studies included in our investigation reduced CPD, CPD improved when *Leucaena leucocephala* was used as TANs' source. This result, together with the higher ruminal concentration of NH_3-N observed in the studies using *L. leucephala*, suggests that TANs from this plant have low capacity for binding to rumen proteins, similar to what has been previously observed in CTs from other plants [57]. It is suggested that TANs with higher molecular weight have a greater capacity to bind to other molecules [21]. Although it has previously been reported that *L. leucocephala* contains CTs with higher molecular weight [58], it is suggested that the molecular weight of CTs is not the only factor influencing the binding capacity of TANs to the proteins.

Ruminants are inefficient animals for converting the ingested protein into animal product because a large part of this protein is lost as NH_3-N in the rumen [54]. In the present meta-analysis, dietary supplementation with TANs reduced rumen NH_3-N concentration, indicating a lower protein degradability in the rumen due to the presence of TANs. However, TANs did not influence ADG, FBW, or FE, probably because CPD also decreased in response to dietary supplementation with TANs. Consequently, the beef cattle seem not to better use the protein ingested even in the presence of TANs in the diet. Similar to our results, a meta-analysis by Herremans et al. [31] reported that dietary supplementation with TANs reduced NH_3-N ruminal concentration in dairy cows. However, it did not improve the nitrogen utilization efficiency. Furthermore, a meta-analysis of 15 in vivo and 15 in vitro studies showed that NH_3-N concentration decreased when increasing TANs' levels in ruminant diets [59]. The free TANs can bind to the soluble protein in the diet and consequently reduce the NH_3-N ruminal concentration [25]. This is to be expected and would partially explain the results observed in this and other studies. However, NH_3-N ruminal concentration also appears to decrease when ruminal protozoa are reduced or elim-

inated [60]. Consequently, in our investigation, the lower NH_3-N ruminal concentration could be associated with the 3.7% reduction observed in the rumen protozoan population.

Supplementation with TANs did not alter the ruminal concentration of total VFA. However, it did improve the concentrations of propionate and butyrate, but this last response variable was subject to publication bias, making it difficult to interpret. The absence of significant changes in ruminal concentration of total VFA can be considered desirable when it is accompanied by a reduction in enteric CH_4 emissions [61], as observed in our meta-analysis. Similar to our results, Dai and Faciola [62] reported that dietary supplementation with TANs improved ruminal concentration of propionate and butyrate in large and small ruminants and also reduced CH_4 production. Because there is a negative correlation between propionate and CH_4 production due to the competition for hydrogen [63], the increase in ruminal concentration of propionate observed in our investigation could be associated with the reduction in enteric CH_4 emissions observed in response to TANs' supplementation.

The type, dose, and source of TANs explained between 6 and 34% of the sources of heterogeneity observed in the ruminal concentration of acetate, propionate, and total VFA. This confirms the hypothesis that the effects of TANs on ruminal fermentation may vary according to the source, dose, and type of TANs supplied in the diets [25]. The subgroup analysis revealed that the ruminal concentration of total VFA increased significantly when CTs were used. However, the ruminal concentration of total VFA only improved significantly when the CTs came from the quebracho tree. This could be related to differences in the molecular weight of the CTs contained in the different sources, since in vitro studies have shown that CTs with different molecular weight can act differently on rumen microbial populations [64,65].

Previous studies have reported that TANs from *Leucaena leucocephala* can reduce the rumen protozoan population [64,66]. However, the mechanisms of action through which these and other TANs act on rumen protozoa are still unknown [67]. Although, in our meta-analysis, the rumen protozoa decreased 3.7% in response to dietary supplementation with TANs, this effect was insignificant, perhaps because only 7.6% of the included studies used *L. leucocephala* as a source of TANs. Similar to our results, a meta-analysis conducted by Jayanegara et al. [59] reported that inclusion of TANs in ruminant diets did not affect counts of protozoa in rumen fluid under in vivo and in vitro conditions. Similarly, Dai and Faciola [62] also did not observe significant effects of dietary supplementation with TANs on the rumen protozoan population in small and large ruminants.

Enteric CH_4 production represents approximately 43% of the greenhouse gases emitted in beef production worldwide [68]. To ensure sustainable livestock production, it is necessary to reduce enteric CH_4 emissions [25]. It has been suggested that TANs can be used to minimize the environmental impact of ruminant production because they can improve ruminal fermentation and mitigate CH_4 emissions [69]. Some studies have reported that TANs decrease rumen methanogenesis directly by reducing methanogenic bacteria populations [63,66,70]. However, often the effects of TANs on CH_4 reduction are more indirect than direct [71]. For example, Fagundes et al. [23] reported that enteric CH_4 emissions from beef cattle decreased in response to dietary supplementation with CTs. However, they attributed the CH_4 reduction to a decrease in feed intake rather than to direct effects of CTs on rumen methanogenic archaea. In addition, some review articles have suggested that enteric CH_4 production may vary depending on the type, dose, and source of TANs employed in the diet [14,25]. However, in our meta-analysis MEDMI was only affected by the source of TANs. This suggests that TANs could improve the environmental sustainability of beef production regardless of the type and dose of TANs used, similar to what has been previously observed in small ruminant production [15].

The period of supplementation with TANs could also contribute to the variability of its effects on methanogenesis [50]. One of the most important problems with the use of phytochemicals in ruminants is the adaptation of ruminal microorganisms to their effects after long periods of supplementation [72]. For example, some essential oils seem to be

more effective in reducing CH_4 production when used for short periods. However, they lose effectiveness over time [40]. In the present meta-analysis, the period of supplementation showed inconsistent effects on MEDMI. In short-term studies (less than 90 days), the reduction in MEDMI was small (SMD = −0.141) but increased (SMD = −0.791) in animals used in long-term studies (91 to 180 days). These results suggest that in beef cattle, ruminal microorganisms related to CH_4 production are not able to adapt to the effects of TANs, even during long periods of supplementation. Similar results were previously reported by Salami et al. [56] in lambs supplemented with different sources of CTs (*Castanea sativa* and *Caesalpinia spinosa*) and HTs (*Acacia negra* and *Uncaria gambir*) during long periods. In their investigation, they observed that all TANs' sources had specific antimicrobial activity against methanogenic bacteria and ruminal protozoa during the whole experimental phase.

Since the content and composition of TANs in plants are highly variable and can be affected by various factors, it has been suggested to use extracts to supply TANs to ruminant diets [25]. About 77% of the studies included in the present meta-analysis used TANs extracts. Nevertheless, the reduction of MEDMI was greater and less heterogeneous when animals were supplied with TANs-rich plants than when extracts were used.

Although most of the studies (34%) included in the present meta-analysis used TANs from quebracho tree, the subgroup analysis revealed that the MEDMI decreased significantly only in response to the use of *Leucaena leucocephala* and tannic acid as TANs' sources. According to Huang et al. [58], *L. leucocephala* contains high-molecular-weight CTs, which varies between 2737 and 2872 Da. On the other hand, tannic acid, although it is a typical HT [5,73], has a molecular weight of 1701 Da [74], which is higher than the weight of 939 Da reported for quebracho tree CTs [75]. TANs with a high molecular weight act better than those with a low molecular weight in suppressing ruminal protozoa populations [64], which are correlated with CH_4 emissions by the equation: methane (g/kg dry matter intake) = $-30.7 + 8.14 \times$ protozoa (log10 cells/mL) [76]. Consequently, the use of *L. leucocephala*, tannic acid, and other high-molecular-weight TANs' sources could have a greater impact on reducing enteric CH_4 emissions compared to other widely studied TANs (e.g., quebracho).

According to Nichols et al. [77], beef cattle production plays an important role in the N cycle as beef cattle excrete up to 80% of the consumed dietary N through urine and feces, and urinary N accounts for approximately 60–80% of the total N excretion [78]. In the present meta-analysis, no changes in NUE were observed in response to dietary supplementation with TANs. However, the observed values for UNE and FNE indicated a reduction in UNE and an increase in FNE, respectively. Similar to our results, a meta-analysis conducted by Herremans et al. [31] reported that dietary supplementation with TANs reduced UNE (−11%) and increased FNE (+10%) without affecting NUE in dairy cows. According to Singh et al. [79], the excreted N might be lost through nitrate (NO_3^-) leaching, emissions of N_2O, and emissions from ammonia volatilization. Compared with feces, urine could rapidly supply available mineral N for nitrification and denitrification through hydrolysis of urea, leading to higher N_2O emissions [80], which has a global warming potential over a 100-year period of 298 times greater than that of carbon dioxide [2]. Therefore, strategies based on changing the composition and concentration of urinary compounds by diet manipulation could be considered potential options to mitigate N_2O emissions from urine [4]. Consequently, the shift from urinary to fecal N observed in this study may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N.

5. Conclusions

One of the most significant findings to emerge from this study is that the environmental impact of beef production systems can be markedly reduced when tannins are included in the diet. The results of the present meta-analysis indicate that TANs reduce enteric CH_4 emissions in beef cattle, particularly when they are supplied naturally as ingredients in the diet, when they are supplemented for long periods, or when *Leucaena leucocephala* and

tannic acid are used as sources of these secondary metabolites. In addition, the shift from urinary to fecal N observed in this study may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N. Therefore, the addition of tannins in the diet of beef cattle could be used as a sustainable natural alternative to reduce the environmental impact of beef production without affecting the economic sustainability. However, several issues need to be addressed before specific recommendations for commercial use of TANs to reduce environmental impact.

Our meta-analysis demonstrates that TANs' supplementation does not affect weight gain, feed intake, or feed efficiency in beef cattle, but reduces diet digestibility at doses above 12 g/kg DM. In addition, TANs' supplementation improves ruminal fermentation characteristics by reducing ruminal NH_3-N concentration and increasing rumen propionate and butyrate concentration. The best result in ruminal propionate and NH_3-N concentration is achieved using TANs from pistachio and HTs, respectively.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su13137410/s1. Figure S1: Flow chart of paper selection process. Figure S2: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on average daily gain (ADG); (b) funnel plot of the effect of dietary supplementation with TANs on ADG obtained using the trim-and-fill method of Duval and Tweedie. Figure S3: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on final body weight (FBW); (b) funnel plot of dietary supplementation with TANs on FBW obtained using the trim-and-fill method of Duval and Tweedie. Figure S4: (a) Funnel plot of the effect of dietary tannins' supplementation (TANs) on organic matter intake (OMI); (b) funnel plot of the effect of dietary supplementation with TANs on OMI obtained using the trim-and-fill method of Duval and Tweedie. Figure S5: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on neutral detergent fiber intake (NDFI); (b) funnel plot of the effect of dietary supplementation with TANs on NDFI obtained using the trim-and-fill method of Duval and Tweedie. Figure S6: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on rumen butyrate concentration; (b) funnel plot of the effect of dietary supplementation with TANs on the ruminal concentration of butyrate obtained using the trim-and-fill method of Duval and Tweedie. Figure S7: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins type on neutral detergent fiber digestibility (NDFD) in beef cattle. Figure S8: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins type on ruminal ammonia nitrogen (NH_3-N) concentration in beef cattle. Figure S9: Forest plot of effect size or standardized mean difference and 95% confidence interval of the chemical or botanical source of tannins on ruminal ammonia nitrogen (NH_3-N) concentration in beef cattle. Figure S10: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins' inclusion method on crude protein digestibility (CPD) in beef cattle.

Author Contributions: Conceptualization, J.F.O.-O., G.D.-I. and A.L.-B.; methodology and data curation, J.F.O.-O., G.D.-I. and G.D.M.-M.; investigation and formal analysis, J.F.O.-O., L.A.M.-R. and P.A.H.-G.; writing—original draft preparation, J.F.O.-O.; writing—review and editing, J.F.O.-O., G.D.-I., A.L.-B., G.D.M.-M., L.A.M.-R. and P.A.H.-G.; supervision and project administration, A.L.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The first author, José Felipe Orzuna, is a Master of Science student of the Program of Animal Production of the Universidad Autonoma Chapingo and thanks the CONACyT Program for the scholarship.

Conflicts of Interest: All other authors declare no conflicts of interest.

Appendix A

Table A1. Summary of the studies included in the meta-analysis.

Author	Country	Tannin Source	Tannin Type	Method of Inclusion
Aboagye et al. [17]	Canada	CH, CH, BL, BL	HT, HT, BL, BL	E, E, E, E
Aboagye et al. [21]	Canada	TA, CH	HT, HT	E, E
Ávila et al. [81]	Brazil	AM, AM	CT, CT	E, E
Avila et al. [20]	Brazil	AM, AM, AM	CT, CT, CT	E, E, E
Caetano et al. [82]	Australia	Grape	CT	NAT
Dickhoefer et al. [83]	Germany	QU, QU, QU, QU	BL, BL, BL, BL	E, E, E, E
Ebert et al. [84]	US	QU (n = 10)	CT (n = 10)	E (n = 10)
Jolazadeh et al. [85]	Iran	PIST, PIST, PIST	BL, BL, BL	E, E, E
Koenig and Beauchemin [86]	Canada	AM	CT	E
Koenig et al. [87]	Canada	AM, AM, AM, AM	CT, CT, CT, CT	E, E, E, E
Krueger et al. [88]	US	CH, AM	HT, CT	E, E
Martello et al. [89]	Brazil	BL, BL	BL, BL	E, E
Mezzomo et al. [90]	Brazil	QU, QU	CT, CT	E, E
Mezzomo et al. [91]	Brazil	BL, BL, BL	BL, BL, BL	E, E, E
Norris et al. [22]	US	QU, QU, QU	CT, CT, CT	E, E, E
Norris et al. [92]	US	QU, QU, QU	CT, CT, CT	E, E, E
Orlandi et al. [11]	Brazil	AM, AM, AM	BL, BL, BL	E, E, E
Piñeiro-Vázquez et al. [19]	Mexico	QU, QU, QU, QU	CT, CT, CT, CT	E, E, E, E
Piñeiro-Vázquez et al. [93]	Mexico	LEU, LEU, LEU, LEU	CT, CT, CT, CT	N, N, N, N
Piñeiro-Vázquez et al. [94]	Mexico	QU, QU, QU, QU	CT, CT, CT, CT	E, E, E, E
Piñeiro-Vázquez et al. [95]	Mexico	LEU, LEU, LEU, LEU	CT, CT, CT, CT	N, N, N, N
Poblete et al. [96]	Philippines	AM, AM	BL, BL	E, E
Rivera-Méndez et al. [97]	Mexico	QU, QU	CT, CT	E, E
Rivera-Méndez et al. [18]	Mexico	QU (n = 4), CH, BL	CT (n = 4), HT, BL	E, E, E, E, E, E
Shakeri et al. [98]	Iran	PIST, PIST, PIST	BL, BL, BL	N, N, N
Shakeri et al. [99]	Iran	PIST (n = 9)	BL (n = 9)	N (n = 9)
Suybeng et al. [24]	Australia	BL, BL, BL	CT, CT, CT	N, N, N
Tabke et al. [100]	US	TA, TA	HT, HT	E, E
Tseu et al. [101]	Brazil	AM, AM, AM	BL, BL, BL	E, E, E
Yang et al. [73]	China	TA, TA, TA	HT, HT, HT	E, E, E
Yuste et al. [102]	Spain	BL	BL	E
Zhou et al. [103]	China	TA, TA	HT, HT	E, E

CH: chestnut (*Castanea sativa*); BL: blend; TA: tannic acid; AM: *Acacia mearnsii*; QU: quebracho (*Schinopsis spp.*); PIST: pistachio (*Pistacia vera*); LEU: *Leucaena leucocephala*; n: number of comparisons; HT: hydrolysable tannin; CT: condensed tannin; E: extract; N: naturally present.

References

1. Min, B.R.; Solaiman, S.; Waldrip, H.M.; Parker, D.; Todd, R.W.; Brauer, D. Dietary mitigation of enteric methane emissions from ruminants: A review of plant tannin mitigation options. *Anim. Nutr.* **2020**. [CrossRef] [PubMed]
2. Nunes, S.M.; Bayer, C.; Lassalas, M.; Michelon, G.M.; Schaitz, L.H.; Biasolo, R.; Civiero, M.; Mendonça, H.; Ribeiro-Filho, N. Effects of ground corn and *Acacia mearnsii* tannin extract supplementation on nitrogen excretion and nitrous oxide emissions from sheep. *Livest. Sci.* **2021**, *246*, 104458. [CrossRef]
3. Batista, E.D.; Detmann, E.; Valadares, F.S.C.; Titgemeyer, E.C.; Valadares, R.F.D. The effect of CP concentration in the diet on urea kinetics and microbial usage of recycled urea in cattle: A meta-analysis. *Animal* **2014**, *11*, 1303–1311. [CrossRef] [PubMed]
4. Gardiner, C.A.; Clough, T.J.; Cameron, K.C.; Di, H.J.; Edwards, G.R.; de Klein, C.A.M. Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N2O emissions: A review. *N. Z. J. Agric. Res.* **2016**, *59*, 301–317. [CrossRef]
5. Huang, Q.; Liu, X.; Zhao, G.; Hu, T.; Wang, Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Anim. Nutr.* **2018**, *4*, 137–150. [CrossRef]
6. Naumann, H.D.; Tedeschi, L.O.; Zeller, W.E.; Huntley, N.F. The role of condensed tannins in ruminant animal production: Advances, limitations and future directions. *Rev. Bras. Zootec.* **2017**, *46*, 929–949. [CrossRef]
7. Adejoro, F.A.; Hassen, A.; Akanmu, A.M. Effect of Lipid-Encapsulated Acacia Tannin Extract on Feed Intake, Nutrient Digestibility and Methane Emission in Sheep. *Animals* **2019**, *9*, 863. [CrossRef]
8. Seoni, E.; Rothacher, M.; Arrigo, Y.; Ampuero Kragten, S.; Bee, G.; Dohme-Meier, F. The Fate of Tannins from Birdsfoot Trefoil and Their Effect on the Nitrogen Balance in Growing Lambs Fed Diets Varying in Protein Level. *Animals* **2021**, *11*, 190. [CrossRef]

9. Zhang, J.; Xu, X.; Cao, Z.; Wang, Y.; Yang, H.; Azarfar, A.; Li, S. Effect of Different Tannin Sources on Nutrient Intake, Digestibility, Performance, Nitrogen Utilization, and Blood Parameters in Dairy Cows. *Animals* **2019**, *9*, 507. [CrossRef]
10. Fagundes, G.M.; Benetel, G.; Santos, K.C.; Welter, K.C.; Melo, F.A.; Muir, J.P.; Bueno, I.C.S. Tannin-Rich Plants as Natural Manipulators of Rumen Fermentation in the Livestock Industry. *Molecules* **2020**, *25*, 2943. [CrossRef]
11. Orlandi, T.; Kozloski, G.V.; Alves, T.P.; Mesquita, F.R.; Ávila, S.C. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract. *Anim. Feed Sci. Technol.* **2015**, *210*, 37–45. [CrossRef]
12. Sarnataro, C.; Spanghero, M. In vitro rumen fermentation of feed substrates added with chestnut tannins or an extract from Stevia rebaudiana Bertoni. *Anim. Nutr.* **2020**, *6*, 54–60. [CrossRef]
13. Santos, J.D.C.d.; Saraiva, E.P.; Gonzaga Neto, S.; Saraiva, C.A.S.; Pinheiro, A.d.C.; Fonsêca, V.d.F.C.; Santos, S.G.C.G.d.; Souza, C.G.d.; Almeida, M.E.V.; Veríssimo, T.N.S.; et al. Feeding Behavior of Lactating Dairy Cattle Fed Sorghum-Based Diets and Increasing Levels of Tannic Acid. *Agriculture* **2021**, *11*, 172. [CrossRef]
14. Verma, S.; Taube, F.; Malisch, C.S. Examining the Variables Leading to Apparent Incongruity between Antimethanogenic Potential of Tannins and Their Observed Effects in Ruminants—A Review. *Sustainability* **2021**, *13*, 2743. [CrossRef]
15. Waghorn, G. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. *Anim. Feed Sci. Technol.* **2008**, *147*, 116–139. [CrossRef]
16. Adejoro, F.A.; Hassen, A.; Akanmu, A.M.; Morgavi, D.P. Replacing urea with nitrate as a non-protein nitrogen source increases lambs' growth and reduces methane production, whereas acacia tannin has no effect. *Anim. Feed Sci. Technol.* **2020**, *259*, 114360. [CrossRef]
17. Aboagye, I.A.; Oba, M.; Castillo, A.R.; Koenig, K.M.; Iwaasa, A.D.; Beauchemin, K.A. Effects of hydrolysable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high forage diet. *J. Anim. Sci.* **2018**, *96*, 5276–5286. [CrossRef]
18. Rivera-Mendez, C.; Plascencia, A.; Torrentera, N.; Zinn, R.A. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. *J. Appl. Anim. Res.* **2017**, *45*, 199–203. [CrossRef]
19. Piñeiro-Vázquez, A.T.; Canul-Solis, J.R.; Alayón-Gamboa, J.A.; Chay-Canul, A.J.; Ayala-Burgos, A.J.; Solorio-Sánchez, F.J.; Aguilar-Pérez, C.F.; Ku-Vera, J.C. Energy utilization, nitrogen balance and microbial protein supply in cattle fed Pennisetum purpureum and condensed tannins. *J. Anim. Physiol. Anim. Nutr.* **2017**, *101*, 159–169. [CrossRef]
20. Avila, A.S.; Zambom, M.A.; Faccenda, A.; Fischer, M.L.; Anschau, F.A.; Venturini, T.; Tinini, R.C.R.; Dessbesell, J.G.; Faciola, A.P. Effects of black wattle (*Acacia mearnsii*) condensed tannins on intake, protozoa population, ruminal fermentation, and nutrient digestibility in Jersey steers. *Animals* **2020**, *10*, 1011. [CrossRef]
21. Aboagye, I.A.; Oba, M.; Koenig, K.M.; Zhao, G.Y.; Beauchemin, K.A. Use of gallic acid and hydrolysable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. *J. Anim. Sci.* **2019**, *97*, 2230–2244. [CrossRef] [PubMed]
22. Norris, A.B.; Tedeschi, L.O.; Foster, J.L.; Muir, J.P.; Pinchak, W.E.; Fonseca, M.A. AFST: Influence of quebracho tannin extract fed at differing rates within a high-roughage diet on the apparent digestibility of dry matter and fiber, nitrogen balance and fecal gas flux. *Anim. Feed Sci. Technol.* **2020**, *260*, 114365. [CrossRef]
23. Fagundes, G.M.; Benetel, G.; Welter, K.C.; Melo, F.A.; Muir, J.P.; Carriero, M.M.; Souza, R.L.M.; Meo-Filho, P.; Frighetto, R.T.S.; Berndt, A.; et al. Tannin as a natural rumen modifier to control methanogenesis in beef cattle in tropical systems: Friend or foe to biogas energy production? *Res. Vet. Sci.* **2020**, *132*, 82–93. [CrossRef] [PubMed]
24. Suybeng, B.; Charmley, E.; Gardiner, C.P.; Malau-Aduli, B.S.; Malau-Aduli, A.E.O. Supplementing northern Australian beef cattle with *Desmanthus* tropical legume reduces *in-vivo* methane emissions. *Animals* **2020**, *10*, 2097. [CrossRef]
25. Aboagye, I.A.; Beauchemin, K.A. Potential of Molecular Weight and Structure of Tannins to Reduce Methane Emissions from Ruminants: A review. *Animals* **2019**, *9*, 856. [CrossRef]
26. Zangeneh, M.; Banaeian, N.; Clark, S. Meta-Analysis on Energy-Use Patterns of Cropping Systems in Iran. *Sustainability* **2021**, *13*, 3868. [CrossRef]
27. Borenstein, M.; Hedges, L.V.; Higgins, J.P.T.; Rothstein, H.R. *Introduction to Meta-Analysis*, 1st ed.; John Wiley and Sons, Ltd.: Chichester, UK, 2009; p. 413.
28. Doré, T.; Makowski, D.; Malézieux, E.; Munier-Jolain, N.; Tchamitchian, M.; Tittonell, P. Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. *Eur. J. Agron.* **2011**, *34*, 197–210. [CrossRef]
29. Higgins, J.P.T.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analysis. *BMJ* **2003**, *327*, 557–560. [CrossRef]
30. Abhijith, A.; Dunshea, F.R.; Warner, R.D.; Leury, B.J.; Ha, M.; Chauhan, S.S. A Meta-Analysis of the Effectiveness of High, Medium, and Low Voltage Electrical Stimulation on the Meat Quality of Small Ruminants. *Foods* **2020**, *9*, 1587. [CrossRef]
31. Herremans, S.; Vanwindekens, F.; Decruyenaere, V.; Beckers, Y.; Froidmont, E. Effect of dietary tannins on milk yield and composition, nitrogen partitioning and nitrogen use efficiency of lactating dairy cows: A meta-analysis. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 1209–1218. [CrossRef]
32. Lean, I.J.; Thompson, J.M.; Dunshea, F.R. A Meta-Analysis of Zilpaterol and Ractopamine Effects on Feedlot Performance, Carcass Traits and Shear Strength of Meat in Cattle. *PLoS ONE* **2014**, *9*, e115904. [CrossRef]

33. Higgins, J.; Thomas, J. *Cochrane Handbook for Systematic Reviews of Interventions*, 2nd ed.; John Wiley and Sons, Ltd.: Chichester, UK, 2019; pp. 143–176.
34. Wallace, B.C.; Lajeunesse, M.J.; Dietz, G.; Dahabreh, I.J.; Trikalinos, T.A.; Schmid, C.H.; Gurevitch, J. OpenMEE: Intuitive, open-source software for metaanalysis in ecology and evolutionary biology. *Methods Ecol. Evol.* **2016**, *8*, 941–947. [CrossRef]
35. Viechtbauer, W. Conducting meta-analysis in R with the metaphor package. *J. Stat. Softw.* **2010**, *36*, 1–48. [CrossRef]
36. Hedges, L.V. Distribution theory for Glass´s estimator of effect size and related estimators. *J. Educ. Stat.* **1981**, *6*, 107–128. [CrossRef]
37. DerSimonian, R.; Laird, N. Meta-analysis in clinical trials. *Control. Clin. Trials* **1986**, *7*, 177–188. [CrossRef]
38. Lean, I.J.; Rabiee, A.R.; Duffield, T.F.; Dohoo, I.R. Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. *J. Dairy Sci.* **2009**, *92*, 3545–3565. [CrossRef]
39. SAS (Statistical Analysis System). *SAS/STAT User's Guide (Release 6.4)*; SAS Institute: Cary, NC, USA, 2017.
40. Torres, R.N.S.; Moura, D.C.; Ghedini, C.P.; Ezequiel, J.M.B.; Almeida, M.T.C. Meta-analysis of the effects of essential oils on ruminal fermentation and performance of sheep. *Small Rumin. Res.* **2020**, *189*. [CrossRef]
41. Higgins, J.P.T.; Thompson, S.G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **2002**, *21*, 1539–1558. [CrossRef]
42. Egger, M.; Smith, G.D.; Altman, D.G. *Systematic Reviews in Health Care*, 2nd ed.; MBJ Publishing Group: London, UK, 2001; pp. 109–121.
43. Littell, J.H.; Corcoran, J.; Pillai, V. *Systematic Reviews and Meta-Analysis*, 1st ed.; Oxford University Press: Oxford, UK, 2008; pp. 111–132.
44. Sterne, J.A.C.; Harbord, R.M. Funnel plots in meta-analysis. *Stata J.* **2004**, *4*, 127–141. [CrossRef]
45. Egger, M.; Smith, G.D.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **1997**, *315*, 629–634. [CrossRef]
46. Begg, C.B.; Mazumdar, M. Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics* **1994**, *50*, 1088–1101. [CrossRef]
47. Ioannidis, J.P.A.; Trikalinos, T.A. The appropriateness of asymmetry tests for publication bias in meta-analyses: A large survey. *CMAJ* **2007**, *176*, 1091–1096. [CrossRef] [PubMed]
48. Duval, S.; Tweedie, R. A nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. *J. Amer. Statist. Assoc.* **2000**, *95*, 89–98. [CrossRef]
49. Capper, J.L.; Hayes, D.J. The environmental and economic impact of removing growth-enhancing technologies from U.S. beef production. *J. Anim. Sci.* **2012**, *90*, 3527–3537. [CrossRef]
50. Hassan, F.; Arshad, M.A.; Ebeid, H.M.; Rehman, M.S.; Khan, M.S.; Shahid, S.; Yang, C. Phytogenic Additives Can Modulate Rumen Microbiome to Mediate Fermentation Kinetics and Methanogenesis Through Exploiting Diet–Microbe Interaction. *Front. Vet. Sci.* **2020**, *7*, 575801. [CrossRef]
51. Mendez-Ortiz, F.A.; Sandoval-Castro, C.A.; Ventura-Cordero, J.; Sarmiento-Franco, L.A.; Torres-Acosta, J.F.J. Condensed tannin intake and sheep performance: A meta-analysis on voluntary intake and live weight change. *Anim. Feed Sci. Technol.* **2018**, *245*, 67–76. [CrossRef]
52. Kenny, D.; Fitzsimons, C.; Waters, S.; McGee, M. Invited review: Improving feed efficiency of beef cattle–the current state of the art and future challenges. *Animal* **2018**, *12*, 1815–1826. [CrossRef]
53. Johnson, K.A.; Johnson, D.E. Methane emissions from cattle. *J. Anim. Sci.* **1995**, *73*, 2483–2492. [CrossRef]
54. Tedeschi, L.O.; Muir, J.P.; Naumann, H.D.; Norris, A.B.; Ramírez-Restrepo, C.A.; Mertens-Talcott, S.U. Nutritional Aspects of Ecologically Relevant Phytochemicals in Ruminant Production. *Front. Vet. Sci.* **2021**, *8*, 628445. [CrossRef]
55. Li, Z.; Deng, Q.; Liu, Y.; Yan, T.; Li, F.; Cao, Y.; Yao, J. Dynamics of methanogenesis, ruminal fermentation and fiber digestibility in ruminants following elimination of protozoa: A meta-analysis. *J. Anim. Sci. Biotech.* **2018**, *9*, 89. [CrossRef]
56. Salami, S.A.; Valenti, B.; Bella, M.; O'Grady, M.N.; Luciano, G.; Kerry, J.P.; Jones, E.; Priolo, A.; Newbold, C.J. Characterisation of the ruminal fermentation and microbiome in lambs supplemented with hydrolysable and condensed tannins. *FEMS Microbiol. Ecol.* **2018**, *94*. [CrossRef]
57. Jayanegara, A.; Goel, G.; Makkar, H.P.; Becker, K. Divergence between Purified Hydrolysable and Condensed Tannin Effects on Methane Emission, Rumen Fermentation and Microbial Population in vitro. *Anim. Feed Sci. Technol.* **2015**, *209*, 60–68. [CrossRef]
58. Huang, X.D.; Liang, J.B.; Tan, H.Y.; Yahya, R.; Khamseekhiew, B.; Ho, Y.W. Molecular weight and protein binding affinity of *Leucaena* condensed tannins and their effects on in vitro fermentation parameters. *Anim. Feed Sci. Technol.* **2010**, *159*, 81–87. [CrossRef]
59. Jayanegara, A.; Leiber, F.; Kreuzer, M. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. *J. Anim. Physiol. Anim. Nutr.* **2012**, *96*, 365–375. [CrossRef]
60. Jouany, J.P. Effect of rumen protozoa on nitrogen utilization by ruminants. *J. Nutr.* **1996**, *126*, 1335S–1346S. [CrossRef]
61. Belanche, A.; Newbold, C.J.; Morgavi, D.P.; Bach, A.; Zweifel, B.; Yáñez-Ruiz, D.R. A meta-analysis describing the effects of the essential oils blend agolin ruminant on performance, rumen fermentation and methane emissions in dairy cows. *Animals* **2020**, *10*, 620. [CrossRef]
62. Dai, X.; Faciola, A.P. Evaluating strategies to reduce ruminal protozoa and their impacts on nutrient utilization and animal performance in ruminants—A meta-analysis. *Front Microbiol.* **2019**, *10*, 2648. [CrossRef]

63. Bhatta, R.; Uyeno, Y.; Tajima, K.; Takenaka, A.; Yabumoto, Y.; Nonaka, I.; Enishi, O.; Kurihara, M. Difference in the Nature of Tannins on in vitro Ruminal Methane and Volatile Fatty Acid Production and on Methanogenic Archaea and Protozoal Populations. *J. Dairy Sci.* **2009**, *92*, 5512–5522. [CrossRef]
64. Saminathan, M.; Gan, H.M.; Abdullah, N.; Wong, C.M.V.L.; Ramiah, S.K.; Tan, H.Y.; Sieo, C.C.; Ho, Y.W. Changes in rumen protozoal community by condensed tannin fractions of different molecular weights from a *Leucaena leucocephala* hybrid in vitro. *J. Appl. Microbiol.* **2017**, *123*, 41–53. [CrossRef]
65. Saminathan, M.; Ramiah, K.S.; Gan, H.M.; Abdullah, N.; Wong, C.M.V.L.; Ho, Y.W.; Idrus, Z. In vitro study on the effects of condensed tannins of different molecular weights on bovine rumen fungal population and diversity. *Ital. J. Anim. Sci.* **2019**, *18*, 1451–1462. [CrossRef]
66. Tan, H.Y.; Sieo, C.C.; Abdullah, N.; Liang, J.B.; Huang, X.D.; Ho, Y.W. Effects of condensed tannins from *Leucaena* on methane production, rumen fermentation and populations of methanogens and protozoa in vitro. *Anim. Feed Sci. Technol.* **2011**, *169*, 185–193. [CrossRef]
67. Patra, A.K.; Saxena, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.* **2011**, *91*, 24–37. [CrossRef]
68. Gerber, P.J.; Steinfeld, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities*; FAO: Rome, Italy, 2013.
69. Ugbogu, E.A.; Elghandour, M.M.M.Y.; Ikpeazu, V.O.; Buendía, G.R.; Molina, O.M.; Arunsi, U.O.; Emmanuel, O.; Salem, A.Z.M. The potential impacts of dietary plant natural products on the sustainable mitigation of methane emission from livestock farming. *J. Clean. Prod.* **2019**, *213*, 915–925. [CrossRef]
70. Saminathan, M.; Sieo, C.C.; Gan, H.M.; Abdullah, N.; Wong, C.M.V.L.; Ho, Y.W. Effects of condensed tannin fractions of different molecular weights on population and diversity of bovine rumen methanogenic archaea in vitro, as determined by high-throughput sequencing. *Anim. Feed Sci. Technol.* **2016**, *216*, 146–160. [CrossRef]
71. Ku-Vera, J.C.; Jiménez-Ocampo, R.; Valencia-Salazar, S.S.; Montoya-Flores, M.D.; Molina-Botero, I.C.; Arango, J.; Gómez-Bravo, C.A.; Aguilar-Pérez, C.F.; Solorio-Sánchez, F.J. Role of Secondary Plant Metabolites on Enteric Methane Mitigation in Ruminants. *Front. Vet. Sci.* **2020**, *7*, 584. [CrossRef]
72. Kholif, A.E.; Olafadehan, O.A. Essential oils and phytogenic feed additives in ruminant diet: Chemistry, ruminal microbiota and fermentation, feed utilization and productive performance. *Phytochem. Rev.* **2021**. [CrossRef]
73. Yang, K.; Wei, C.; Zhao, G.Y.; Xu, Z.W.; Lin, S.X. Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. *J. Anim. Physiol. Anim. Nutr.* **2016**, *101*, 302–310. [CrossRef]
74. Kardel, M.; Taube, F.; Schulz, H.; Schütze, W.; Gierus, M. Di_erent approaches to evaluate tannin content and structure of selected plant extracts–review and new aspects. *J. Appl. Bot. Food Qual.* **2013**, *86*, 154–166. [CrossRef]
75. Venter, P.B.; Sisa, M.; Van Der Merwe, M.J.; Bonnet, S.L.; Van Der Westhuizen, J.H. Analysis of commercial proanthocyanidins. Part 1: The chemical composition of quebracho (*Schinopsis lorentzii* and *Schinopsis balansae*) heartwood extract. *Phytochemistry* **2012**, *73*, 95–105. [CrossRef]
76. Guyader, J.; Eugène, M.; Nozière, P.; Morgavi, D.P.; Doreau, M.; Martin, C. Influence of rumen protozoa on methane emission in ruminants: A meta-analysis approach. *Animal* **2014**, *8*, 1816–1825. [CrossRef]
77. Nichols, K.L.; Del Grosso, S.J.; Derner, J.D.; Follett, R.F.; Archibeque, S.L.; Stewart, C.E.; Paustian, K.H. Nitrous oxide and methane fluxes from cattle excrement on C3 pasture and C4-dominated shortgrass steppe. *Agric. Ecosyst. Environ.* **2016**, *225*, 104–115. [CrossRef]
78. Erickson, G.; Klopfenstein, T. Nutritional and management methods to decrease nitrogen losses from beef feedlots. *J. Anim. Sci.* **2010**, *88*, E172–E180. [CrossRef]
79. Singh, B.P.; Cowie, A.L.; Chan, K.Y. *Soil Health and Climate Change*, 1st ed.; Springer: Berlin, Germany, 2011; p. 403.
80. Wecking, A.R.; Wall, A.M.; Liáng, L.L.; Lindsey, S.B.; Luo, J.; Campbell, D.I.; Schipper, L.A. Reconciling annual nitrous oxide emissions of an intensively grazed dairy pasture determined by eddy covariance and emission factors. *Agric. Ecosyst. Environ.* **2020**, *287*, 106646. [CrossRef]
81. Avila, S.C.; Kozloski, G.V.; Orlandi, T.; Mezzomo, M.P.; Stefanello, S. Impact of a tannin extract on digestibility, ruminal fermentation and duodenal flow of amino acids in steers fed maize silage and concentrate containing soybean meal or canola meal as protein source. *J. Agric. Sci.* **2015**, *153*, 943–953. [CrossRef]
82. Caetano, M.; Wilkes, M.J.; Pitchford, W.S.; Lee, S.J.; Hynd, P.I. Effect of ensiled crimped grape marc on energy intake, performance and gas emissions of beef cattle. *Anim. Feed Sci. Technol.* **2019**, *247*, 166–172. [CrossRef]
83. Dickhoefer, U.; Ahnert, S.; Susenbeth, A. Effects of quebracho tannin extract on rumen fermentation and yield and composition of microbial mass in heifers. *J. Anim. Sci.* **2016**, *94*, 1561–1575. [CrossRef]
84. Ebert, P.J.; Bailey, E.A.; Shreck, A.L.; Jennings, J.S.; Cole, N.A. Effect of condensed tannin extract supplementation on growth performance, nitrogen balance, gas emissions, and energetic losses of beef steers. *J. Anim. Sci.* **2017**, *95*, 1345–1355. [CrossRef]
85. Jolazadeh, A.; Dehghan-Banadaky, M.; Rezayazdi, K. Effects of soybean meal treated with tannins extracted from pistachio hulls on performance, ruminal fermentation, blood metabolites and nutrient digestion of Holstein bulls. *Anim. Feed Sci. Technol.* **2015**, *203*, 33–40. [CrossRef]

86. Koenig, K.M.; Beauchemin, K.A. Effect of feeding condensed tannins in high protein finishing diets containing corn distillers' grains on ruminal fermentation, nutrient digestibility, and route of nitrogen excretion in beef cattle. *J. Anim. Sci.* **2018**, *96*, 4398–4413. [CrossRef] [PubMed]
87. Koenig, K.M.; Beauchemin, K.A.; McGinn, S.M. Feeding condensed tannins to mitigate ammonia emissions from beef feedlot cattle fed high-protein finishing diets containing distillers grains. *J. Anim. Sci.* **2018**, *96*, 4414–4430. [CrossRef] [PubMed]
88. Krueger, W.K.; Gutierrez-Bañuelos, H.; Carstens, G.E.; Min, B.R.; Pinchak, W.E.; Gomez, R.R.; Anderson, R.C.; Krueger, N.A.; Forbes, T.D.A. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet. *Anim. Feed Sci. Technol.* **2010**, *159*, 1–9. [CrossRef]
89. Martello, H.F.; De Paula, N.F.; Teobaldo, R.W.; Zervoudakis, J.T.; Fonseca, M.A.; Cabral, L.S.; Rocha, J.K.L.; Mundim, A.T.; Moraes, E.H.B.K. Interaction between tannin and urea on nitrogen utilization by beef cattle grazing during the dry season. *Livest. Sci.* **2020**, *234*, 103988. [CrossRef]
90. Mezzomo, R.; Paulino, P.V.R.; Detmann, E.; Valadares Filho, S.C.; Paulino, M.F.; Monnerat, J.P.I.S.; Duarte, M.S.; Silva, L.H.P.; Moura, L.S. Influence of condensed tannin on intake, digestibility, and efficiency of protein utilization in beef steers fed high concentrate diet. *Livest. Sci.* **2011**, *141*, 1–11. [CrossRef]
91. Mezzomo, R.; Paulino, P.V.R.; Barbosa, M.M.; da Silva-Martins, T.; Paulino, M.F.; Alves, K.S.; Gomes, D.I.; Monnerat, J.P.I.S. Performance and carcass characteristics of young cattle fed with soybean meal treated with tannins. *Anim. Sci. J.* **2016**, *87*, 775–782. [CrossRef]
92. Norris, A.B.; Crossland, W.L.; Tedeschi, L.O.; Foster, J.L.; Muir, J.P.; Pinchak, W.E.; Fonseca, M.A. Inclusion of quebracho tannin extract in a high-roughage cattle diet alters digestibility, nitrogen balance, and energy partitioning. *J. Anim. Sci.* **2020**, *3*, 1–12. [CrossRef]
93. Pineiro-Vázquez, A.T.; Canul-Solis, J.R.; Jiménez-Ferrer, G.O.; Alayón-Gamboa, J.A.; Chay-Canul, A.J.; Ayala-Burgos, A.J.; Aguilar-Pérez, C.F.; Ku-Vera, J.C. Effect of condensed tannins from *Leucaena leucocephala* on rumen fermentation, methane production and population of rumen protozoa in heifers fed low-quality forage. *Asian Australas J. Anim. Sci.* **2018**, *31*, 1738–1746. [CrossRef]
94. Piñeiro-Vázquez, A.T.; Jiménez-Ferrer, G.; Alayon-Gamboa, J.A.; Chay-Canul, A.J.; Ayala-Burgos, A.J.; Aguilar-Pérez, C.F.; Ku-Vera, J.C. Effects of quebracho tannin extract on intake, digestibility, rumen fermentation, and methane production in crossbred heifers fed low-quality tropical grass. *Trop. Anim. Health Prod.* **2018**, *50*, 29–36. [CrossRef]
95. Piñeiro-Vázquez, A.T.; Jiménez-Ferrer, G.O.; Chay-Canul, A.J.; Casanova-Lugo, F.; Díaz-Echeverría, V.F.; Ayala-Burgos, A.J.; Solorio-Sánchez, F.J.; Aguilar-Pérez, C.F.; Ku-Vera, J.C. Intake; digestibility; nitrogen balance and energy utilization in heifers fed low-quality forage and *Leucaena leucocephala*. *Anim. Feed Sci. Technol.* **2017**, *228*, 194–201. [CrossRef]
96. Poblete, J.B.; Angeles, A.A.; Agbisit, J.E.M.; Espaldon, M.V.O. Response of Growing Dairy Bulls to Dietary Tannin in Rations with Varying Energy Levels. *Trop. Anim. Sci. J.* **2020**, *43*, 50–56. [CrossRef]
97. Rivera-Méndez, C.R.; Plascencia, A.; Torrentera, N.; Zinn, R.A. Influence of tannins supplementation on growth performance, dietary net energy and carcass characteristics of yearling steers fed finishing diet containing dried distillers grains with solubles. *Indian J. Anim. Sci.* **2016**, *86*, 108–111.
98. Shakeri, P.; Riasi, A.; Alikhani, M.; Fazaeli, H.; Ghorbani, G.R. Effects of feeding pistachio by-products silage on growth performance, serum metabolites and urine characteristics in Holstein male calves. *J. Anim. Physiol. Anim. Nutr.* **2013**, *97*, 1022–1029. [CrossRef] [PubMed]
99. Shakeri, P.; Riasi, A.; Alikhani, M. Effects of long period feeding pistachio by-product silage on chewing activity, nutrient digestibility and ruminal fermentation parameters of Holstein male calves. *Animal* **2014**, *8*, 1826–1831. [CrossRef]
100. Tabke, M.C.; Sarturi, J.O.; Galyean, M.L.; Trojan, S.J.; Brooks, J.C.; Johnson, B.J.; Martin, J.; Baggerman, J.; Thompson, J. Effects of tannic acid on growth performance, carcass characteristics, digestibility, nitrogen volatilization, and meat lipid oxidation of steers fed steam-flaked corn–based finishing diets. *J. Anim. Sci.* **2017**, *11*, 5124–5136. [CrossRef]
101. Tseu, R.J.; Junior, F.P.; Carvalho, R.F.; Sene, G.A.; Tropaldi, C.B.; Peres, A.H.; Rodrigues, P.H.M. Effect of tannins and monensin on feeding behaviour, feed intake, digestive parameters and microbial efficiency of nellore cows. *Ital. J. Anim. Sci.* **2020**, *19*, 262–273. [CrossRef]
102. Yuste, S.; Amanzougarene, Z.; de la Fuente, G.; de Vega, A.; Fondevila, M. Rumen protozoal dynamics during the transition from milk/grass to high-concentrate based diet in beef calves as affected by the addition of tannins or medium-chain fatty acids. *Anim. Feed Sci. Technol.* **2019**, *257*, 114273. [CrossRef]
103. Zhou, K.; Bao, Y.; Zhao, G. Effects of dietary crude protein and tannic acid on rumen fermentation, rumen microbiota and nutrient digestion in beef cattle. *Arch. Anim. Nutr.* **2019**, *73*, 30–43. [CrossRef]

This chapter was published in *Agriculture*

3. PRODUCTIVE PERFORMANCE, CARCASS TRAITS, AND MEAT QUALITY IN FINISHING LAMBS SUPPLEMENTED WITH A POLYHERBAL MIXTURE

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara- Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1], Rufino López-Ordaz [1] and Pedro Abel Hernández-García [3]

[1]Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo 56230, México; jforzuna@gmail.com (J.F.O.-O.); griseldi0993@gmail.com (G.D.-I.); albertomiranda@correo.chapingo.mx (L.A.M.-R.): rlopezor@yahoo.com (R.L.-O.)

[2]Departamento de Producción Agrícola y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, México City 04960, México; gmendoza@correo.xoc.uam.mx

[3]Centro Universitario UAEM Amecameca, Universidad Autónoma del Estado de México, Amecameca 56900, México; pedro_abel@yahoo.com

Corresponding author.

E-mail address: alarab_11@hotmail.com (A. Lara-Bueno).

agriculture

MDPI

Article

Productive Performance, Carcass Traits, and Meat Quality in Finishing Lambs Supplemented with a Polyherbal Mixture

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1], Rufino López-Ordaz [1] and Pedro Abel Hernández-García [3]

[1] Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo CP 56230, Mexico; al19132949@chapingo.mx (J.F.O.-O.); al20130495@chapingo.mx (G.D.-I.); lmirandar@chapingo.mx (L.A.M.-R.); rlopezo@chapingo.mx (R.L.-O.)
[2] Departamento de Producción Agrícola Y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, Mexico City CP 04960, Mexico; gmendoza@correo.xoc.uam.mx
[3] Centro Universitario UAEM Amecameca, Universidad Autónoma Del Estado De México, Amecameca CP 56900, Mexico; pahernandezg@uaemex.mx
* Correspondence: alarab@chapingo.mx

Abstract: The objective of this study was to evaluate the effects of dietary supplementation of a polyherbal mixture (HM) containing saponins, flavonoids, and polysaccharides on productive performance, carcass characteristics and meat quality of lambs during the final fattening period. Thirty-six Dorper × Katahdin lambs (23.27 ± 1.23 kg body weight (BW)) were housed in individual pens and were assigned to four treatments ($n = 9$) with different doses of HM: 0 (CON), 1 (HM1), 2 (HM2) and 3 (HM3) g of HM kg^{-1} of DM for 56 days. Data were analysed as a completely randomized design using the MIXED and GLM procedures of statistical analysis system (SAS), and linear and quadratic effects were tested to evaluate the effects of the HM level. DM digestibility decreased in lambs fed HM3 ($p < 0.05$). There was no effect of HM on daily weight gain, dry matter intake, final BW, feed conversion, carcass characteristics, colour (L* and a*) and meat chemical composition. Meat pH, cooking loss and drip loss increased linearly ($p < 0.05$) when the HM dose was increased. The Warner-Bratzler shear force (WBSF) of meat was lower ($p < 0.05$) in lambs fed HM3. In conclusion, dietary inclusion of 3 g HM kg^{1} of DM improves meat tenderness. However, high doses of HM in the diet may decrease the digestibility of DM and increase the cooking loss and drip loss of lamb meat during the final fattening period.

Keywords: fattening lamb; saponins; bioactive compounds; mutton tenderness

Citation: Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; López-Ordaz, R.; Hernández-García, P.A. Productive Performance, Carcass Traits, and Meat Quality in Finishing Lambs Supplemented with a Polyherbal Mixture. *Agriculture* **2021**, *11*, 942. https://doi.org/10.3390/agriculture11100942

Academic Editors: Lubomira Gresakova and Emilio Sabia

Received: 11 August 2021
Accepted: 26 September 2021
Published: 29 September 2021

1. Introduction

Antibiotics have been commonly used as growth promoters in animals. However, the emergence of bacteria resistant to these drugs has led to the search for alternative products with similar effects to antibiotics, but of natural origin [1]. Dietary supplementation with herbal products seems to be a promising strategy to improve the productive performance, carcass characteristics and meat quality of small ruminants [2]. Some polyherbal mixtures (HM) prepared with medicinal plants have shown positive effects on productive performance, meat and carcass quality characteristics of steers and lambs during the final fattening period [3–5]. On the other hand, in calves, it has been reported that the use of HM can improve growth and health status during the pre-ruminant period until weaning by modifying gene expression [6]. However, the effects of bioactive compounds (for example, saponins and flavonoids) of HM in biological systems, may depend on the efficiency of their absorption and extensive metabolic transformation [7].

Previous studies [8,9] have shown that some plants containing saponins, flavonoids and polysaccharides can improve antioxidant status, ruminal fermentation, immune response and productive performance in sheep. Likewise, some HM containing saponins,

flavonoids and tannins have been shown to have a positive impact on nutrient utilization efficiency in goats [10]. Other products containing saponins have shown positive impact on energy metabolism and on the duodenal flux of amino acids [11], ruminal fermentation rate [12,13], rumen microbial populations [14], and production of volatile fatty acids [12–14]. Similarly, flavonoids can modulate the ruminal microbiome, improve rumen fermentation and metabolic status to improve the productive performance and health of ruminants [15]. Some HM containing flavonoids have shown positive impact on antioxidant status [7], and ruminal microbial populations of lambs [16]. In addition, flavonoid supplementation modifies the expression of genes in the rumen epithelium that could be related to inflammation and animal behaviour modulation [17].

Some plant parts containing saponins have also been used to improve the meat quality of adult goats and kids [18,19]. However, there is limited information on the effects of plants or HM containing saponins, flavonoids, and polysaccharides on the productivity, carcass characteristics, and meat quality of lambs. The botanical origin, the dose, and the composition of the diet used can influence the biological response that saponins have on ruminants [20]. Although, the effects of using saponins in ruminant feed have been investigated in animals fed diets containing a high proportion of forage [11,13]; information on the effects of these bioactive metabolites in ruminants fed high concentrate diet is limited and inconsistent [19,21]. Some saponin extracts improve ruminal fermentation and increase the efficiency of energy use in the animals, which could result in better productive performance [11]. However, the effects of saponins on ruminal fermentation may differ depending on the ruminal pH [22], which varies according to the dietary level of concentrate. Due to the beneficial effects of herbal products and their secondary metabolites, it has been hypothesized that supplementation with HM as a source of saponins, flavonoids and polysaccharides can contribute to improving the productivity of the lambs during the final fattening period, without affecting the quality of the meat or the characteristics of the carcass. The objective of this study was to evaluate the effects of increasing doses of an HM containing saponins, flavonoids, and polysaccharides on the productive performance, carcass characteristics, and meat quality of lambs fed high-concentrate diets.

2. Materials and Methods

2.1. Location

The experiment was conducted at the Teaching and Research Unit of Small Ruminants located at the Experimental Farm of the Universidad Autónoma Chapingo, Mexico, which is located at 19°22′ north latitude and 98°35′ west longitude, with an altitude of 2250 m. The climate is temperate subhumid, with rain during the summer and dry during the winter, with average annual precipitation and temperatures of 665 mm and 15.2 °C, respectively [23]. The study was conducted during the summer, under hot and rainy conditions. The care and handling procedures for the lambs were carried out following the guidelines of the Official Mexican Standard (NOM-062-ZOO-1995).

2.2. Polyherbal Mixture Characteristics

The HM used was Peptasan® (Nuproxa S. de RL. de CV. Querétaro, México), which is a commercial polyherbal formula labelled to contain 150 g kg^{-1} of saponins. In addition, Peptasan® is composed of parts from the *Saccharum officinarum*, *Balanites roxburghi* and *Acacia concinna* plants. *S. officinarum* contains polysaccharides with immunostimulating effects [24]; *B. roxburghii* contains saponins and flavonoids with antioxidant, anti-inflammatory, antimicrobial and antiviral properties [25]; and *A. concinna* contains saponins with immunomodulatory properties [26].

2.3. Diet Composition

HM was fed to the lambs through diets formulated to have weight gains of 300 g d^{-1} [27]. HM (1, 2 or 3 g kg^{-1} of diet DM basis) was premixed with minor ingredients (vitamin and mineral supplement, limestone and salt) before incorporation into complete mixed

diets. The lambs were fed a finishing diet (total mixed ration) comprised 30.3% ground corn, 24.1% ground sorghum, 8.1% soybean meal, 7.1% wheat bran, 7.4% corn gluten, 2.3% bypass fat, 19.4% oat straw, 0.5% vitamin and mineral supplement, 0.5% salt, and 0.3% limestone (DM basis). Oat straw was ground in a hammer mill (Azteca 20, Molinos Azteca, Guadalajara, México) with a 3.8 cm screen before incorporation into total mixed ration. The nutrient composition of the basal diet was 15.53% crude protein, 2.58% ether extract, 13.57% acid detergent fiber, 26.14% neutral detergent fiber, 5.47% ash and 2.8 Mcal of metabolizable energy according to NRC [27] DM basis.

2.4. Animals and Experimental Design

Thirty-six male Dorper × Katahdin lambs (23.27 ± 1.23 kg BW, 4–5 months old) were randomly distributed in four treatments: (1) basal diet without HM (CON); (2) HM1, CON + 1 g of HM kg^{-1} dry matter (DM); (3) HM2, CON + 2 g of HM kg^{-1} DM; and (4) HM3, CON + 3 g of HM kg^{-1} DM. The lambs were placed in individual pens (2.6 m × 0.8 m) equipped with automatic drinkers and individual feeders. Prior to the start of the experimental phase, lambs were vaccinated against *Clostridium* and *Pasteurella* (2.5 mL $lamb^{-1}$, Bobact® 8 MSD-Merck, Kenilworth, NJ, USA), and dewormed through an oral administration of Koptisin ovine® (10 mg kg^{-1} BW, Chinoin, Labs, Mexico City, Mexico). Additionally, 1 mL $lamb^{-1}$ of vitamins containing 500,000 IU of vitamin A, 75,500 IU of vitamin D and 50 mg of vitamin E (Vigantol® Bayer, Mexico City, Mexico) was provided on day 1 of the adaptation period. The lambs had an adaptation period to the basal diet of 14 days, and the experimental phase lasted 56 days. During the adaptation period, the lambs received oat straw as a ruminal pH buffer, and the experimental diets were administered at increasing levels (20, 40, 60, 80 and 100% of the total ration) for 14 days (3 days per level, except for 100%), until the oat straw was reduced to 0%. The feed was provided at 09:00 and 17:00 h, and the drinking water was supplied ad libitum. Individual BW was recorded before the morning feeding on days 1, 14, 28, 42 and 56, of the experimental phase. The amount of diet offered and refused was recorded daily to estimate dry matter intake (DMI, kg d^{-1}). The amount of feed offered was always 10% higher than the previous intake to ensure ad libitum intake. Daily weight gain (DWG, kg d^{-1}) was calculated between feeding period intervals. The feed conversion ratio (FCR) was expressed as feed consumption per unit of body weight gain. Figure 1 shows the experimental procedure.

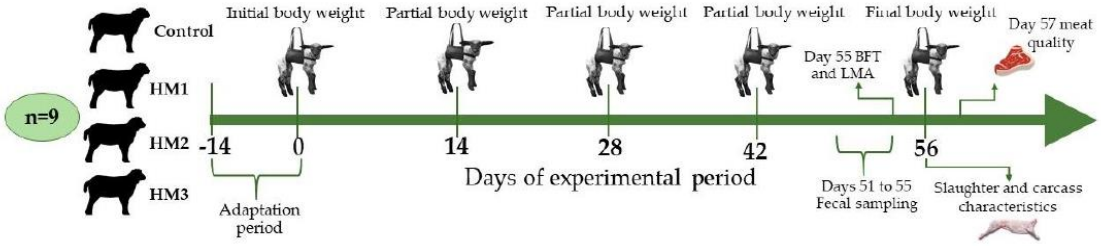

Figure 1. Completely randomized design and sampling times of lambs supplemented with a polyherbal mixture (HM) during the final fattening period; *n* = 9—indicate the number of animals sampled in each treatment; Control—basal diet without HM; HM1—basal diet + 1 g of HM kg^{-1} of dry matter (DM); HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; BFT—backfat thickness; LMA—*longissimus* muscle area.

2.5. Sampling and Analyses of Feeds

Samples of feed provided and rejected were collected daily to determine the chemical composition. Prior to the analysis, the food samples were dried at 55 °C in a forced air oven and then ground in a Wiley mill (model 4, Arthur Thomas Co. Philadelphia, PA, USA). The variables determined were dry matter, crude protein, ether extract and ash [28]. Acid

detergent fibre and neutral detergent fibre was determined using the procedures described by Van Soest et al. [29].

2.6. Apparent Dry Matter Digestibility

Faecal samples were collected from each animal during five consecutive days (in the morning at 08:00 a.m. and in the afternoon at 16:00 p.m. before feed delivery) starting on day 51, directly from the rectum [5]. Feed and orts were collected daily during the same period. Acid-insoluble ash was employed as a marker of internal tract digestibility to analyse the apparent total tract DM digestibility [30].

2.7. Carcass Characteristics

The *longissimus* muscle area (LMA) and the backfat thickness (BFT) located between the 12th and 13th ribs of the lamb were measured on day 55 of the experiment using a Sonovet 600 (Medison, Inc., Cypress, CA, USA) with a 7.5 Mhz transducer [31]. After the last weighing (day 56 of the experiment) the lambs were fasted for 18 h before being slaughtered. All lambs were slaughtered on the same day. The slaughter process was conducted in a commercial slaughterhouse in accordance with standard procedures of the Official Mexican Standard (NOM-033-SAG/ZOO-2014). Lambs were stunned (captive bolt), exsanguinated and skinned. Immediately after the slaughter, the hot carcass weight was registered (HCW). The hot carcass yield (HCY) was determined through HCY = (HCW/FBW) $*$ 100, as it was described by Zimerman et al. [32]. In addition, the skin, head, legs, testicles, rumen (empty), liver, spleen, kidneys, heart, lungs, small intestine (empty), and large intestine (empty) were each weighed separately.

2.8. Meat Quality

After 1 h post-mortem, the right *Longissimus thoracis* (LT) muscle between the 7th and 11th ribs was removed from the carcass with a scalpel and used for pH, colour, Warner-Bratzler shear force (WBSF), chemical composition, drip loss and cooking loss analysis. Samples of *LT* muscle (approximately 600 g) were collected from the carcass and then frozen at −20 °C for a subsequent meat quality analysis.

Prior to the analysis of cooking losses (CL) and Warner-Bratzler shear force (WBSF), the samples were thawed for 24 h at 4 °C in a cooler protected from drafts and the meat samples were analysed in triplicate. CL was determined according by Vazquez-Mendoza et al. [33]; for this purpose, fillets with 2.5 cm thick were roasted on a grill (Toastmaster cool-edge-grill, Macon, MO, USA) until they reached an internal temperature of 70 °C, which was monitored with a thermometer (Brannan & Sons, Cleator Moor, Cumbria, UK). When the temperature reached 70 °C, the fillets were removed from the grill and allowed to cool to room temperature (20–25 °C). To calculate the percentage of CL, each fillet was weighed before and after the procedure (weight of raw meat−weight of cooked meat)/weight of raw meat $\times$100), as it was described by Vazquez-Mendoza et al. [33]. In order to measure the WBSF, 2.5 cm thick meat fillets (three per lamb) were cooked at 70 °C using the CL method, as sited above. WBSF was measuring using an Instron® universal testing machine (model 1132, Instron, Canton, MA, USA) with a Warner-Bratzler accessory [34]. Meat colour was determined on cuts of the *longissimus dorsi* muscle 24 h after slaughter using a Minolta CM-2006d spectrophotometer (Konica model, Minolta Holdings Inc., Osaka, Japan). Lightness (L*), redness (a*) and yellowness (b*) as meat quality attributes were evaluated using the procedure described by Miltenburg et al. [35]. With the values of a* and b*, the Chroma (C*) and Hue (H*) indices were calculated using the equations: Chroma = $(a^* 2 + b^* 2)^{0.5}$ and Hue = $\tan^{-1}$ (b*/a*) $\times$ 57.29 both expressed in degrees [36]. Colour coordinate values were obtained using the average of three measurements of colour for each sample. Meat pH was measured following the procedure described by Negrete et al. [37]. This was measured in triplicate on 3 g of *longissimus dorsi* muscle homogenized in 20 mL of deionized water using a blender Waring 51BL32 (model 700, Torrington, CT, USA), and using a Hanna® pH meter (Model HI 98127, Waterproof Tester, Woonsocket, RI, USA). Drip loss value was calculated

as weight loss of the fresh meat sample (90 g) placed in a plastic bag after storage for 24 h at 4 °C. Drip loss was determined in triplicate as percentage of water released from fresh muscle [38].

Prior to the proximate analysis of meat, the samples were thawed for 24 h at 4 °C. The subcutaneous fat and connective tissue were separated from the muscle using a scalpel, and the meat was ground and homogenized for 5 min with a mixer. Meat samples were analysed in triplicate to determine the moisture, lipid, protein and ash content as a percentage of the muscle sample following AOAC procedures [28].

2.9. Statistical Analysis

All statistical analyses were performed using the SAS statistical program [39]. First, it was performed the normality test on all variables using the UNIVARIATE procedure. BW, DMI, DWG and FCR data were analysed for each period with a completely randomized design with repeated measures over time, using the MIXED procedure. Initially, initial BW was included as a covariate to adjust the variables DWG, DMI and final BW. However, this covariate was removed from the model because it was not significant ($p > 0.05$). Different variance–covariance structures were verified to fit the statistical model, and the compound symmetry structure showed the best fit according to the criteria of the lowest values of BIC and AIC [40]. The full statistical model used was:

$$Y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + A_k + e_{ijk} \quad (1)$$

where Y_{ijk} represents the value measured at period j and treatment i for the lamb k, μ represents the overall mean, T_i represents the fixed effect of HM treatments (i = 1, 2, 3, 4), P_j represents the fixed effect of the period within four feeding periods (j = period 1: 1–14, period 2: 15–28, period 3: 29–42 and period 4: 43–56 d), $(T \times P)_{ij}$ represents the fixed effect of interaction between treatment and period, A_k represents the random effect of lambs provided different diets (k = 1, 2, 3, . . . 36), and e_{ijk} represents the random residual error.

On the other hand, data on carcass characteristics, animal organs and meat quality were analysed using the GLM procedure. Each lamb was considered an experimental unit. Initially, final BW was included as a covariate to adjust all variables (carcass characteristics, organs and meat quality). However, this covariate was removed from the model because it was not significant ($p > 0.05$). The statistical model used was: $Y_{ijk} = \mu + T_i + e_{ij}$, in which μ is the mean value, T_i is the treatment effect (fixed), and e_{ij} is the error term.

Linear and quadratic orthogonal polynomials were used to evaluate the effects of HM level on all variables evaluated. Means of treatments were compared using the Tukey test, and significant differences were considered when $p \leq 0.05$. In addition, a trend was considered when $p > 0.05$ and ≤ 0.10.

3. Results

3.1. Productive Performance and Digestibility

Final body weight (FBW) was not affected by treatments (Table 1). For dry matter intake (DMI), no significant differences were found among the treatments during the experimental period. On the other hand, DWG showed a tendency of linear decrease ($p = 0.06$), and the lambs that were supplemented with HM3 performed lower than the lambs fed with the other diets. However, the feed conversion ratio was not affected by the level of HM added to the diet. On the other hand, the dry matter digestibility (DMD) decreased linearly ($p = 0.03$) as the dose of HM in the diet increased. The lowest digestibility of DM was observed in lambs fed HM3 diet (Table 1).

Table 1. Productive performance of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Initial body weight (IBW) kg	23.15	23.45	22.93	23.55	1.233	0.90	0.90
Final body weight (FBW) kg	41.93	39.88	40.13	38.80	1.608	0.21	0.82
Dry matter intake (DMI) kg d^{-1}	1.161	1.083	1.059	1.034	0.056	0.12	0.64
Daily weight gain (DWG) kg d^{-1}	0.335 *	0.293	0.307	0.272 *	0.020	0.06	0.85
Feed conversion ratio (FCR) DMI/DWG	3.49	3.74	3.54	3.91	0.196	0.23	0.76
Dry matter Digestibility (DMD) %	75.71 ^{a}	74.72 ^{ab}	72.31 ^{ab}	70.39 ^{b}	1.528	0.03	0.76

[1] Peptasan® based on *Saccharum officinarum, Balanites roxburghi and, Acacia concinna.* CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$; *—indicates a tendency.

3.2. Carcass Traits

No differences were observed in hot carcass weight, hot carcass yield, backfat thickness, *longissimus dorsi* muscle area, weight of internal organs (empty rumen, small intestine, large intestine, lungs and trachea, heart, liver, kidneys, spleen), nor in the weight of testicles, skin, feet and head by the effect of supplementation with the HM (Table 2).

Table 2. Carcass traits and organ weights of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Hot carcass weight kg	20.73	19.43	19.38	18.88	0.757	0.11	0.22
Hot carcass yield %	49.47	48.72	48.28	48.95	0.767	0.57	0.54
Backfat thickness mm	3.00	3.11	3.00	3.11	0.114	0.67	0.99
Muscle area *longissimus dorsi* cm^2	11.24	10.90	10.92	10.66	0.312	0.22	0.90
Rumen (empty) kg	1.188	1.152	1.134	1.139	0.047	0.43	0.47
Small intestine (empty) kg	0.882	0.839	0.896	0.913	0.046	0.47	0.34
Large intestine (empty) kg	1.046	1.042	1.024	1.045	0.053	0.93	0.86
Lungs and Trachea kg	0.699	0.686	0.679	0.638	0.040	0.30	0.41
Heart kg	0.198	0.172	0.176	0.192	0.009	0.74	0.92
Liver, kg	0.823	0.842	0.839	0.800	0.034	0.64	0.71
Kidneys kg	0.337	0.352	0.328	0.316	0.019	0.31	0.24
Spleen kg	0.076	0.079	0.083	0.078	0.006	0.70	0.60
Testicles kg	0.690	0.717	0.718	0.634	0.055	0.50	0.62
Skin kg	2.914	2.718	2.834	2.527	0.159	0.15	0.40
Feet kg	0.882	0.824	0.833	0.807	0.041	0.25	0.44
Head kg	1.967	2.025	1.986	1.937	0.072	0.69	0.64

[1] Peptasan® based on *Saccharum officinarum, Balanites roxburghi* and, *Acacia concinna*. CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means.

3.3. Meat Quality

Meat pH, cooking loss and drip loss increased linearly ($p < 0.05$) as the dose of HM in the diet increased (Table 3). The WBSF of meat decreased linearly as the level of HM in the diet increased ($p = 0.02$). On the other hand, no significant changes were observed in meat colour variables, with the exception of yellowness (b*), which decreased as dietary HM dose increased ($p = 0.04$). The chemical composition (moisture, protein, fat and ash) of the meat was not affected by the dose of HM in the diet.

Table 3. Meat characteristics of lambs supplemented with a polyherbal mixture [1] during the final fattening period.

Parameter	Treatment					*p*-Value	
	CON	HM1	HM2	HM3	EEM	Linear	Quadratic
Meat pH (24 h)	5.50 ^{ab}	5.36 ^{b}	5.69 ^{a}	5.84 ^{a}	0.14	0.04	0.32
WBSF kg cm^{-2}	6.47 ^{a}	6.29 ^{a}	5.53 ^{ab}	4.73 ^{b}	0.57	0.02	0.58
Cooking loss (%)	16.89	18.72	19.28	20.13	1.09	0.04	0.65
Dripp loss (%)	3.55 ^{b}	4.07 ^{ab}	4.84 ^{a}	4.81 ^{a}	0.38	0.01	0.48
Lightness (L^*)	36.22	36.20	33.45	34.77	1.27	0.22	0.60
Redness (a^*)	9.23	8.45	9.05	9.23	0.44	0.75	0.28
Yellowness (b^*)	10.28 ^{a}	9.11 ^{b}	9.45 ^{ab}	8.73 ^{b}	0.45	0.04	0.62
Chroma	13.87	12.46	13.12	12.74	0.51	0.25	0.33
Hue $^{\circ}$	47.81 ^{a}	47.12 ^{ab}	46.48 ^{ab}	43.40 ^{b}	1.65	0.07	0.47
Moisture, g 100 g^{-1}	73.70	73.69	73.69	73.58	0.48	0.97	0.99
Crude protein, g 100 g^{-1}	20.38	20.47	20.59	20.48	0.38	0.94	0.88
Fat, g 100 g^{-1}	2.45	2.46	2.45	2.49	0.07	0.99	0.98
Ash, g 100 g^{-1}	1.34	1.33	1.33	1.32	0.03	0.82	0.98

[1] Peptasan® based on *Saccharum officinarum, Balanites roxburghi* and, *Acacia concinna*. WBSF—Warner-Bratzler shear force; CON—basal diet without polyherbal mixture (HM); HM1—basal diet + 1 g of HM kg^{-1} of DM; HM2—basal diet + 2 g of HM kg^{-1} of DM; HM3—basal diet + 3 g of HM kg^{-1} of DM; EEM—standard error of the treatment means; ^{a,b}—means within a row with different subscripts differ when $p \leq 0.05$.

4. Discussion

Some plants containing saponins, polysaccharides and flavonoids have shown positive effects on antioxidant capacity and immune status in ruminants [8,41]. In addition, saponins have been reported to improve the energy utilization efficiency and increase the duodenal flux of amino acids and microbial protein [11]. Consequently, lambs supplemented with herbal products containing saponins, polysaccharides, and flavonoids would be expected to have higher growth rates. However, although in our study FBW and DWG were not affected by HM, a linear reduction trend was observed in DWG of lambs fed the HM3 diet, which could be a consequence of the lower dry matter digestibility observed with HM3. This suggests that high doses of HM in the diet could affect the growth rate of lambs when it is used for prolonged periods. Similar results were previously reported by Liu et al. [42] in lambs supplemented with *Medicago sativa* saponin extracts (0, 0.5, 1, 2 and 4 g kg^{-1} DM for 90 days); and by Nasri et al. [43] who examined the effects of increasing doses of *Quillaja saponaria* saponin extracts (0, 30, 60 and 90 mg kg^{-1} DM for 57 days) in lambs fed high concentrate diets. In the latter investigation, BW and DWG was similar among treatments, regardless of the dose of saponins used. In another study, Wang et al. [9] investigated the effects of supplementing lambs with *Astragalus membranaceus* roots (0, 20, 50 and 80 g kg^{-1} DM for 56 days) containing saponins, polysaccharides and flavonoids. In that study, BW was not affected, but DWG was higher in the treatments supplemented with *Astragalus membranaceus*, perhaps as a consequence of the beneficial effects that the saponins, flavonoids and polysaccharides of the plant had on the antioxidant and immune status, and on the serum concentration of growth hormone in the animals.

Some plants containing saponins, polysaccharides and flavonoids increase the relative abundance of fibre-degrading bacteria in the rumen [41]. This could result in higher fibre and feed digestibility and could also increase ruminal passage rate and dry matter intake. However, in our study, DMI was similar among lambs of all treatments during the experimental period. Although HM could increase the rate of passage, saponins are natural surfactant glycosides, which may have a bitter and astringent taste for animals [44]. This can cause low palatability of the diet, which would partially explain the absence of changes observed in DMI. In a similar study, Liu et al. [42] investigated the effects of extracts of *Medicago sativa* saponins (0, 0.5, 1, 2 and 4 g kg^{-1} DM for 90 days) on the productive performance of lambs. In that study, DMI increased linearly as the dose of saponins in the diet increased. This suggests that the lambs are able to adapt to consume saponins, but this adaptation could require long periods of supplementation.

Some plant-extracted saponins have shown promising effects on improving feed utilization efficiency because they can suppress enteric methane emissions through direct effects on ruminal microorganisms [12,14]. In the present study, FCR was similar among treatments, suggesting that HM did not affect feed utilization efficiency. The absence of significant changes in FCR could be explained by the fact that DMI and DWG were also not affected by the treatments. Similar results were previously reported by Mandal et al. [18] in goats supplemented with 5 g d^{-1} of *Acacia concinna* pods for 90 days, and by Liu et al. [42] in lambs supplemented with alfalfa saponin extracts (0.5, 1, 2 and 4 g kg^{-1} DM for 90 days). In their study, they observed that FCR was similar among treatments, even though feed digestibility was higher in lambs supplemented with saponins.

Previous studies have reported that digestion and utilization of nutrients in the diet of ruminants could be improved by dietary supplementation of saponins [12,45], and plants containing saponins, polysaccharides and flavonoids [41]. However, in our study, a negative effect of HM on DM digestibility was observed. Similar results were previously reported by Nasri et al. [43] in lambs supplemented with saponin extracts from *Quillaja saponaria* at dietary concentrations of 30, 60 and 90 mg kg^{-1} DM; and by Nasehi et al. [21] in lambs supplemented with increasing doses (0, 6.1, 8.7 and 11.3 g kg^{-1} DM) of saponins from the green tea plant (*Camellia sinensis)*. Their results showed that saponins reduced the digestibility of DM of the lambs but did not affect their productive performance.

Regarding carcass characteristics, HCW and HCY were not affected by dietary supplementation of HM. No information is available on the effects of HM containing saponins, polysaccharides and flavonoids on sheep or goat carcass characteristics. However, results that are congruent with our findings were previously reported by Nasri et al. [43] on lambs supplemented with increasing doses of saponin extracts from *Quillaja saponaria* (0, 30, 60 and 90 mg kg^{-1} DM for 57 days); and by Abdallah et al. [46] on sheep supplemented with 10 and 15% dried *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. Their results showed that HCW and HCY were not affected by dietary supplementation of saponins, and neither were they affected by the mixture of saponins, polysaccharides and flavonoids from *Astragalus membranaceus*. The limited information on the effects of HM on ruminant carcass characteristics makes it difficult to explain the results observed in this and other studies. However, the similarity of BFT in the carcass of lambs from all treatments may partially explain the absence of changes in HCY in the present study.

BFT and LMA were also not affected by the HM dietary supplementation. The mechanism of action of herbal products and their bioactive compounds on lipogenesis has not been studied in lambs [4]. However, Liang et al. [47] observed that, in beef cattle fed with high-grain rations, supplementation of flavonoid extracts in the diet increased BFT through changes in the differential expression of genes involved in lipid metabolism. In the present study BFT was not affected by the inclusion of HM in the diet, even though it contains parts of the plant *Balanites roxburghii*, which contains flavonoids [25]. This suggests that the effects of flavonoids on BFT are dependent on botanical origin. Given that fat deposition, physical and chemical carcass characteristics of lambs are influenced by breed, sex, age and weight [48,49], the homogeneity of these characteristics in the lambs used in the present study partially explains the absence of changes in LMA and BFT.

Regarding the internal and external organs of lambs, similar results were previously reported by Hundal et al. [19] in goats supplemented with 2% of *Macrotyloma uniflorum* seeds containing saponins; and by Abdallah et al. [46] in sheep supplemented with 10 and 15% of *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. They observed that the weight of the kidneys on sheep supplemented with the highest dose of saponins, flavonoids and polysaccharides from *A. membranaceus* was higher than that on sheep from the other treatments, but there was no effect on the other internal organs. Information on the effects of herbal products or their bioactive compounds on the size and weight of internal organs in ruminants is still limited, which makes it difficult to explain the results observed in this study. However, differences in the internal organs of sheep are

influenced by the breed, sex and age of the animals [50], and by the feeding regime [51]. In the present study all these factors were controlled, which would partially explain the absence of significant changes.

The lowest pH of the meat was observed in the lambs with the HM1 treatment, while in the animals of the other treatments the pH was similar, within the normal range of 5.5 to 5.8 suggested by Sañudo et al. [52]. Abdallah et al. [46] did not observe pH changes in the meat of lambs supplemented with 10 and 15% *Astragalus membranaceus* roots containing saponins, flavonoids and polysaccharides. In another study, Nasri et al. [43] also did not observe pH changes in the meat of lambs supplemented with saponin extracts from *Quillaja saponaria* at concentrations of 30, 60 and 90 mg kg^{-1} DM. However, it was observed that the pH of the meat in lambs of all treatments was below the normal range, similar to what was observed in our study with the HM1 treatment. Therefore, the effects of HM on the pH of the meat observed in the present study could be related to the presence of bioactive compounds. The pH is important for preserving meat during storage. A low pH has a bacteriostatic effect, while a pH above the normal range favours the growth of proteolytic microorganisms [53,54]. This suggests that supplementation of low doses of HM in the diet could promote favourable bacteriostatic effects in lamb meat, and thus increase its shelf life.

Ponnampalam et al. [55] mentioned that an ultimate pH > 5.8 is associated with alterations in drip loss and WBSF. In addition, in sheep meat, Watanabe et al. [56] reported a curvilinear association between ultimate pH and WBSF values, with a toughness peak at pH around 6.0 and improvements in tenderness at pH below and above 6.0. In our study, WBSF decreased as the dose of HM increased; however, this result must be carefully interpreted considering the low number of replicates used and the high coefficient of variation observed (30.74%, data not shown). Similar results were previously reported by Qin et al. [57] in lambs fed pomace (7.8 and 16% for 80 days) obtained from *Hippophae rhamnoides* fruits, which contained 0.69 and 1.02% flavonoids, respectively. In that experiment, WBSF decreased when the flavonoid dose increased. In another study, Abdalla et al. [46] observed no significant changes in WBSF of meat from lambs supplemented with saponins, flavonoids and polysaccharides from *Astragalus membranaceus* roots. WBSF is a well-known method for estimating the meat tenderness [57], consequently, the lower WBSF observed in the present study suggests that dietary supplementation of HM could improve the lamb meat tenderness. Although the exact mechanism is unknown, the changes in WBSF observed in this and other studies suggest that bioactive compounds contained in some plants facilitate the activation of some peptidases such as calpains and cathepsins, which help prevent and delay post-mortem muscle fibre stiffening [58]. It is also possible that these bioactive compounds act by reducing calpastatin activity, allowing a higher rate of myofibril protein degradation [59]. This hypothesis is supported by the observed linear increase in drip loss as WBSF decreased, because drip water losses may increase when calpastatin activity decreases [60]. Furthermore, Webb and Agbeniga [61] reported a linear relationship between WBSF and drip loss, in which higher drip loss was associated with rapid tenderisation and lower WBSF of the meat.

Drip loss is associated with the capacity to retain water in the muscle, with the juiciness and the tenderness of the meat [46,49]. In the present study, the drip loss of meat increased when the dose of HM increased, indicating that high doses of HM could affect the water retention capacity, tenderness and juiciness of meat. Abdallah et al. [46] investigated the effects of dietary supplementation with dried *Astragalus membranaceus* roots containing saponins, flavonoids, and polysaccharides, and observed that meat drip loss decreased in response to *A. membranaceus* supplementation. However, WBSF was similar in the meat of lambs from all treatments. Although the exact mechanism involved is unknown, the higher drip loss observed in the meat analysed in the present study could be related to the observed changes in WBSF, as previously discussed.

Colour is an important attribute of meat quality because it is the first aspect that attracts consumers when choosing fresh meat [62]. A variety of secondary compounds

from plants can improve oxidative stability and prevent discolouration of meat of small ruminants [2]. In the present study, HM did not affect the values of L*, a*, Chroma and Hue °. However, b* decreased in response to supplementation of HM in the diet. This result could be positive because consumers generally do not expect to find high b* in fresh meat [63]. Similarly, previous studies [46,64] reported that supplementation with medicinal plants containing saponins, polysaccharides, and flavonoids also did not affect the colouration of meat from lambs and goats.

There is little information on the use of HM containing saponins, polysaccharides and flavonoids as a colour preservative in ruminant meat. The pigment content of meat can modify its colouration [35]. Likewise, the inclusion of some medicinal plants containing flavonoids increases the hypertrophy of muscle fibres in lambs [57], which could dilute the content of muscle pigments and consequently alter meat colour [65,66]. These findings suggest that the HM used could increase muscle hypertrophy, which would partially explain the observed reduction in b*. On the other hand, Luo et al. [64] reported that dietary supplementation of medicinal plants containing saponins, polysaccharides and flavonoids altered the pigment content on the meat of small ruminants. Similar effects of consumption of these metabolites would partially explain the b* changes in the meat of lambs supplemented with HM in the present study.

In the present study, the chemical composition of lamb meat was similar in all treatments, perhaps as a consequence of the low impact of HM supplementation on the nutritional composition of the diet. In a similar study, Abdallah et al. [46] investigated the effects of *Astragalus membranaceus* roots (0, 10 and 15% for 47 days) containing saponins, polysaccharides and flavonoids on sheep meat quality. In that research, the moisture, protein and ash content of the meat was similar among treatments. However, they observed that fat content decreased in sheep that ate *A. membranaceus* roots. Furthermore, in our study, HM supplementation had little impact on the final BW of the lambs, all being of the same breed and age, which partially explains the absence of significant changes in the chemical composition of the meat [48,49,54].

5. Conclusions

The results of this study indicate that dietary supplementation with HM reduces dry matter digestibility (linear effect). However, the inclusion of up to 3 g HM kg^{-1} DM does not affect productive performance, carcass characteristics, chemical composition, and meat colouration (lightness and redness) of lambs fed high concentrate diets during the final fattening period. Meat yellowness decreases (linear effect) in response to HM supplementation in the diet, which could be positive because consumers, in general, do not expect to find high yellowness in fresh meat. On the other hand, meat pH, cooking loss and drip loss increase linearly as the dose of HM in the diet increases (linear effect). In addition, Warner-Bratzler shear force decreases as the dose of HM increases (linear effect). Thus, Peptasan® HM could be used to improve meat tenderness of lambs fed high concentrate diets. However, this result must be carefully interpreted considering the low number of replicates used. In addition, the increased drip loss in response to HM supplementation could be a risk of microbial spoilage during meat storage. Therefore, it is convenient to carry out meat quality analyses at the muscle level to evaluate the impact of other doses of this HM in rations with different proportion of concentrate for lambs in different experimental periods and physiological stages.

Author Contributions: Conceptualization, J.F.O.-O., G.D.-I., A.L.-B., G.D.M.-M.; methodology, J.F.O.-O., G.D.-I., A.L.-B. and L.A.M.-R.; software, L.A.M.-R., R.L.-O. and P.A.H.-G.; validation, A.L.-B., L.A.M.-R. and P.A.H.-G.; formal analysis, J.F.O.-O., G.D.-I. and A.L.-B.; research, J.F.O.-O., G.D.-I. and A.L.-B.; investigation, J.F.O.-O., G.D.-I. and A.L.-B.; resources, A.L.-B. and G.D.M.-M.; data curation, L.A.M.-R., R.L.-O. and P.A.H.-G.; writing-original draft preparation, J.F.O.-O., G.D.-I. and A.L.-B.; writing-review and editing, L.A.M.-R., R.L.-O. and P.A.H.-G.; project administration, A.L.-B.; funding acquisition, A.L.-B. and G.D.M.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Animal care and handling procedures were conducted according to the technical specifications for the production, care and use of laboratory animals established in the Official Mexican Standard (NOM-062-ZOO-1995). The approval for this study was granted prior to commencement of the work by, Comisión Académica del Doctorado en Ciencias Agropecuarias de la Universidad Autónoma Metropolitana, Unidad Xochimilco.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The first author, José Felipe Orzuna Orzuna is a Master of Science student of the Program of Animal Production of the Universidad Autónoma Chapingo and thanks the CONACyT Program for the scholarship. We appreciate the assistance of Nuproxa México S. de RL. de CV. Querétaro, México for providing the Herbal Plant Additive.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lillehoj, H.; Liu, Y.; Calsamiglia, G.; Fernandez-Miyakawa, M.E.; Chi, F.; Cravens, R.L.; Oh, S.; Gay, C.G. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. *Vet. Res.* **2018**, *49*, 76. [CrossRef] [PubMed]
2. Vasta, V.; Luciano, G. The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. *Small Rumin. Res.* **2011**, *101*, 150–159. [CrossRef]
3. Lee, S.J.; Kim, D.H.; Le, L.G.; Ahn, S.K.; Cho, K.W.; Lee, S.S. Effect of medicinal plant by-products supplementation to total mixed ration on growth performance, carcass characteristics and economic efficacy in the late fattening period of Hanwoo steers. *Asian-Australas. J. Anim. Sci.* **2015**, *28*, 1729–1735. [CrossRef] [PubMed]
4. Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Hernández-García, P.A. Growth Performance, Carcass Characteristics, and Blood Metabolites of Lambs Supplemented with a Polyherbal Mixture. *Animals* **2021**, *11*, 955. [CrossRef] [PubMed]
5. Razo, O.P.B.; Mendoza, M.G.D.; Vázquez, S.G.; Osorio, T.A.I.; González, S.J.F.; Hernández, G.P.A.; Torre, H.M.E.; Espinosa, A.E. Polyherbal feed additive for lambs: Effects on performance, blood biochemistry and biometry. *J. Appl. Anim. Res.* **2020**, *48*, 419–424. [CrossRef]
6. Díaz, G.C.; Méndez, O.E.T.; Martínez, G.D.; Gloria, T.A.; Hernández, G.P.A.; Espinosa, A.E.; Palacios, M.M.; Lara, B.A.; Mendoza, M.G.D.; Velázquez, C.L.A. Influence of a polyherbal mixture in dairy calves: Growth performance and gene expression. *Front. Vet. Sci.* **2021**, *7*, 623710. [CrossRef] [PubMed]
7. Čobanová, K.; Váradyová, Z.; Grešáková, L.; Kucková, K.; Mravčáková, D.; Várady, M. Does herbal and/or zinc dietary supplementation improve the antioxidant and mineral status of lambs with parasite infection? *Antioxidants* **2020**, *9*, 1172. [CrossRef]
8. Hao, X.; Wang, P.; Ren, Y.; Liu, G.; Zhang, J.; Leury, B.; Zhang, C. Effects of Astragalus membranaceus roots supplementation on growth performance, serum antioxidant and immune response in finishing lambs. *Asian-Australas. J. Anim.* **2020**, *33*, 965–972. [CrossRef]
9. Wang, X.J.; Ding, L.M.; Wei, H.Y.; Jiang, C.X.; Yan, Q.; Hu, C.S.; Jia, G.X.; Zhou, Y.Q.; Henkin, Z.; Degen, A.A. Astragalus membranaceus root supplementation improves average daily gain, rumen fermentation, serum immunity and antioxidant indices of Tibetan sheep. *Animal* **2021**, *15*, 100061. [CrossRef]
10. Choubey, M.; Pattanaik, A.K.; Baliyan, S.; Dutta, N.; Jadhav, S.E.; Sharma, K. Dietary supplementation of a novel phytogenic feed additive: Effects on nutrient metabolism, antioxidant status and immune response of goats. *Anim. Prod. Sci.* **2016**, *56*, 1612–1621. [CrossRef]
11. McMurphy, C.P.; Sexten, A.L.; Mourer, G.L.; Sharman, E.D.; Trojan, S.J.; Rincker, M.J.; Coblentz, W.K.; Lalman, D.L. Effects of including saponins (Micro-Aid®) on intake, rumen fermentation and digestibility in steers fed low-quality prairie hay. *Anim. Feed Sci. Technol.* **2014**, *190*, 47–58. [CrossRef]
12. Liu, Y.; Ma, T.; Chen, D.; Zhang, N.; Si, B.; Deng, K.; Tu, Y.; Diao, Q. Effects of tea saponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in Dorper crossbred ewe. *Animals* **2019**, *9*, 29. [CrossRef]
13. Molina-Botero, I.C.; Arroyave-Jaramillo, J.; Valencia-Salazar, S.; Barahona-Rosales, R.; Aguilar-Pérez, C.F.; Ayala, B.A.; Arango, J.; Ku-Vera, J.C. Effect of tannins and saponins contained in foliage of Gliricidia sepium and pods of Enterolobium cyclocarpum on fermentation, methane emissions and rumen microbial population in crossbred heifers. *Anim. Feed Sci. Technol.* **2019**, *251*, 1–11. [CrossRef]
14. Mao, H.L.; Wang, J.K.; Zhou, Y.Y.; Liu, J.X. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. *Livest. Sci.* **2010**, *129*, 56–62. [CrossRef]

15. Hassan, F.U.; Arshad, M.A.; Li, M.; Rehman, M.S.U.; Loor, J.J.; Huang, J. Potential of mulberry leaf biomass and its flavonoids to improve production and health in ruminants: Mechanistic insights and prospects. *Animals* **2020**, *10*, 2076. [CrossRef] [PubMed]
16. Petrič, D.; Mravčáková, D.; Kucková, K.; Kišidayová, S.; Cieslak, A.; Szumacher-Strabel, M.; Huang, H.; Kolodziejski, P.; Lukomska, A.; Slusarczyk, S.; et al. Impact of zinc and/or herbal mixture on ruminal fermentation, microbiota, and histopathology in lambs. *Front. Vet. Sci.* **2021**, *8*, 630971. [CrossRef] [PubMed]
17. Paniagua, M.; Crespo, F.J.; Arís, A.; Devant, M. Effects of flavonoids extracted from citrus aurantium on performance, behavior, and rumen gene expression in holstein bulls fed with high-concentrate diets in pellet form. *Animals* **2021**, *11*, 1387. [CrossRef] [PubMed]
18. Mandal, G.P.; Roy, A.; Patra, A.K. Effects of feeding plant additives rich in saponins and essential oils on the performance carcass traits and conjugated linoleic acid concentration in muscle and adipose tissues of Black Bengal goats. *Anim. Feed Sci. Technol.* **2014**, *197*, 76–84. [CrossRef]
19. Hundal, J.S.; Wadhwa, M.; Bakshi, M.P.S.; Chatli, M.K. Effect of herbal feed additive containing saponins on the performance of goat kids. *Indian J. Anim. Sci.* **2020**, *90*, 229–236.
20. Wina, E.; Muetzel, S.; Hoffmann, E.; Makkar, H.P.S.; Becker, K. Saponins containing methanol extract of Sapindus rarak affect microbial fermentation, microbial activity and microbial community structure in vitro. *Anim. Feed Sci. Technol.* **2005**, *121*, 159–174. [CrossRef]
21. Nasehi, M.; Torbatinejad, N.M.; Rezaie, M.; Ghoorchi, T. Effects of partial substitution of alfalfa hay with green tea waste on growth performance and in vitro methane emission of fat-tailed lambs. *Small Rumin. Res.* **2018**, *168*, 52–59. [CrossRef]
22. Cardozo, P.W.; Calsamiglia, S.; Ferret, A.; Kamel, C. Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. *J. Anim. Sci.* **2005**, *83*, 2572–2579. [CrossRef] [PubMed]
23. García, E. *Modificaciones del Sistema de Clasificación Climática de Köppen*, 5th ed.; Instituto de Geografía, Universidad Nacional Autónoma de México: México City, Mexico, 2004; p. 51. (In Spanish)
24. Awais, M.M.; Akhtar, M.; Anwar, M.I.; Khaliq, K. Evaluation of Saccharum officinarum L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. *Vet. Parasitol.* **2018**, *249*, 74–81. [CrossRef] [PubMed]
25. Chothani, D.L.; Vaghasiya, H.U. A review on Balanites aegyptiaca Del (desert date): Phytochemicals constituents, traditional uses, and pharmacological activity. *Phcog. Rev.* **2011**, *5*, 55–62. [CrossRef] [PubMed]
26. Kukhetpitakwong, R.; Hahnvajanawong, C.; Homchampa, P.; Leelavatcharamas, V.; Satra, J.; Khunkitti, W. Immunological adjuvant activities of saponin extracts from the pods of Acacia concinna. *Int. Immunopharmacol.* **2006**, *6*, 1729–1735. [CrossRef]
27. NRC. *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*; National Academies Press: Washington, DC, USA, 2007.
28. AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists, The William Byrd Press Inc.: Richmond, VA, USA, 1990.
29. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef]
30. Van Keulen, J.V.; Young, B.A. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* **1977**, *44*, 282–287. [CrossRef]
31. Silva, S.R.; Gomes, M.J.; Dias-da-Silva, A.; Gil, L.F.; Azevedo, J.M.T. Estimation in vivo of the body and carcass chemical composition of growing lambs by real-time ultrasonography. *J. Anim. Sci.* **2005**, *83*, 350–357. [CrossRef]
32. Zimerman, M.; Domingo, E.; Lanari, M.R. Carcass characteristics of Neuquén Criollo kids in Patagonia region, Argentina. *Meat Sci.* **2008**, *79*, 453–457. [CrossRef]
33. Vazquez-Mendoza, O.V.; Aranda-Osorio, G.; Huerta-Bravo, M.; Kholif, A.E.; Elghandour, M.M.Y.; Salem, A.Z.M.; Maldonado-Simán, E. Carcass and meat properties of six genotypes of young bulls finished under feedlot tropical conditions of Mexico. *Anim. Prod. Sci.* **2017**, *57*, 1186–1192. [CrossRef]
34. Wheeler, T.L.; Shackelford, S.D.; Johnson, L.P.; Miller, M.F.; Miller, R.K.; Koohmaraie, M. A comparison of Warner-Bratzler shear force assessment within and among institutions. *J. Anim. Sci.* **1997**, *75*, 2423–2432. [CrossRef] [PubMed]
35. Miltenburg, G.A.; Wensing, T.; Smulders, F.J.M.; Breukink, H.J. Relationship between blood hemoglobin, plasma and tissue iron, muscle heme pigment, and carcass color of veal. *J. Anim. Sci.* **1992**, *70*, 2766–2772. [CrossRef] [PubMed]
36. Ripoll, G.; Albertí, P.; Joy, M. Influence of alfalfa grazing based feeding systems on carcass fat colour and meat quality of light lambs. *Meat Sci.* **2012**, *90*, 457–464. [CrossRef] [PubMed]
37. Negrete, L.O.; Pinos-Rodríguez, J.M.; Grajales-Lagunes, A.; Morales, J.A.; García-López, J.C.; Lee-Rangel, H.A. Effects of increasing amount of dietary Prosopis laevigata pods on performance, meat quality and fatty acid profile in growing lambs. *J. Anim. Physiol. Anim. Nutr.* **2017**, *101*, 303–311. [CrossRef]
38. Honikel, K.O. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* **1998**, *49*, 447–457. [CrossRef]
39. SAS (Statistical Analysis System). *SAS/STAT User's Guide (Release 6.4)*; SAS Inst.: Cary, NC, USA, 2017.
40. Littell, R.C.; Henry, P.R.; Ammerman, C.B. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **1998**, *76*, 1216–1231. [CrossRef] [PubMed]
41. Wei, H.; Ding, L.; Wang, X.; Yan, Q.; Jiang, C.; Hu, C.; Wang, G.; Zhou, Y.; Henkin, Z.; Degen, A.A. Astragalus root extract improved average daily gain, immunity, antioxidant status and ruminal microbiota of early weaned yak calves. *J. Sci. Food Agric.* **2020**, *101*, 82–90. [CrossRef] [PubMed]

42. Liu, C.; Qu, Y.-H.; Guo, P.-T.; Xu, C.-C.; Ma, Y.; Luo, H.-L. Effects of dietary supplementation with alfalfa (*Medicago sativa* L.) saponins on lamb growth performance, nutrient digestibility, and plasma parameters. *Anim. Feed Sci. Technol.* **2018**, *236*, 98–106. [CrossRef]
43. Nasri, S.; Ben Salem, H.; Vasta, V.; Abidi, S.; Makkar, H.P.S.; Priolo, A. Effect of increasing levels of Quillaja saponaria on digestion, growth and meat quality of Barbarine lamb. *Anim. Feed Sci. Technol.* **2011**, *164*, 71–78. [CrossRef]
44. Cheok, C.Y.; Salman, H.A.K.; Sulaiman, R. Extraction and quantification of saponins: A review. *Food Res. Int.* **2014**, *59*, 16–40. [CrossRef]
45. Wang, B.; Ma, M.P.; Diao, Q.Y.; Tu, Y. Saponin-induced shifts in the rumen microbiome and metabolome of young cattle. *Front. Microbiol.* **2019**, *10*, 356. [CrossRef]
46. Abdallah, A.; Zhang, P.; Elemba, E.; Zhong, Q.; Sun, Z. Carcass characteristics, meat quality, and functional compound deposition in sheep fed diets supplemented with Astragalus membranaceus by-product. *Anim. Feed Sci. Technol.* **2020**, *259*, 114346. [CrossRef]
47. Liang, H.; Xu, L.; Zhao, X.; Pan, K.; Yi, Z.; Bai, J.; Qi, X.; Xin, J.; Li, M.; Ouyang, K.; et al. RNA-Seq analysis reveals the potential molecular mechanisms of daidzein on adipogenesis in subcutaneous adipose tissue of finishing Xianan beef cattle. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 1–11. [CrossRef]
48. de Lima, J.D.M.; de Carvalho, F.F.; da Silva, F.J.; Rangel, A.H.D.N.; Novaes, L.P.; Difante, G.D.S. Intrinsic factors affecting sheep meat quality: A review. *Rev. Colomb. Cienc. Cienc.* **2016**, *29*, 3–15. [CrossRef]
49. Corazzin, M.; Del Bianco, S.; Bovolenta, S.; Piasentier, E. Carcass characteristics and meat quality of sheep and goat. In *More than Beef, Pork and Chicken-The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet*; Lorenzo, J.M., Munekata, P.E.S., Barba, F., Toldrá, F., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 119–165, ISBN 978-3-030-05483-0.
50. Riley, R.R.; Savell, J.W.; Shelton, M.; Smith, G.C. Carcass and offal yields of sheep and goats as influenced by market class and breed. *Small Rumin. Res.* **1989**, *2*, 265–272. [CrossRef]
51. Fluharty, F.L.; McClure, K.E. Effects of dietary energy intake and protein concentration on performance and visceral organ mass in lambs. *J. Anim. Sci.* **1997**, *75*, 604–610. [CrossRef] [PubMed]
52. Sañudo, C.; Santolaria, M.P.; Maria, G.; Osorio, M.; Sierra, I. Influence of carcass weight on instrumental and sensory lamb meat quality in intensive production systems. *Meat Sci.* **1996**, *42*, 195–202. [CrossRef]
53. Dalle, Z.O. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livest. Prod. Sci.* **2002**, *75*, 11–32. [CrossRef]
54. Toldrá, F. *Lawrie's Meat Science*, 8th ed.; Woodhead Publishing Limited: Cambridge, UK, 2017; p. 713.
55. Ponnampalam, E.N.; Hopkins, D.L.; Bruce, H.; Li, D.; Baldi, G.; El-din Bekhit, A. Causes and contributing factors to dark cutting meat: Current trends and future directions: A review. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 400–430. [CrossRef] [PubMed]
56. Watanabe, A.; Daly, C.; Devine, C. The effects of the ultimate pH of meat on tenderness changes during ageing. *Meat Sci.* **1996**, *42*, 67–78. [CrossRef]
57. Qin, X.; Zhang, T.; Cao, Y.; Deng, B.; Zhang, J.; Zhao, J. Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. *Meat Sci.* **2020**, *166*, 108141. [CrossRef]
58. Zamora, F.; Aubry, L.; Sayd, T.; Lepetit, J.; Lebert, A.; Sentandreu, M.A.; Ouali, A. Serine peptidase inhibitors, the best predictor of beef ageing amongst a large set of quantitative variables. *Meat Sci.* **2005**, *71*, 730–742. [CrossRef]
59. Whipple, G.; Koohmaraiae, M.; Dikeman, M.E.; Crouse, J.D.; Hunt, M.C.; Klemm, R.D. Evaluation of attributes that affect Longissimus muscle tenderness in Bos taurus and Bos indicus cattle. *J. Anim. Sci.* **1990**, *68*, 2716–2728. [CrossRef]
60. Wiklund, E.; Dobbie, P.; Stuart, A.; Littlejohn, R.P. Seasonal variation in red deer (Cervus elaphus) venison (M. longissimus dorsi) dripp loss, calpain activity, colour and tenderness. *Meat Sci.* **2010**, *86*, 720–727. [CrossRef] [PubMed]
61. Webb, E.C.; Agbeniga, B. Timing and duration of low voltage electrical stimulation on selected meat quality characteristics of light and heavy cattle carcasses. *Anim. Prod. Sci.* **2020**, *60*, 967–977. [CrossRef]
62. Cimmino, R.; Barone, C.M.A.; Claps, S.; Varricchio, E.; Rufrano, D.; Caroprese, M.; Albenzio, M.; De Palo, P.; Campanile, G.; Neglia, G. Effects of dietary supplementation with polyphenols on meat quality in Saanen goat kids. *BMC Vet. Res.* **2018**, *14*, 181. [CrossRef]
63. Garcia-Galicia, I.A.; Arras-Acosta, J.A.; Huerta-Jimenez, M.; Rentería-Monterrubio, A.L.; Loya-Olguin, J.L.; Carrillo-Lopez, L.M.; Tirado-Gallegos, J.M.; Alarcon-Rojo, A.D. Natural oregano essential oil may replace antibiotics in lamb diets: Effects on meat quality. *Antibiotics* **2020**, *9*, 248. [CrossRef] [PubMed]
64. Luo, Y.; Su, L.; Su, R.; Wang, B.; Liu, C.; Wang, Z.; Zhao, L.; Jin, Y. Effects of Astragalus Membranaceus supplementation on oxidative stability of Cashmere goat. *Food Sci. Nutr.* **2020**, *8*, 5550–5556. [CrossRef]
65. Lopez-Baca, M.A.; Contreras, M.; González-Ríos, H.; Macías-Cruz, U.; Torrentera, N.; Valenzuela-Melendres, M.; Muhlia-Almazán, A.; Soto-Navarro, S.; Avendaño-Reyes, L. Growth, carcass characteristics, cut yields and meat quality of lambs finished with zilpaterol hydrochloride and steroid implant. *Meat Sci.* **2019**, *158*, 107890. [CrossRef] [PubMed]
66. Dávila-Ramírez, J.L.; Avendaño-Reyes, L.; Macías-Cruz, U.; Torrentera-Olivera, N.G.; Zamorano-García, L.; Peña-Ramos, A.; González-Ríos, H. Effects of zilpaterol hydrochloride and soybean oil supplementation on physicochemical and sensory characteristics of meat from hair lambs. *Small Rumin. Res.* **2013**, *114*, 253–257. [CrossRef]

This chapter was published in *Animals*

4. GROWTH PERFORMANCE, MEAT QUALITY AND ANTIOXIDANT STATUS OF SHEEP SUPPLEMENTED WITH TANNINS: A META-ANALYSIS

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1] and Héctor Aarón Lee-Rangel [3]

[1]Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo 56230, México; jforzuna@gmail.com (J.F.O.-O.); griseldi0993@gmail.com (G.D.-I.); albertomiranda@correo.chapingo.mx (L.A.M.-R.)

[2]Departamento de Producción Agrícola y Animal, Unidad Xochimilco, Universidad Autónoma Metropolitana, México City 04960, México; gmendoza@correo.xoc.uam.mx

[3]Centro de Biociencias, Facultad de Agronomía y Veterinaria, Instituto de Investigaciones en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, San Luis Potosí CP 78321, México; hector.lee@uaslp.mx (H.A.L.-R.)

Corresponding author.

E-mail address: alarab_11@hotmail.com (A. Lara-Bueno).

animals

MDPI

Review

Growth Performance, Meat Quality and Antioxidant Status of Sheep Supplemented with Tannins: A Meta-Analysis

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Germán David Mendoza-Martínez [2], Luis Alberto Miranda-Romero [1] and Héctor Aarón Lee-Rangel [3]

[1] Departamento de Zootecnia, Universidad Autónoma Chapingo, Chapingo CP 56230, Mexico; jforzuna@gmail.com (J.F.O.-O.); griseldi0993@gmail.com (G.D.-I.); albertomiranda@correo.chapingo.mx (L.A.M.-R.)
[2] Unidad Xochimilco, Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Mexico City CP 04960, Mexico; gmendoza@correo.xoc.uam.mx
[3] Centro de Biociencias, Facultad de Agronomía y Veterinaria, Instituto de Investigaciones en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, San Luis Potosí CP 78321, Mexico; hector.lee@uaslp.mx
* Correspondence: alarab_11@hotmail.com

Simple Summary: Tannins can be used to improve productive performance, meat quality and antioxidant status of ruminants. The objective of this study was to evaluate the effects of dietary tannin supplementation on productive performance, carcass characteristics, meat quality and blood serum antioxidant status of sheep through a meta-analysis. Only studies with weaned or older sheep were included. The sheep included in the present study were between 2 and 6 months old, and between 12 and 31 kg of body weight. Tannin supplementation improved productive performance, carcass yield, meat oxidative stability and blood serum antioxidant capacity. This suggests that the inclusion of tannins in sheep diets could be used to improve growth and reduce oxidative stress in animals, and to improve meat quality and shelf life.

Abstract: The objective of this study was to evaluate the effects of dietary supplementation with tannins (TANs) on productive performance, carcass characteristics, meat quality, oxidative stability, and blood serum antioxidant capacity of sheep through a meta-analysis. Using Scopus, Web of Science, ScienceDirect, and PubMed databases, a systematic search was performed for studies published in scientific journals that investigated the effects of TANs supplementation on the variables of interest. Only studies with weaned or older sheep were included. The data analyzed were extracted from 53 peer-reviewed publications. The sheep included in the present study were between 2 and 6 months old, and between 12 and 31 kg of body weight. The effects of TANs were analyzed using random-effects statistical models to examine the standardized mean difference (SMD) between treatments with TANs and control (no TANs). Heterogeneity was explored by meta-regression and a subgroup analysis was performed for covariates that were significant. Supplementation with TANs did not affect dry matter intake, pH, color (L* and b*), Warner–Bratzler shear force, cooking loss and meat chemical composition ($p > 0.05$). Supplementation with TANs increased daily weight gain (SMD = 0.274, $p < 0.05$), total antioxidant capacity (SMD = 1.120, $p < 0.001$), glutathione peroxidase enzyme activity (SMD = 0.801, $p < 0.001$) and catalase (SMD = 0.848, $p < 0.001$), and decreased malondialdehyde (MDA) concentration in blood serum (SMD = −0.535, $p < 0.05$). Supplementation with TANs decreased feed conversion rate (SMD = −0.246, $p < 0.05$), and the concentration of MDA (SMD = −2.020, $p < 0.001$) and metmyoglobin (SMD = −0.482, $p < 0.05$) in meat. However, meat redness (SMD = 0.365), hot carcass yield (SMD = 0.234), cold carcass yield (SMD = 0.510), backfat thickness (SMD = 0.565) and the *Longissimus dorsi* muscle area (SMD = 0.413) increased in response to TANs supplementation ($p < 0.05$). In conclusion, the addition of tannins in sheep diets improves productive performance, antioxidant status in blood serum, oxidative stability of meat and some other characteristics related to meat and carcass quality.

Keywords: oxidative stability; natural antioxidants; polyphenolic compounds; meta-regression

Citation: Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Lee-Rangel, H.A. Growth Performance, Meat Quality and Antioxidant Status of Sheep Supplemented with Tannins: A Meta-Analysis. *Animals* **2021**, *11*, 3184. https://doi.org/10.3390/ani11113184

Academic Editors: Susana Chamorro and Carlos Romero

Received: 5 October 2021
Accepted: 5 November 2021
Published: 8 November 2021

1. Introduction

Antibiotics (e.g., monensin) have been used for several decades as growth promoters in animals [1]. However, the inappropriate use of these products results in an accumulation of toxic residues in meat, which can affect the health of the consumer [2,3]. In addition, the emergence of bacterial strains resistant to the antibiotic effects [4] as well as the prohibition of these compounds in some countries [5] have led the industry and researchers to search for alternative products with similar effects as antibiotics, but of natural origin. Tannins (TANs), which are derived from plants, have received special attention and are among the most studied bioactive compounds, particularly in ruminants [1]. TANs are a group of polyphenolic compounds present in a wide variety of plants, which can be grouped into hydrolysable tannins (HTs) and condensed tannins (CTs) based on their chemical structure [6,7]. TANs can produce positive effects in animals, such as those of the antioxidant, antimicrobial, antiparasitic, immunomodulatory, and anti-inflammatory varities [1,6].

TANs can play an important role in the nutritional value of the feed, the quality of the products obtained, and the health and welfare of the animals [8]. Dietary inclusion of TANs at low to moderate concentrations (20 to 45 g kg^{-1} DM) can improve growth rate and feed utilization efficiency in ruminants, mainly due to a reduction in protein degradation in the rumen and a subsequent increase in the flow of amino acids to the small intestine [9–11]. However, large amounts (>55 g kg^{-1} DM) of TANs in the diet reduce feed intake, rumen microbial activity, nutrient digestibility and endogenous digestive enzyme activity [1,9,10,12], resulting in lower feed efficiency and growth rate.

Particularly in sheep, several studies have been conducted to evaluate the effects of dietary supplementation with extracts of TANs and TANs-rich plants on productive performance [13,14], carcass characteristics [15,16], oxidative stability and physicochemical characteristics of meat [17–20], and blood serum antioxidant status [21,22]. However, the results are still not consistent, probably as a consequence of variability among the studies regarding feeding conditions, age of the animals, type of product used, dosage and source of TANs [1,8]. Therefore, identifying the factors that contribute to this variability is a key aspect in the development of products containing TANs that can be used to improve productive performance, meat and carcass quality, and antioxidant status of sheep.

Some review articles [1,8,23,24] have suggested that dietary supplementation with TANs can improve productive performance, meat quality and antioxidant status of livestock. However, these reviews did not use a meta-analysis approach and also did not focus only on sheep. In addition, narrative reviews can lead to biased conclusions because they lack a methodological approach and are subjective to the author's interpretation of previous research [25]. In contrast, meta-analysis (MA) is a statistical tool that allows synthesizing data published in different studies in a quantitative way [26,27]. Furthermore, MA allows us to explore the heterogeneity sources among the diverse studies, which helps to obtain additional information about the factors contributing to the variability of the observed outcomes in response to a specific treatment [28]. MA has been frequently used in biomedical and clinical research, but its use in research related to secondary plant metabolites and meat science is still limited [29]. The objective of this meta-analysis was to evaluate the effect of dietary supplementation with tannins on productive performance, carcass characteristics, meat quality and oxidative stability, and antioxidant status of sheep blood plasma. The heterogeneity of responses was also examined using meta-regression analysis with the purpose of identifying factors contributing to the variability in the response variables.

2. Materials and Methods

2.1. Literature Search and Study Selection

To perform a robust meta-analysis, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [30] were used in the identification, selection, choice and inclusion of information, as shown in Figure S1. To identify studies that evaluated the effect of supplementation with TANs on productive performance, carcass and meat quality characteristics, and antioxidant status of sheep blood serum, a systematic

literature search was performed in the scientific databases of Scopus, ScienceDirect, Web of Science, and PubMed. The following keywords were used in all databases: tannin, lamb, sheep, growth performance, intake, carcass characteristics, meat quality, antioxidant status, and oxidative stability. The search and selection process was limited to the results of papers published between January 2010 and June 2021, where 1157 scientific publications were identified (Figure S1). These publications went through a two-step selection process as previously described by other authors [31,32]. First, a selection of titles and abstracts was performed excluding simulation studies, review articles, studies not conducted in sheep, in-vitro studies, and articles that did not include the variables of interest. Subsequently, to be considered, studies had to meet inclusion criteria previously used by other authors [31–33]: (1) Use of sheep and specify the procedure used to randomly assign animals within treatments; (2) data on productive performance, oxidative stability of meat, meat and/or carcass quality characteristics, or blood serum antioxidant status; (3) similarity between control and experimental groups except for the presence of TANs; (4) quantification or possible determination of the amount of TANs in the diet; (5) peer-reviewed journal articles written in English; (6) least squares means of the control and experimental groups with measures of variability (standard deviation or standard error); and (7) sample size.

2.2. Data Extraction

After exclusion of duplicate papers and selection of titles and abstracts, 99 full-text articles were evaluated; of these, only 53 articles met the inclusion criteria (Table A1) and were used to obtain the quantitative data for the meta-analysis. To be considered, variables had to be reported in at least three studies [32,34]. Consequently, the response variables included in the meta-analysis were: daily weight gain, dry matter intake, feed conversion rate (feed intake/weight gain), hot and cold carcass yield, backfat thickness, *Longissimus dorsi* muscle area, meat quality characteristics (pH, color, chemical composition, malondialdehyde content, among others), as well as total antioxidant capacity, malondialdehyde content (as an indicator of lipid oxidation) and antioxidant enzyme activity (superoxide dismutase, catalase and glutathione peroxidase) in blood serum. In addition, when available, additional data were collected, such as: characteristics of the published study (author and year of publication), chemical composition of the diet, amount of forage in the diet (g kg^{-1} DM), number of replicates, amount of TANs in the diet (g kg^{-1} DM), period of supplementation with TANs (days), type of TANs (HTs, CTs or mixture of both), source of botanical origin of the TANs, and method of inclusion of the TANs (extract or naturally present in the diet). The references of the articles included in the dataset are listed in Table A1. Averages, standard deviation (SD), and number of replicates for each treatment were extracted from these articles. When the articles presented the SD of each experimental group, these values were used directly in the meta-analysis. Where the SD was not reported, it was calculated by multiplying the standard error of the means (SEM) by the square root of the sample size, using the equation: $SD = SEM \times \sqrt{n}$, as previously reported by Higgins and Thomas [35], where n = number of replicates.

2.3. Calculations and Statistical Analysis

Meta-analysis and meta-regression data were analyzed using the Open Meta-analyst for Ecology and Evolution software [36]. Response variables were analyzed using the standardized mean difference (SMD), also referred to as effect size (ES), in which the difference between the means of the experimental and control groups was standardized using the SD of the groups with and without TANs [37]. SMDs were calculated using methods previously described by DerSimonian and Laird [38] for random-effects models. The SMD is a more robust estimate of ES when heterogeneity exists in the data set [39]. The variables of the chemical composition of the diets were analyzed with the MEANS procedure using the SAS statistical program [40] to obtain descriptive statistics values. Differences in the composition of the diets of the control and TANs-supplemented treatments were evaluated

by the MIXED procedure, using the studies as random effect and Tukey's test to detect differences between treatments, as previously reported by other authors [32,41].

2.4. Heterogeneity

Heterogeneity was measured using the I^2 statistic and the chi-square (Q) test [42]. Because of the relatively low capacity of the Q test to detect heterogeneity among a small number of treatment comparisons, an α level of 0.10 was used [39,43]. I^2 (percentage of variation) values range from 0 to 100%, where values close to 25% indicate low heterogeneity, close to 50% indicate moderate heterogeneity, and close to 75% indicate high heterogeneity between studies [26,28]. Likewise, I^2 values greater than 50% indicate significant heterogeneity [33].

2.5. Publication Bias

Since a visual inspection of funnel plots (generally used to assess publication bias) is subjective and must be balanced with additional analyses [44], three methods were used to assess evidence of publication bias: (1) the funnel plot [45]; (2) Begg's adjusted rank correlation [46]; and (3) Egger's regression asymmetry test [47]. Bias was considered to be present when the funnel plot showed asymmetry, or when at least one of the statistical methods (Begg's test and Egger's test) was significant ($p < 0.10$). Tests to assess publication bias are inappropriate when the variable to be assessed is not reported in at least 10 studies, and when significant heterogeneity (Q) is detected with an $\alpha \leq 0.10$, because it may lead to false-positive claims [48]. Consequently, funnel plots, Begg's test, and Egger's test were only performed for variables that met the aforementioned criteria. In cases where statistical evidence of publication bias was found, the "trim-and-fill" method of Duval and Tweedie was used to estimate the number of possible missing observations [49].

2.6. Meta-Regression

Sources of parameter heterogeneity that showed Q with an α level of $\leq$0.10 [43] or I^2 greater than 50% [26] were assessed by meta-regression analysis. Similar to the publication bias tests, meta regression analysis was only performed for response variables that were reported in at least 10 studies [44]. The meta regression was estimated using the DerSimonian and Laird method of moments, which is well known for estimating variance among studies [26]. Continuous and categorical variables were used in the meta-regression. The continuous variables included: differences in neutral detergent fiber (NDF) and ether extract (EE) content in the diets (g kg^{-1} DM), TANs dose (g kg^{-1} DM), and duration of the experimental phase (days). Categorical variables included: source of botanical origin of TANs, method by which TANs were supplied (extract or as part of some dietary ingredient), type of TANs (CTs, HTs or mixture of both), and age of animals ($\leq$3 months of age or >3 months of age). When categorical covariates were significant at an α level of $\leq$0.05, SMD was assessed by subgroup analysis [32,41]. Likewise, when the meta regression was significant ($p \leq 0.05$) for supplementation level and experimental period, these covariates were evaluated by subgroup analysis by dividing the covariates as follows: dietary TANs supplementation level ($\leq$20 or >20 g kg^{-1} DM); and experimental period ($\leq$70 or >70 days).

3. Results

3.1. Study Attributes and Excluded Studies

Descriptive statistics and mean test for diet composition are presented in Table 1. Except for NDF, EE and organic matter (OM) content, no significant differences were observed between the control and TANs treatment for the rest of the nutritional components of the diet. Among the nutritional components, only fiber and fat content seem to have considerable effects on productive performance, carcass characteristics and meat quality [50]. Thus, it is possible to exclude the effects of the rest of the diet components on the response of animals to TANs supplementation for the data set.

Table 1. Descriptive statistics of the complete data set for the effect of tannins supplementation to sheep diets.

Parameter		Mean		Median		Minimum		Maximum		SD	
Dietary Features	**NC**	**Control**	**Tannin**	**Control**	**Tannin**	**Control**	**Tannin**	**Control**	**Tannin**	**Control**	**Tannin**
Forage, g kg^{-1} DM	122	428.5	432.2	400.0	400.0	0	0	1000	1000	265.6	261.3
DM, g kg^{-1}	100	874.8	871.7	900.0	903.4	160.0	156.0	953.9	947.5	122.8	130.8
OM, g kg^{-1} DM	46	857.5 ^{b}	866.8 ^{a}	910.0	922.0	148.0	146.0	957.2	984.5	193.7	188.7
CP, g kg^{-1} DM	124	156.3	156.9	157.0	156.5	84.0	89.0	251.0	255.0	26.9	26.5
EE, g kg^{-1} DM	106	35.5 ^{b}	40.7 ^{a}	29.3	36.3	13.0	12.8	81.0	98.3	16.6	18.3
NDF, g kg^{-1} DM	122	388.8 ^{a}	375.9 ^{b}	385.7	365.5	156.0	152.0	731.1	704.9	179.6	108.7
ADF, g kg^{-1} DM	94	211.2	212.1	190.0	179.5	81.0	69.8	516.0	496.5	94.1	94.2
Ca, g kg^{-1} DM	24	7.0	7.2	6.1	6.7	3.4	3.6	17.0	18.0	3.1	3.4
P, g kg^{-1} DM	22	4.0	4.0	4.4	4.4	2.0	2.0	5.8	6.2	1.2	1.3
ME, MJ kg^{-1} DM	56	10.6	10.6	10.5	10.7	9.2	8.8	12.5	12.6	0.8	0.9
Tannin, g kg^{-1} DM	135	-	20.2	-	15.5	-	0.02	-	132.0	-	20.6
Duration, days	135	70.0		70.0		28.0		180.0		30.0	

NC: number of comparisons; SD: standard deviation; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; P: phosphorus; MJ: megajoule; ^{a},^{b}: in the same row, means followed by different letters differ significantly by the Tukey test ($p < 0.05$).

The studies included in the present meta-analysis were conducted in 21 different countries, predominantly in Brazil (17%) and Iran (13.2%). The experimental doses of TANs ranged from 0.02 to 132 g kg^{-1} DM, and the duration of the experimental periods varied from 28 to 180 days (Table 1). The TANs used were divided into: CTs, HTs and mixture of both. Of the treatments, 54.7% used mixtures of CTs and HTs, 39.6% used CTs and the remaining 5.7% used HTs. Moreover, 79.3% of the treatments used plant parts, forages or by-products containing TANs in natural form, and 20.7% used extracts of TANs in the diets. The studies included in the meta-analysis used a total of 36 different sources of TANs, the majority of treatments (13.2%) used TANs from Vitis vinifera, 9.4% from Cistus ladanifer, 7.5% from plant mixtures and 9.4% from pomegranate (Punica granatum) and in the other 60.5% of the treatments, 32 other different sources of TANs were used.

3.2. Growth Performance and Carcass Characteristics

Table 2 shows that dietary supplementation with TANs increased ($p < 0.05$) daily weight gain (DWG), hot carcass yield (HCY), cold carcass yield (CCY), backfat thickness (BFT) and *Longissimus dorsi* muscle area (LMA). There was no observed a significant impact of the inclusion of TANs in the diet on dry matter intake (DMI; $p > 0.05$). In addition, feed conversion ratio (FCR) decreased in response to dietary supplementation with TANs ($p < 0.05$).

Table 2. Growth performance and carcass characteristics of sheep supplemented with tannins.

					95% CI			Heterogeneity		
Parameter	**N**	**NC**	**SMD**	**SE**	**Lower**	**Upper**	***p*-Value**	**Q**	***p*-Value**	**I^2 (%)**
Daily weight gain (DWG)	42	104	0.274	0.116	0.046	0.501	0.018	472.57	<0.001	78.20
Dry matter intake (DMI)	42	104	0.090	0.124	−0.152	0.333	0.466	524.508	<0.001	80.36
Feed conversion ratio (FCR)	27	60	−0.308	0.127	−0.556	−0.060	0.015	197.05	<0.001	70.06
Hot carcass yield (HCY)	26	59	0.234	0.108	0.023	0.445	0.030	142.03	<0.001	59.16
Cold carcass yield (CCY)	9	23	0.510	0.228	0.063	0.957	0.025	86.09	<0.001	74.45
Backfat thickness (BFT)	9	24	0.565	0.193	0.188	0.943	0.003	77.94	<0.001	70.49
Longissimus dorsi muscle area (LMA)	10	22	0.413	0.170	0.080	0.747	0.015	52.40	<0.001	59.92

N: number of studies; NC: number of comparisons; SMD: standardized mean difference; CI: confidence interval of SMD; SE: standard error. Q: chi-squared statistic and associated significance level (*p*-value); I^2: percentage of variation.

3.3. Meat Quality Characteristics

No significant effects of TANs inclusion in the sheep diet ($p > 0.05$) were observed on meat pH, meat lightness (L*) and yellowness (b*), Warner–Bratzler shear force (WBSF), meat cooking loss (CL), protein content, intramuscular fat (IMF) and meat ash (Table 3). Dietary supplementation with TANs decreased drip loss (DL) and meat moisture ($p < 0.05$). While meat redness (a*) increased in response to dietary supplementation with TANs

($p < 0.05$). In addition, malondialdehyde content in raw meat (MDAc) and metmyoglobin (MetMb) content of meat decreased in response to dietary supplementation with TANs ($p < 0.05$).

Table 3. Meat characteristics of sheep supplemented with tannins.

					95% CI			Heterogeneity		
Parameter	**N**	**NC**	**SMD**	**SE**	**Lower**	**Upper**	***p*-Value**	**Q**	***p*-Value**	**I^2 (%)**
Meat pH	19	52	0.037	0.098	−0.156	0.230	0.706	89.29	<0.001	42.88
Lightness (L*)	20	54	0.008	0.128	−0.243	0.260	0.950	151.88	<0.001	65.10
Redness (a*)	20	54	0.365	0.120	0.129	0.601	0.002	133.39	<0.001	62.27
Yellowness (b*)	20	54	0.048	0.145	−0.236	0.332	0.742	186.70	<0.001	71.61
WBSF	15	42	−0.027	0.093	−0.210	0.155	0.769	53.74	0.088	23.71
Drip loss (DL)	4	17	−2.828	0.516	−3.839	−1.817	<0.001	149.57	<0.001	89.30
Cooking loss (CL)	14	42	0.105	0.216	−0.317	0.528	0.626	243.00	<0.001	83.13
Moisture	5	16	−0.693	0.333	−1.345	−0.041	0.037	77.25	< 0.001	80.58
Protein	8	23	0.249	0.282	−0.304	0.802	0.378	114.45	<0.001	80.78
Intramuscular fat (IMF)	16	40	−0.168	0.186	−0.532	0.196	0.366	172.04	<0.001	77.33
Ash	6	20	0.507	0.332	−0.144	1.158	0.127	108.41	<0.001	82.47
Malondialdehyde (MDAc)	10	29	−2.020	0.326	−2.659	−1.380	<0.001	195.96	<0.001	85.65
Metmyoglobin (MetMb)	3	6	−0.482	0.222	−0.916	−0.047	0.030	5.25	0.387	4.68

N: number of studies; NC: number of comparisons; SMD: standardized mean difference; CI: confidence interval of SMD; SE: standard error. Q: chi-squared statistic and associated significance level (*p*-value); I^2: percentage of variation; WBSF: Warner–Bratzler shear force.

3.4. Antioxidant Status

Table 4 shows that dietary supplementation with TANs increased total antioxidant capacity (TAC), and catalase (CAT) and glutathione peroxidase (GPx) enzyme activity in blood serum ($p < 0.05$). On the other hand, the concentration of malondialdehyde in blood serum (MDAs) decreased ($p < 0.05$; Table 2) in response to TANs' supplementation. Moreover, no significant impact was observed for blood serum superoxide dismutase (SOD) enzyme activity ($p > 0.05$).

Table 4. Oxidative status of lambs supplemented with tannins.

					95% CI			Heterogeneity		
Parameter	**N**	**NC**	**SMD**	**SE**	**Lower**	**Upper**	***p*-Value**	**Q**	***p*-Value**	**I^2 (%)**
Total antioxidant capacity (TAC)	9	17	1.120	0.222	0.686	1.555	<0.001	43.661	<0.001	63.35
Superoxide dismutase (SOD)	6	14	−0.122	0.328	−0.766	0.521	0.709	61.306	<0.001	78.79
Catalase (CAT)	5	12	0.848	0.239	0.380	1.315	<0.001	22.963	0.018	52.10
Glutathione peroxidase (GPx)	3	6	0.801	0.209	0.392	1.211	<0.001	2.267	0.811	0
Malondialdehyde (MDAs)	7	17	−0.535	0.244	−1.014	-0.056	0.029	54.824	<0.001	70.81

N: number of studies; NC: number of comparisons; SMD: standardized mean difference; CI: confidence interval of SMD; SE: standard error. Q: chi-squared statistic and associated significance level (*p*-value); I^2: percentage of variation.

3.5. Analysis of Publication Bias

DWG, DMI, FCR, HCY, LMA, meat pH, meat color (L*, a* and b*), WBSF, CL, IMF and MDAc had significant heterogeneity (Q) with an $\alpha \leq 0.10$. Whereas CCY, BFT, DL, moisture, protein, ash, MetMb, TAC, SOD, CAT, GPx and MDAs were reported in less than 10 studies. Therefore, tests to assess publication bias were not performed for any variable, because under these conditions they may result in false positive claims [48].

3.6. Meta-Regression

Significant heterogeneity (Q; $p < 0.10$) was observed for DWG, DMI, FCR, HCY, LMA (Table 2), meat pH, meat color (L*, a* and b*), WBSF, DL, CL, moisture content, protein, IMF, meat ash, MDAc, MetMb (Table 3), TAC, SOD, CAT, and MDAs (Table 4). Since it is not advisable to use meta-regression when there are fewer than 10 studies that reported

the response variable of interest [44], this analysis was only performed for the variables: DWG, DMI, FCR, HCY, LMA, meat pH, L*, a*, b*, CL, IMF, and MDAc.

Table 5 shows that the dose of TANs explained ($p < 0.05$) 0.54, 3.02, 8.84, 14.48, 1.26, 9.56, and 17.0% of the heterogeneity observed for DWG, DMI, FCR, HCY, CL, IMF, and MDAc, respectively. The period of TANs supplementation had a significant relationship with DWG, DMI, FCR, LMA, a*, and MDAc ($p < 0.05$); however, it only explained between 1.15 and 26.30% of the observed heterogeneity in these variables. Animal age was significantly related to FCR, LMA, a*, b*, IMF and MDAc, explaining 6.57, 66.18, 28.53, 2.80, 14.55 and 56.60% of the observed heterogeneity, respectively ($p < 0.05$). The type of TANs explained between 11.65 and 54.54% of the observed heterogeneity for FCR, meat pH, L*, b* and MDAc ($p < 0.01$). The method of inclusion of TANs in the diet explained 1.17, 23.06 and 5.11 of the observed heterogeneity in DWG, meat pH and MDAc, respectively ($p < 0.05$). The botanical origin of TANs had a significant relationship with DMI, FCR, HCY, LMA, meat pH, L*, b*, CL, IMF and MDAc, explaining between 1.12 and 100% of the observed heterogeneity in these variables ($p < 0.01$). A significant relationship ($p < 0.001$) was observed between a* and MDAc with dietary ether extract content (EED), where the variation in EED explained 19.28 and 4.60% of the heterogeneity observed in a* and MDAc, respectively. A significant relationship ($p < 0.05$) was observed between HCY and a* with the neutral detergent fiber content of the diets (NDFD), where variation in NDFD explained 6.27 and 21.86% of the heterogeneity observed in HCY and a*, respectively.

3.7. Subgroup Analysis

Table S1 shows that DWG increased when doses of TANs lower than 20 g kg^{-1} DM were used (SDM = 0.485; $p < 0.001$), but doses higher than 20 g kg^{-1} DM did not affect DWG (SMD = −0.282; $p > 0.05$). Similarly, DMI increased when doses of TANs lower than 20 g kg^{-1} DM were used (SDM = 0.324; $p < 0.05$), but doses higher than 20 g kg^{-1} DM did not affect DMI (SMD = −0.416; $p > 0.05$). Additionally, animals from studies using doses lower than 20 g kg^{-1} DM had lower FCR (SMD = −0.423; $p < 0.001$), but no differences were observed with respect to FCR in animals from studies using doses higher than 20 g kg^{-1} DM (SMD = 0.640; $p > 0.05$). HCY was higher in animals supplemented with doses of TANs lower than 20 g kg^{-1} DM (SMD = 0.276; $p < 0.05$), whereas doses higher than 20 g kg^{-1} DM did not modify HCY (SMD = 0.152; $p > 0.05$). CL increased when TANs doses lower than 20 g kg^{-1} DM were used (SMD = 0.501; $p < 0.05$) but decreased with doses higher than 20 g kg^{-1} DM (SMD = −1.158; $p < 0.05$). Low doses of TANs (<20 g kg^{-1} DM) did not affect IMF content (SMD = 0.207; $p > 0.05$), but doses higher than 20 g kg^{-1} DM reduced IMF content (SMD = −0.691; $p < 0.001$). MDAc decreased regardless of the dose of TANs used ($p < 0.01$); however, the effect was greater when doses lower than 20 g kg^{-1} DM were used (SMD = −2.735) compared to doses higher than 20 g kg^{-1} DM (SMD = −1.058).

Table S2 shows that DWG increased in response to dietary supplementation with TANs regardless of the supplementation period ($p < 0.05$). However, the effect was greater when TANs were offered for more than 70 days (SMD = 0.515) compared to periods up to 70 days (SMD = 0.256). On the other hand, DMI increased when dietary supplementation with TANs lasted more than 70 days (SMD = 0.590; $p < 0.05$) but was not affected when TANs were offered for up to 70 days (SMD = −0.142; $p > 0.05$). In contrast, FCR decreased when dietary supplementation with TANs lasted more than 70 days (SMD = −0.549; $p < 0.05$) but was not affected when TANs were offered for up to 70 days (SMD = −0.197; $p > 0.05$). Additionally, a* of meat increased when dietary supplementation with TANs lasted more than 70 days (SMD = 0.981; $p < 0.001$) but was not affected when TANs were offered for up to 70 days (SMD = −0.029; $p > 0.05$). Higher LMA was observed when supplementation periods longer than 70 days were used (SMD = 0.870; $p < 0.01$), but when the period lasted less than 70 days no significant effects were observed in LMA (SMD = 0.063; $p > 0.05$). MDAc decreased in response to dietary supplementation with TANs regardless of the supplementation period used ($p < 0.001$). However, the effect was greater when TANs

were offered for more than 70 days (SMD = −2.706) compared to periods up to 70 days (SMD = −0.784).

Table 5. Meta-regression of the effects of dietary tannins supplementation on growth performance, meat quality and antioxidant status of sheep.

Parameter		Tannins Dose	Supplementation Period	Lamb's Age	Tannins Type	Tannins Source	Method of Inclusion	EED	NDFD
DWG	QM	6.943	8.263	0.378	1.070	43.329	5.786	1.092	0.240
	df	1	1	1	2	32	1	1	1
	p-Value	0.008	0.004	0.989	0.586	0.087	0.016	0.296	0.624
	R^2 (%)	0.54	2.85	0	0	0	1.17	0.92	0
DMI	QM	4.800	10.206	0.927	2.503	113.649	0.892	0.033	2.752
	df	1	1	1	2	32	1	1	1
	p-Value	0.028	0.001	0.336	0.286	<0.001	0.345	0.856	0.097
	R^2 (%)	3.02	2.49	0	0	14.37	0	0	0
FCR	QM	7.711	3.716	5.348	9.193	48.362	0.129	0.006	0.335
	df	1	1	1	2	22	1	1	1
	p-Value	0.005	0.050	0.021	0.010	<0.001	0.720	0.940	0.563
	R^2 (%)	8.84	3.35	6.57	13.11	29.30	0	0	0
HCY	QM	7.401	2.618	1.168	0.017	65.118	0.226	3.348	4.094
	df	1	1	1	2	22	1	1	1
	p-Value	0.007	0.106	0.280	0.992	<0.001	0.634	0.067	0.043
	R^2 (%)	14.48	4.97	1.39	0	68.77	0	7.78	6.27
LMA	QM	0.004	5.968	13.647	0.100	36.514	0.625	2.658	2.251
	df	1	1	1	1	6	1	1	1
	p-Value	0.947	0.015	<0.001	0.751	<0.001	0.429	0.103	0.134
	R^2 (%)	0	26.30	66.18	0	97.92	0	8.86	8.61
Meat pH	QM	0.453	1.354	0.076	17.572	68.102	6.236	0.852	0.016
	df	1	1	1	2	18	1	1	1
	p-Value	0.501	0.245	0.783	<0.001	<0.001	0.013	0.356	0.899
	R^2 (%)	0	2.62	0	54.54	100	23.06	0	0
L*	QM	0.132	1.913	0.728	9.171	38.080	2.731	3.146	0.126
	df	1	1	1	2	19	1	1	1
	p-Value	0.716	0.167	0.393	0.010	0.006	0.098	0.076	0.723
	R^2 (%)	0	0	0	11.65	26.61	1.34	0	0
a*	QM	0.003	13.834	16.603	4.698	29.143	0.435	12.199	10.968
	df	1	1	1	2	19	1	1	1
	p-Value	0.956	<0.001	<0.001	0.095	0.064	0.509	<0.001	<0.001
	R^2 (%)	0	18.51	28.53	2.86	13.73	0	19.28	21.86
b*	QM	1.982	0.257	5.999	19.021	37.939	1.091	0.590	0.014
	df	1	1	1	2	19	1	1	1
	p-Value	0.159	0.612	0.014	<0.001	0.009	0.296	0.442	0.906
	R^2 (%)	0	0	2.80	12.91	1.12	0	0	0
CL	QM	4.339	0.121	0.471	4.947	52.306	2.199	0.121	0.036
	df	1	1	1	2	14	1	1	1
	p-Value	0.037	0.728	0.492	0.084	<0.001	0.138	0.728	0.849
	R^2 (%)	1.26	0	0	8.34	16.48	3.11	0	0
IMF	QM	3.967	0.419	7.676	0.866	80.997	2.191	1.462	3.435
	df	1	1	1	2	18	1	1	1
	p-Value	0.047	0.517	0.006	0.649	<0.001	0.139	0.227	0.064
	R^2 (%)	9.56	0.38	14.55	0	54.24	0.33	1.96	2.56
MDAc	QM	9.33	11.05	56.60	29.38	143.390	9.83	13.23	0.31
	df	1	1	1	2	12	1	1	1
	p-Value	0.002	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.574
	R^2 (%)	17.00	1.15	56.60	32.50	93.81	5.11	4.60	4.73

QM: coefficient of moderators; QM was considered significant at $p < 0.05$; R^2: amount of heterogeneity accounted for; df: degree of freedom; DWG: daily weight gain; DMI: dry matter intake; FCR: feed conversion ratio; HCY: hot carcass yield; LMA: *Longissimus dorsi* muscle area; L*: lightness; a*: redness: b*: yellowness; CL: cooking loss; IMF: intramuscular fat content; MDAc: malondialdehyde content in raw meat; EED: variation in ether extract content of the diets; NDFD: variation in neutral detergent fiber content of the diets.

Table S3 shows that supplementation with TANs reduced FCR in animals older than three months of age (SMD = −0.519; $p < 0.01$), but no effect was observed in lambs up to

three months of age (SMD = 0.099; $p > 0.05$). In contrast, LMA increased in sheep older than three months of age (SMD = 1.108; $p < 0.001$); however, supplementation with TANs did not affect LMA when lambs were up to three months of age (SMD = −0.245; $p > 0.05$). On the other hand, a* of meat increased when TANs were offered to sheep older than three months of age (SMD = 0.844; $p < 0.001$); however, supplementation with TANs did not affect a* of meat when sheep were up to three months of age (SMD = −0.061; $p > 0.05$). In contrast, meat b* decreased when TANs were offered to sheep up to three months of age (SMD = −0.246; $p < 0.05$), but no significant effects were observed in sheep older than three months of age (SMD = 0.502; $p > 0.05$). Dietary supplementation with TANs decreased IMF content in animals up to three months of age (SMD = −0.498; $p < 0.01$); nevertheless, when TANs were offered to sheep older than three months of age IMF content was not affected (SMD = 0.553; $p > 0.05$). Dietary supplementation with TANs reduced MDAc in animals older than three months of age (SMD = −4.489; $p < 0.001$), but no effect was observed in lambs up to three months of age (SMD = −0.320; $p > 0.05$).

Table S4 shows that FCR decreased in response to dietary supplementation of CTs (SMD = −0.563; $p < 0.05$) and HTs (SMD = −2.000; $p < 0.001$), but there was no change in FCR in sheep supplemented with mixtures of CTs and HTs (SMD = −0.093; $p > 0.05$). Meat pH decreased in response to dietary supplementation with HTs (SMD = −1.556; $p < 0.001$). However, there was no significant change in meat pH ($p > 0.05$) when CTs (SMD = −0.111) and mixtures of CTs and HTs (SMD = 0.128) were used. L* of meat increased when HTs were used (SMD = 1.373; $p < 0.05$), but there was no change ($p > 0.05$) when CTs (SMD = −0.072) and mixtures of CTs and HTs (SMD = −0.114) were used. Similarly, b* of meat increased when HTs were used (SMD = 3.312; $p < 0.05$) but decreased when CTs were used (SMD = −0.500; $p < 0.001$). However, meat b* was not affected in sheep supplemented with mixtures of CTs and HTs (SMD = 0.123; $p > 0.05$). Dietary supplementation with mixtures of CTs and HTs decreased MDAc (SMD = −3.666; $p < 0.001$), but there was no change ($p > 0.05$) of MDAc in sheep supplemented with CTs (SMD = −0.313) and HTs (SMD = −0.106).

Table S5 shows that DWG increased when ingredients containing TANs were supplied naturally in the diets (SMD = 0.422; $p < 0.002$) but DWG was not affected when TANs extracts were used (SMD = −0.233; $p > 0.05$). Meat pH was not affected by the method of inclusion of TANs in the diet ($p > 0.05$). MDAc decreased when ingredients containing TANs were supplied naturally in the diets (SMD = −2.664; $p < 0.001$) but was not affected when TANs extracts were used (SMD = −0.159; $p > 0.05$).

Figure S2 shows that DMI increased only when TANs came from *Mimosa tenuiflora* (SMD = 4.531; $p = 0.001$), *Hedysarum coronarium* (SMD = 7.809; $p < 0.001$), *Ficus infectoria* (SMD = 1.448; $p < 0.001$), *Schinopsis* spp. (SMD = 0.862; $p = 0.026$), *Pistacia vera* (SMD = 0.638; $p = 0.038$), *Passiflora edulis* (SMD = 0.872; $p = 0.044$) and *Prunus amygdalus* (SMD = 0.860; $p = 0.036$). However, it decreased when TANs came from *Cistus ladanifer* (SMD = −0.363; $p = 0.050$) and was not affected when TANs came from other plants ($p > 0.05$). Figure 1 shows that FCR decreased when TANs came from plant mixtures (SMD = −1.766; $p = 0.017$), *Hedysarum coronarium* (SMD = −1.140; $p = 0.003$), *Castanea sativa* (SMD = −2.000; $p < 0.001$); *Vitis vinifera* (SMD = −2.581; $p = 0.008$) and *Sorghum bicolor* (SMD = −1.071; $p = 0.008$). FCR was not affected in sheep supplemented with other sources of TANs ($p > 0.05$).

Figure 2 shows that HCY only increased when TANs were from *Hedysarum coronarium* (SMD = 1.675; $p < 0.001$), *Vitis vinifera* (SMD = 0.830; $p = 0.008$) *and Sorghum bicolor* (SMD = 1.440; $p < 0.001$). However, HCY decreased when TANs were from *Mimosa tenuiflora* (SMD = −1.044; $p = 0.037$). HCY was not affected when other plants were used as sources of TANs ($p > 0.05$).

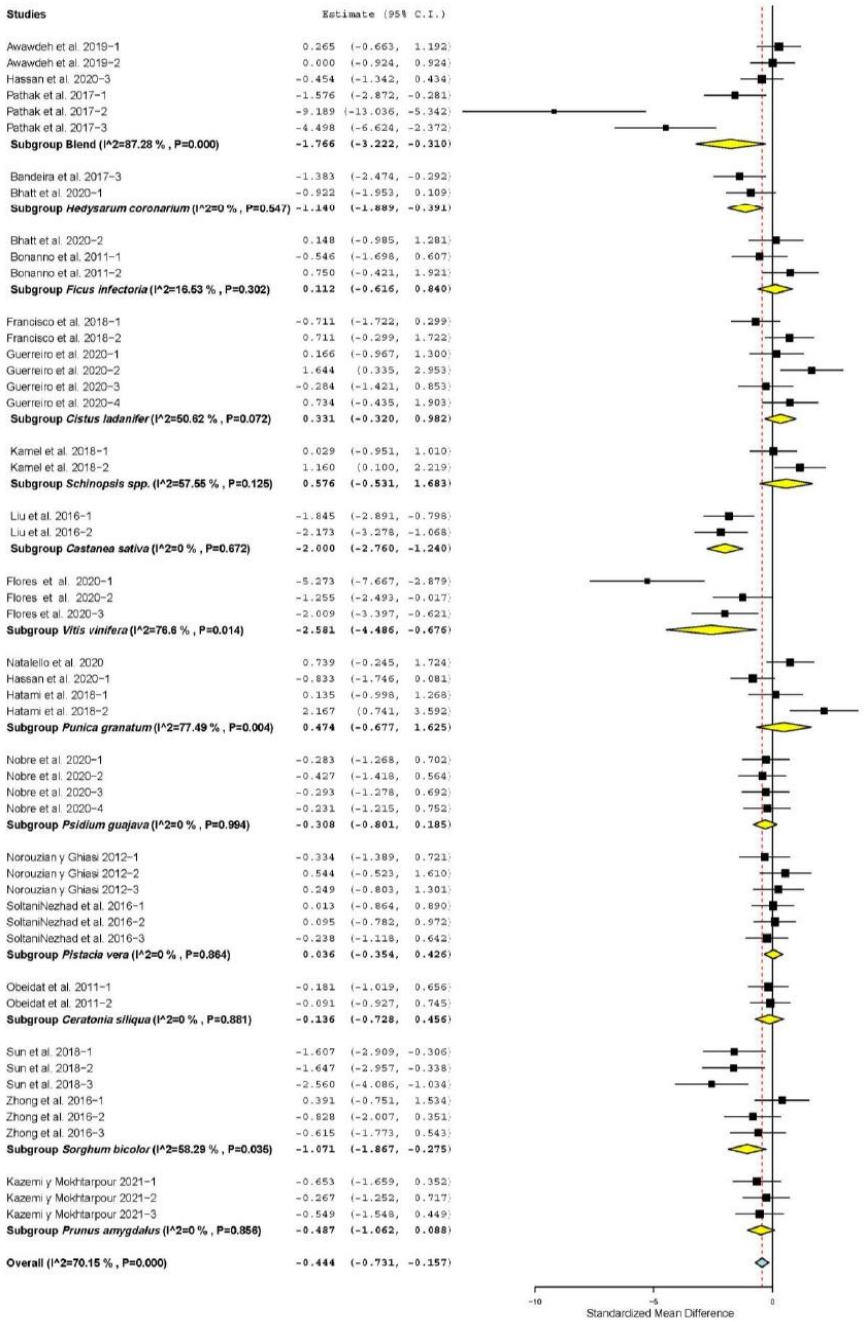

Figure 1. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on feed conversion ratio (FCR) in sheep. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction in FCR, while points to the right of the line indicate increase in FCR.

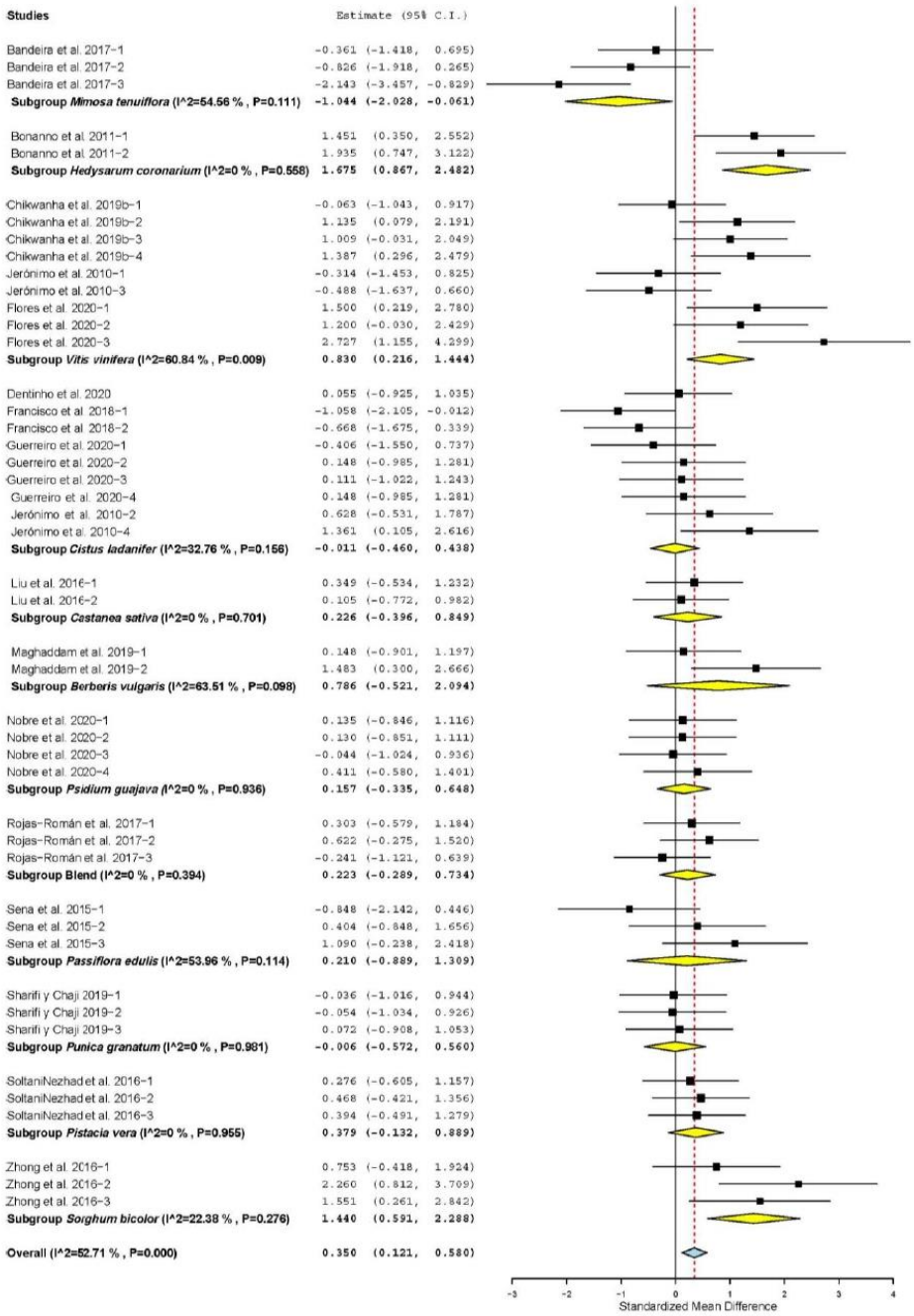

Figure 2. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on hot carcass yield (HCY) of sheep. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction in HCY, while points to the right of the line indicate increase in HCY.

Figure 3 shows that LMA increased only when *Pisum sativum* (SMD = 0.642; $p = 0.014$), *Vitis vinifera* (SMD = 1.110; $p = 0.021$) and *Pistacia vera* (SMD = 1.774; $p < 0.001$) were used as sources of TANs. However, LMA was not affected when other plants were used as sources of TANs ($p > 0.05$).

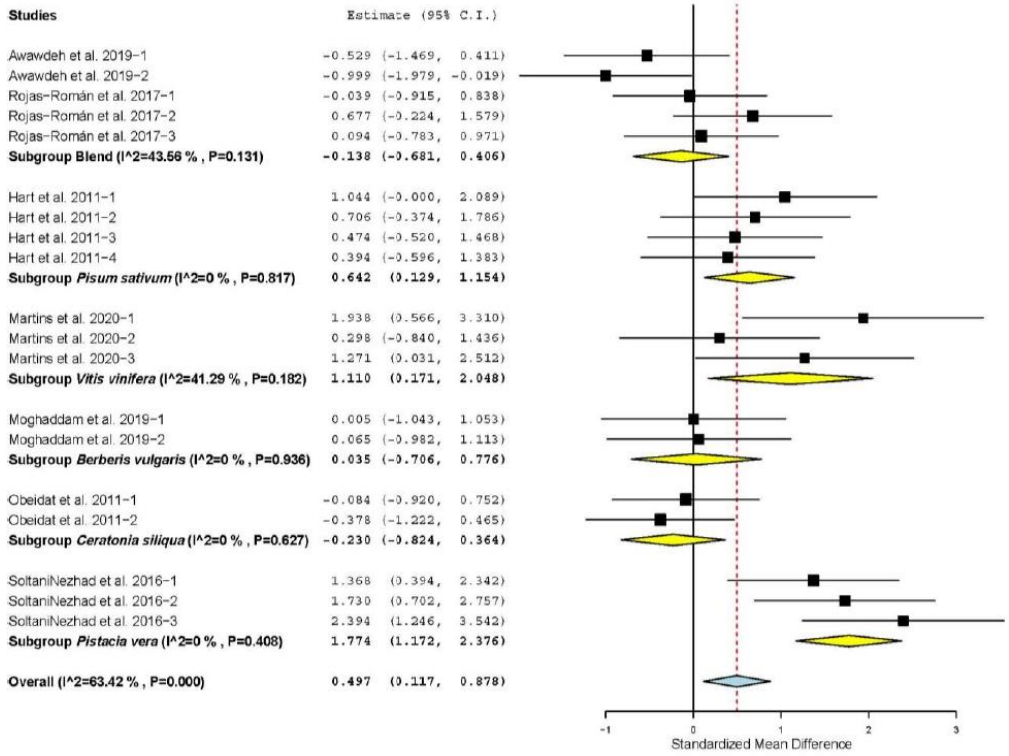

Figure 3. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on sheep *Longissimus dorsi* muscle area (LMA). The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction in LMA, while points to the right of the line indicate increase in LMA.

Figure 4 shows that meat pH increased only when TANs came from *Psidium guajava* (SMD = 1.324; $p < 0.001$), *Rosmarinus officinalis* (SMD = 0.866; $p = 0.013$) and *Nigella sativa* (SMD = 0.667; $p = 0.050$). Meat pH decreased when TANs were from *Castanea sativa* (SMD = −1.556; $p < 0.001$), and it was not affected when TANs were from other sources ($p > 0.05$).

Figure S3 shows that L* of meat increased only when *Cistus ladanifer* (SMD = 0.470; $p = 0.028$), *Castanea sativa* (SMD = 1.803; $p = 0.036$) and *Mimosa tenuiflora* (SMD = 0.851; $p = 0.009$) were used as sources of TANs. L* decreased when TANs were from *Sorghum bicolor* (SMD = −0.947; $p = 0.007$), and it was not affected by other sources of TANs ($p > 0.05$). In contrast, Figure S4 shows that b* of meat decreased only when *Acacia mearnsii* (SMD = −0.884; $p = 0.042$), *Ceratonia siliqua* (SMD = −0.618; $p = 0.045$) and *Sorghum bicolor* (SMD = −1.358; $p < 0.001$) were used as sources of TANs. However, b* increased when TANs were from *Mimosa tenuiflora* (SMD = 1.903; $p = 0.033$), and it was not affected when TANs were from other sources ($p > 0.05$).

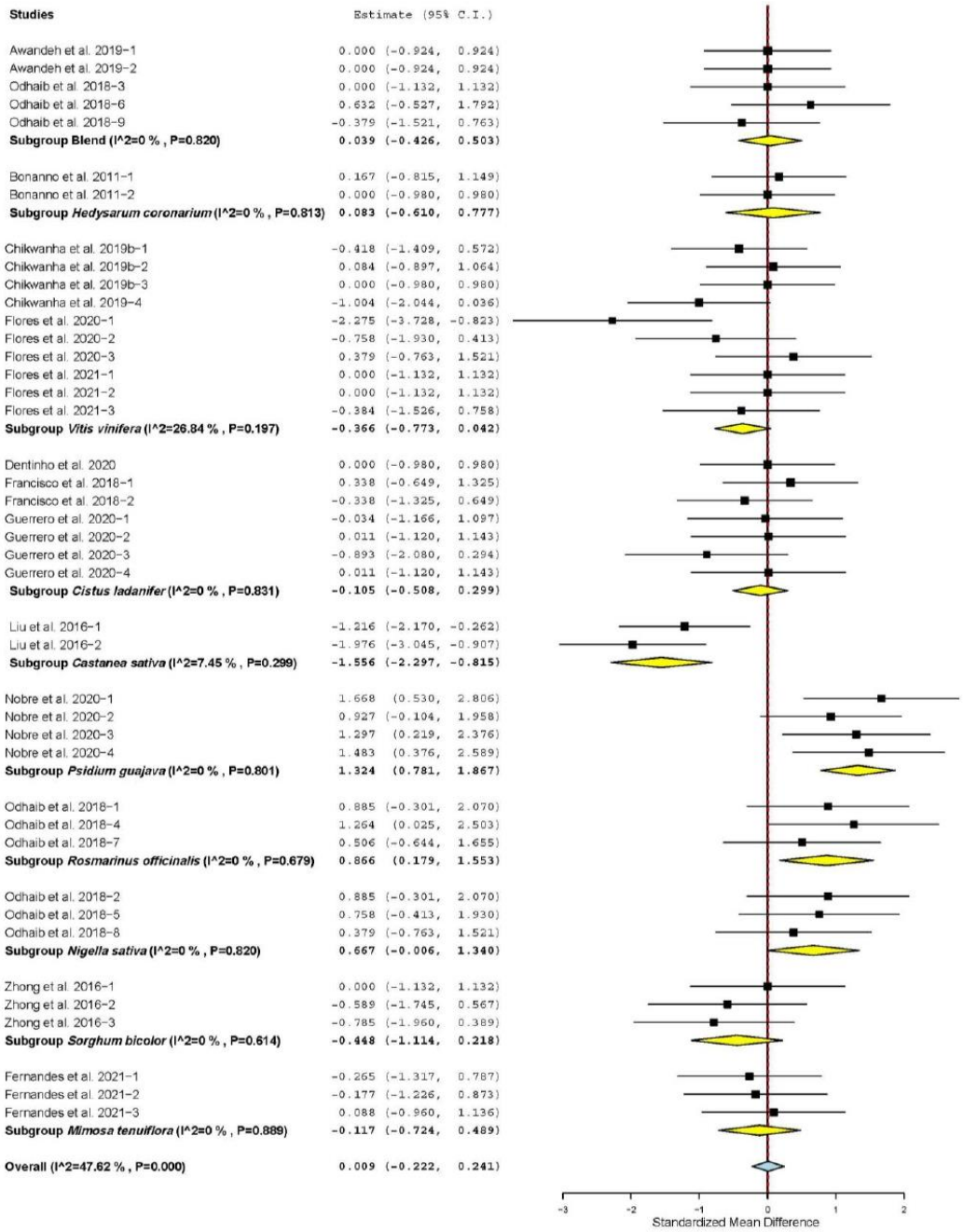

Figure 4. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on meat pH of sheep. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction in meat pH, while points to the right of the line indicate increase in meat pH.

Figure 5 shows that the IMF content of meat decreased only when *Cesalpinia spinosa* (SMD = −0.870; p = 0.028) and *Onobrychis viciifolia* (SMD = −1.319; p = 0.042) were used as sources of TANs. However, IMF increased when TANs were from *Vitis vinifera* (SMD = 0.893; p = 0.050), and it was not affected when TANs were from other plants ($p > 0.05$). Additionally, Figure S5 shows that CL decreased only when TANs came from *Vitis vinifera* (SMD = −1.584; p = 0.047) and *Rosmarinus officinalis* (SMD = −0.691; p = 0.045). CL increased when TANs came from *Castanea sativa* (SMD = 1.949; $p < 0.001$) and *Psidium guajava* (SMD = 6.842; $p < 0.001$), and it was not affected when TANs came from other sources ($p > 0.05$).

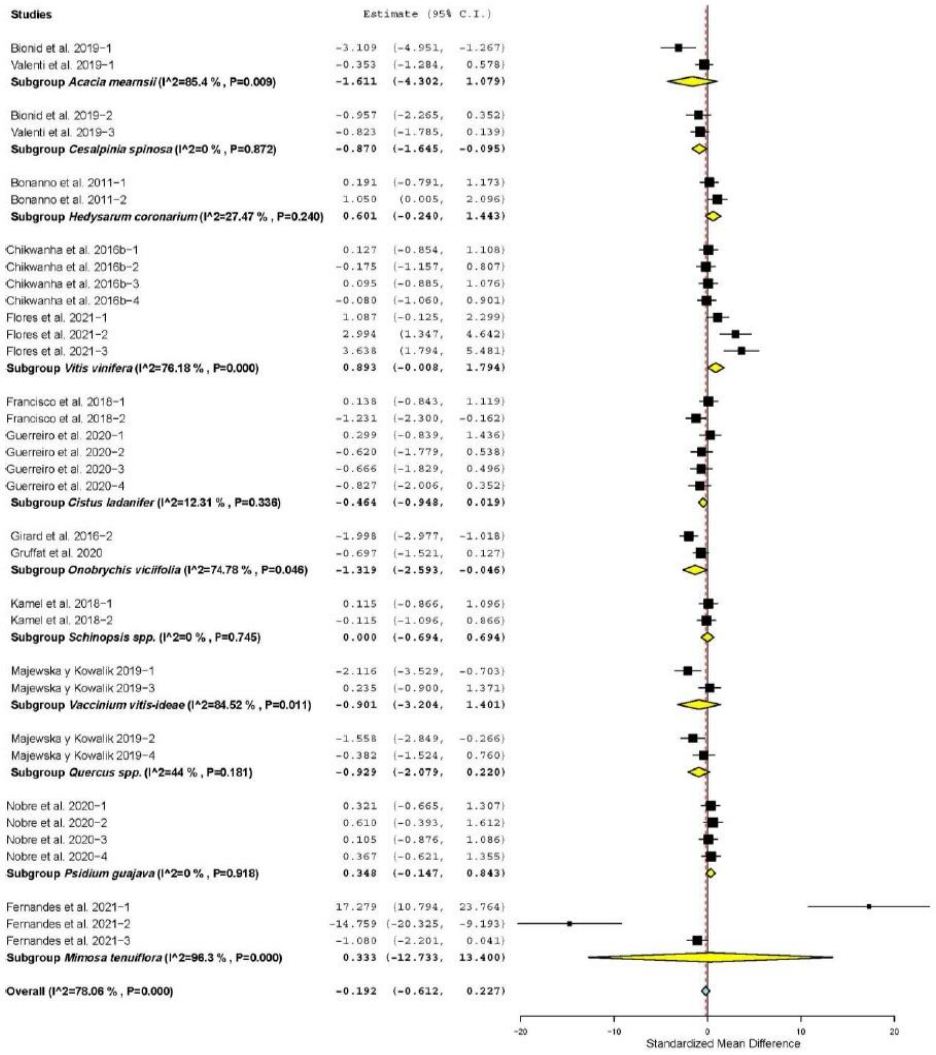

Figure 5. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical origin of tannin on intramuscular fat (IMF) content in sheep meat. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction in IMF, while points to the right of the line indicate increase in IMF.

Figure 6 shows that MDAc content decreased only when TANs came from *Vitis vinifera* (SMD = −3.106; $p < 0.001$), *Rosmainus officinalis* (SMD = −5.479; $p < 0.001$), *Nigella sativa* (SMD = −6.022; $p < 0.001$), *Sorghum bicolor* (SMD = −0.843; $p = 0.017$) and plant mixtures (SMD = −5.184; $p < 0.001$). While MDAc was not affected when TANs were from other plants ($p > 0.05$).

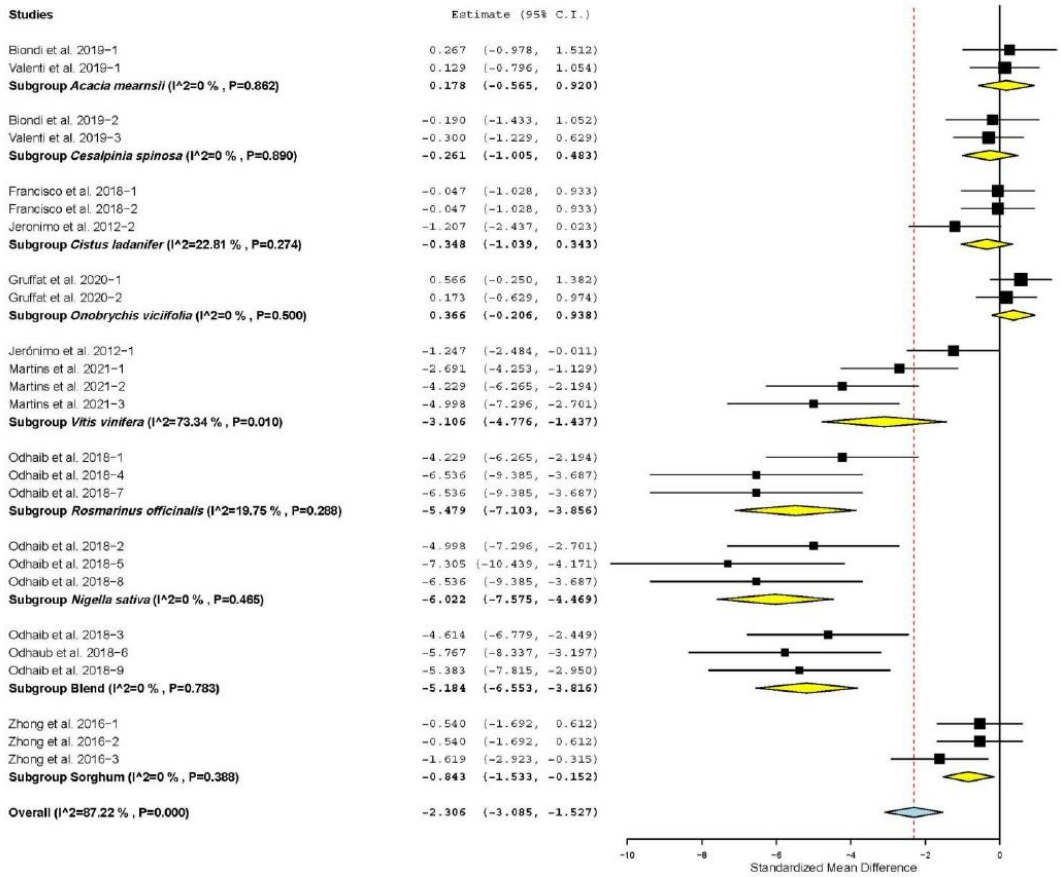

Figure 6. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical origin of tannin on the malondialdehyde content of raw sheep meat (MDAc). The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical black line represent reduction of MDAc, while points to the right of the line indicate increase of MDAc.

4. Discussion

It has been suggested that high doses of TANs in the diet (>55 g kg^{-1} DM) may have negative effects on feed utilization efficiency and growth rate of ruminants; however, low-moderate levels (20 to 40 g kg^{-1} DM) could result in neutral or even positive effects [9,12]. In this regard, a meta-analysis conducted by Orzuna-Orzuna et al. [32] reported that dietary supplementation with TANs at average doses of 14.61 g kg^{-1} DM did not affect DWG or feed efficiency in beef cattle. However, in the present meta-analysis, higher DWG and lower FCR were observed in response to dietary supplementation with TANs. This suggests that TANs improve growth rate and feed utilization efficiency in sheep. The lower FCR observed could be explained because TANs supplementation reduces infection by

gastrointestinal nematodes, which decrease feed efficiency in livestock [1]. According to Pimentel et al. [12], dietary inclusion of TANs in moderate doses can increase the efficiency of nutrient utilization of the diet, due to the ability of TANs to form complexes with macromolecules. In addition, the presence of TANs in diets consumed by ruminants has been reported to reduce enteric methane production [32], increase duodenal flux of amino acids and microbial protein [51], and reduce energy losses due to urea excretion [32,52]. This would partially explain the positive effects observed in DWG and FCR for sheep supplemented with TANs. On the other hand, reactive oxygen species (ROS) oxidize and destroy cellular biological molecules and impair the integrity of the intestinal membrane, resulting in reduced nutrient absorption [53,54]. Antioxidant enzymes and exogenous antioxidants can help restore oxidative balance and maintain healthy intestinal mucosa [21,54,55]. Consequently, the positive effects observed for DWG and FCR in the present study suggest that TANs reduced the presence of ROS and intestinal membrane damage in sheep. This hypothesis is supported by the observed increase in CAT and GPx in sheep supplemented with TANs, because CAT and GPx can convert ROS into less harmful compounds for the organism and prevent lesions in the gastrointestinal mucosa [53,56].

Although some review articles have suggested that the presence of TANs in the diet may negatively affect DMI in ruminants [1,9,57], in the present meta-analysis no changes in DMI were observed in response to dietary supplementation with TANs. This absence of changes in DMI probably occurred because the average dose of TANs used was 19.29 g kg^{-1} DM and negative effects of TANs on intake seem to occur with doses higher than 50 g kg^{-1} DM [4,9,12]. Similar to our results, two previously elaborated meta-analyses reported that dietary supplementation with TANs at average concentrations of 9.5 and 14.61 g kg^{-1} DM did not significantly affect DMI of dairy cows in production and beef cattle, respectively [31,32]. These results together suggest that TANs can be used in sheep and cattle without negative effects on feed intake.

It has been suggested that the reduction of feed palatability could be produced by a reaction between dietary TANs and salivary mucoproteins, or by a direct reaction of TANs with taste receptors, causing an astringent sensation [10,58,59]. However, prolonged exposure to dietary TANs can induce adaptive mechanisms in ruminants, such as changes in the amount of proline and other salivary proteins with high affinity for TANs [8,59–61]. Unlike other protein complexes and TANs formed, protein complexes rich in proline and TANs are stable over the entire pH range of the digestive tract, which could eliminate or reduce their negative effect on palatability and feed intake [10,62–64]. Regarding this, in the present study a subgroup analysis revealed that DMI was not affected when TANs were offered for up to 70 days but increased when supplementation lasted more than 70 days.

With respect to carcass characteristics, dietary supplementation with TANs increased HCY and CCY. There is limited information on the effects of TANs on ruminant carcass characteristics, which makes it difficult to explain the results observed in the present meta-analysis. However, variation in carcass fat and muscle deposition can modify carcass performance [65]. Consequently, the observed increase in LMA and BFT in the carcasses of sheep supplemented with TANs could partially explain the higher HCY and CCY obtained in the present meta-analysis.

BFT and LMA also increased in response to dietary supplementation with TANs. The mechanism of action of TANs on lipogenesis and muscle development has not been studied in sheep. Nevertheless, some polyphenolic compounds have been reported to increase BFT in beef cattle by changing the differential expression of genes involved in lipid metabolism [66]. Similarly, dietary supplementation from plants with phenolic compounds can increase muscle fiber size and increase skeletal muscle mass in lambs [67]. Similar effects of TANs consumption in the present meta-analysis would partially explain the observed increases in BFT and LMA.

Meat with high pH has higher microbial deterioration, which reduces its quality and shelf life [50]. Although dietary supplementation with TANs did not affect meat pH, subgroup analysis revealed that meat pH decreased significantly when HTs were used.

This suggests that HTs could reduce microbial spoilage of meat, and consequently improve its quality and increase its shelf life. In this regard, Biondi et al. [68] observed lower overall load of pathogenic bacteria (*Escherichia coli*, *Enterobacteriaceae* and *Pseudomonas* spp.) in meat from lambs supplemented with HTs (40 g kg^{-1} DM) of *Caesalpinia spinosa* and concluded that HTs may have antimicrobial activity within muscle tissue.

Color is one of the most important characteristics that determines meat quality, because it is the first attribute that attracts consumers when choosing fresh meat [50,69]. The pH, IMF content, the amount of myoglobin, and the formation and accumulation of muscle metmyoglobin are the main factors that influence the color of small ruminant meat [65]. Particularly, L* of meat depends on the IMF content [70] and is negatively correlated (r = −0.63) with muscle myoglobin concentration [71]. Similarly, b* of meat is correlated with pH [72], and with the IMF content of meat [70]. In the present meta-analysis, pH and meat IMF content were similar between treatments. In addition, previous studies [17,18] have reported that dietary supplementation of TANs does not affect muscle myoglobin content in sheep. These findings could partially explain the absence of L* and b* changes observed in sheep consuming TANs in the present study. Additionally, compounds originating in meat as a consequence of lipid oxidation have been reported to promote the formation of metmyoglobin, which reduces a* values in meat [73]. In the present meta-analysis, MDAc (as an indicator of lipid oxidation in meat) and the metmyoglobin content of meat decreased in response to dietary supplementation with TANs, which would partially explain the observed increase in a* in meat from sheep supplemented with TANs.

Meat tenderness is one of the main characteristics that influences consumers' meat choice and can be evaluated by WBSF [65]. It has been hypothesized that some natural antioxidants, such as polyphenols extracted from citrus fruits, might contain tenderizing compounds because their use can increase meat tenderness [74]. However, although TANs are polyphenolic compounds with antioxidant activity [75], in the present study the addition of TANs in the sheep diet did not affect WBSF. These results suggest that TANs do not affect the tenderness of sheep meat. Malheiros et al. [76] reported that ROS production can improve meat tenderness by increasing the degradation of toughness-related structural proteins. It has been reported that TANs can improve the antioxidant status of small ruminant meat by increasing mRNA and protein expression levels of SOD and GPx in skeletal muscle cells [77]. This effect could reduce structural and functional damage in muscle cells and tissues caused by ROS [78], which would partially explain the absence of changes observed for WBSF in the present study.

DL and CL are parameters used to evaluate the water holding capacity (WHC) of meat [65]. In the present meta-analysis, the values observed for DL suggest that dietary supplementation with TANs can improve WHC. However, these results should be interpreted carefully due to the low number of studies that reported on this variable. There is a strong negative correlation (r = −0.894) between WHC and CL [79]. Therefore, the results observed for CL suggest that TANs do not affect WHC of sheep meat. Meat pH is also related to WHC [50,65], and variation in IMF content can alter muscle structure and modify water retention in meat [65]. This suggests that the similarity of IMF content and meat pH between treatments could be related to the lack of changes observed for CL in the present meta-analysis.

Meat moisture content decreased in response to dietary supplementation with TANs, suggesting that TANs might affect WHC of meat. However, these results should be interpreted carefully due to the low number of studies that reported on this variable. Moreover, the results observed for protein, IMF and ash content of meat indicate that supplementation with TANs does not affect the nutritional composition of sheep meat.

Subgroup analysis revealed that doses of TANs higher than 20 g kg^{-1} DM can reduce IMF content. The mechanism of action of TANs on IMF deposition has not been studied in sheep. However, the number and size of intramuscular adipocytes have been reported to be related to the process of IMF deposition or reduction in livestock [80,81]. Gallic acid (a typical isomer of HTs) can inhibit bovine adipocyte proliferation and adipogenesis under

in-vitro conditions [81]. Similarly, in-vitro studies have reported that CTs and HTs inhibit preadipocyte differentiation [82,83], and CTs induce apoptosis in mature adipocytes and inhibit lipid accumulation in maturing preadipocytes [82]. This would partly explain the lower IMF content in meat from sheep supplemented with more than 20 g kg^{-1} DM of TANs. The lower IMF content could be related to the reduced moisture content of meat from sheep supplemented with TANs, because there is a negative correlation ($r = -0.47$) between moisture content and IMF content of meat [84].

Oxidation reactions during the processing, distribution and storage of meat products can cause physicochemical changes and undesirable odors that affect the quality of the final product [85]. For example, oxidation of myoglobin and lipids can cause discoloration and off-flavor development in meat, respectively [86]. In the present meta-analysis, lipid oxidation and myoglobin oxidation of meat decreased in response to dietary supplementation with TANs. This suggests that TANs may induce antioxidant enzyme gene expression in sheep muscle [87], similar to what was previously observed by Zhong et al. [77] in skeletal muscle cells from goat treated with *Camellia sinensis* TANs under in-vitro conditions. These results also suggest that TANs could be used to delay the discoloration and appearance of off-flavor in meat, and consequently improve the quality and shelf life of meat products.

TANs are polyphenolic compounds with antioxidant activity [75]. It has been reported that phenols can switch from an antioxidant to a prooxidant state when used in high doses [88]. In the present meta-analysis, lipid oxidation of meat decreased in response to dietary supplementation with TANs regardless of the dose used. However, the reduction was greater at doses below 20 g kg^{-1} DM. These results suggest that TANs may improve the oxidative stability of meat when supplied to the diet at low doses, but at high concentrations they could have prooxidant effects on meat, as previously reported for some essential oils rich in phenolic compounds [88].

Subgroup analysis revealed that lipid oxidation of meat decreased significantly only when TANs were fed to animals older than 3 months of age. This probably occurred because the presence of microorganisms in the rumen increases with the age of the animals [89], and the action of ruminal microorganisms increases the bioavailability of ingested TANs in sheep [90].

The type and source of TANs explained most (between 30 and 90%) of the heterogeneity observed in MDAc. These results suggest that the effects of TANs on oxidative stability of meat depend on the type and source of TANs used rather than the dose, period and method of supplementation. Subgroup analyses revealed that MDAc was significantly reduced only when mixtures of CTs and HTs were used. These results suggest a synergistic effect between both types of TANs in reducing lipid oxidation of meat. Although TANs have been shown to have high antioxidant activity [75], their effect on the antioxidant capacity of muscle tissues seems to depend on how effectively they can be absorbed through the gastrointestinal tract [91]. In this regard, it has been reported that some CTs can neither be degraded nor absorbed in the gastrointestinal tract of sheep [91]. In contrast, some HTs act for a short time because they are rapidly transferred to the blood plasma in sheep [90]. Similar effects of individual use of CTs and HTs in sheep would partially explain the results observed in the present meta-analysis.

TAC is an integrated parameter that considers all antioxidants present in blood plasma [92]. In the present study, TAC was observed to increase in response to dietary supplementation with TANs, suggesting that TANs intake may improve the total antioxidant status of sheep. Although there are no reference values for TAC in ruminants [93], TAC changes in blood plasma following supplementation with antioxidant-rich foods or purified antioxidants provide information on the absorption and bioavailability of ingested antioxidant compounds [92]. Therefore, although the metabolic fate of TANs ingested by ruminants is not yet fully understood [22], the results observed in the present meta-analysis suggest that TANs ingested by sheep may be degraded and absorbed in the gastrointestinal tract and subsequently transferred to the bloodstream to serve as exogenous antioxidants.

Excessive accumulation of prooxidant substances, such as ROS, can cause oxidative stress in ruminants [93,94]. Some antioxidant enzymes, such as GPx, SOD and CAT are important because they can convert ROS into less harmful compounds for the organism [56], and consequently can reduce ROS-mediated damage on biological macromolecules [95,96]. On the other hand, although ROS can attack any of the major biomolecules, lipids are particularly susceptible, so biomarkers of lipid peroxidation (e.g., malondialdehyde) are considered the best indicators of oxidative stress [97]. In the present meta-analysis, dietary supplementation with TANs increased the activity of antioxidant enzymes (CAT and GPx) and reduced the concentration of malondialdehyde in sheep blood serum. This suggests that inclusion of TANs in the diet could be used as a dietary strategy to mitigate oxidative stress in sheep, which could improve animal health [94].

5. Conclusions

The results of the present meta-analysis indicate that dietary supplementation with TANs does not affect dry matter intake, but improves daily weight gain, feed conversion ratio, total antioxidant capacity and antioxidant enzyme activity in sheep blood serum. The best result for daily weight gain is achieved using doses of TANs lower than 20 g kg^{-1} DM, with supplementation periods longer than 70 days and when TANs are supplied naturally as ingredients in the diet. The best results for feed conversion ratio are achieved using doses of TANs lower than 20 g kg^{-1} DM, with supplementation periods longer than 70 days, in animals older than three months of age and using CTs.

In addition, TANs reduce lipid oxidation in blood plasma and meat. The best results of lipid oxidation of meat are observed using mixtures of CTs and HTs, with supplementation periods longer than 70 days, using doses lower than 20 g kg^{-1} DM, in animals older than three months of age, and when TANs are supplied naturally as ingredients in the diet. Supplementation with TANs does not affect meat tenderness, chemical composition, pH and color (L* and b*). However, it increases hot and cold carcass yield, backfat thickness and *Longissimus dorsi* muscle area. The best hot carcass yield is obtained with *Vitis vinifera*, *Hedysarium coronarium* and *Sorghum bicolor* as sources of TANs. In contrast, the best *Longissimus dorsi* muscle area result is observed in animals older than three months of age, with supplementation periods longer than 70 days, and using *Vitis vinifera*, *Pisum sativum* and *Pistacia vera* as sources of TANs. In addition, supplementation with TANs improves meat a*, particularly with supplementation periods longer than 70 days and in animals older than three months of age.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11113184/s1, Figure S1: A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis. Table S1: Subgroup analysis of the effect of dietary tannin dose in sheep. Table S2: Subgroup analysis of the effect of tannin supplementation period in sheep. Table S3: Subgroup analysis of the effect of age on the response to tannin supplementation in sheep. Table S4: Subgroup analysis of the effect of tannin type in sheep. Table S5: Subgroup analysis of the effect of tannin inclusion method in sheep. Figure S2: Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical origin of tannin on dry matter intake (DMI) in sheep. Figure S3: Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on the lightness (L*) of sheep meat. Figure S4: Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on the yellowness (b*) of sheep meat. Figure S5: Forest plot of the effect size or standardized mean difference and 95% confidence interval of the botanical source of tannin on cooking loss (CL) of sheep meat.

Author Contributions: Conceptualization, J.F.O.-O., G.D.-I. and A.L.-B.; methodology and data curation, J.F.O.-O., G.D.-I. and G.D.M.-M.; investigation and formal analysis, J.F.O.-O., L.A.M.-R. and H.A.L.-R.; writing—original draft preparation, J.F.O.-O.; writing—review and editing, J.F.O.-O., G.D.-I., A.L.-B., G.D.M.-M., L.A.M.-R. and H.A.L.-R.; supervision and project administration, A.L.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: The first author, José Felipe Orzuna Orzuna is a Master of Science student of the Program of Animal Production of the Universidad Autónoma Chapingo and thanks the CONACyT Program for the scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Summary of the studies included in the meta-analysis.

Author	Country	Tannin Source	Tannin Type	Method of Inclusion
Awawdeh et al. [98]	Jordan	B, B	B, B	N, N
Bandeira et al. [99]	Brazil	*Mimosa tenuiflora* (*n* = 3)	CT, CT, CT	N, N, N
Bhatt et al. [100]	India	PA, ER	B, B	N, N
Biondi et al. [68]	Spain	AM, CS	B, B	E, E
Bonanno et al. [101]	Italy	*Hedysarum coronarium*	CT, CT	N, N
Buccioni et al. [102]	Italy	CH, QU	HT, CT	E, E
Chikwanha et al. [103]	South Africa	VV (*n* = 4)	B, B, B, B, B	N, N, N, N, N
Chikwanha et al. [104]	South Africa	VV (*n* = 4)	B, B, B, B, B	N, N, N, N, N
Costa et al. [105]	Brazil	AM (*n* = 4)	CT, CT, CT, CT, CT	E, E, E, E, E
Dentinho et al. [20]	Brazil	CL	CT	E
Dey et al. [106]	India	*Ficus infectoria* (*n* = 3)	B, B, B	N, N, N
Abdalla et al. [107]	Brazil	OP, Clep	B, B	N, N
Fernandes et al. [14]	Brazil	*Mimosa tenuiflora* (*n* = 3)	B, B, B	N, N, N
Francisco et al. [108]	Portugal	CL (*n* = 2)	CT, CT	N, N
Girard et al. [109]	Switzerland	LC and OV	B, B	N, N
Guerreiro et al. [110]	Portugal	CL (*n* = 4)	CT, CT, CT, CT, CT	N, N, N, N, N
Gruffat et al. [111]	France	OV (*n* = 2)	CT, CT	N, N
Hart et al. [112]	United Kingdom	*Pisum sativum* (*n* = 4)	CT, CT, CT, CT, CT	N, N, N, N, N
Hassan et al. [113]	Egypt	*Punica granatum*, MI, B	B, B, B	N, N, N
Hatami et al. [114]	Iran	*Punica granatum* (*n* = 3)	B, B	N, N
Jerónimo et al. [115]	Portugal	VV (*n* = 2), CL (*n* = 2)	CT, CT, CT, CT, CT	E, N, E, N
Jerónimo et al. [116]	Portugal	VV (*n* = 2), CL (*n* = 2)	CT, CT, CT, CT, CT	E, N, E, N
Kamel et al. [117]	Saudi Arabia	QU (*n* = 2)	CT, CT	E, E
Kazemi and Mokhtarpour [118]	Iran	*Prunus amygdalus* (*n* = 3)	B, B, B	N, N, N
Leparmarai et al. [119]	Switzerland	VV	B	E
Lima et al. [120]	Brazil	*Macrotyloma axillare*	B	N
Liu et al. [21]	China	CH (*n* = 2)	HT, HT	E, E
López-Andrés et al. [91]	Italy	QU	CT	E
Majewska and Kowalik [121]	Poland	VAC, *Quercus* sp.	B, B	N, N
Flores et al. [122]	Brazil	VV (*n* = 3)	B, B, B	N, N, N
Flores et al. [123]	Brazil	VV (*n* = 3)	B, B, B	N, N, N
Moghaddam et al. [124]	Iran	*Berberis vulgaris* (*n* = 2)	B, B	N, N
Natalello et al. [18]	Italy	*Punica granatum*	B	N
Nobre et al. [125]	Brazil	*Psidium guajava* (*n* = 4)	B, B, B, B, B	N, N, N, N, N
Norouzian and Ghiasi [126]	Iran	*Pistacia vera* (*n* = 3)	B, B, B	N, N, N
Obeidat et al. [127]	Jordan	*Ceratonia siliqua* (*n* = 2)	CT, CT	N, N
Odhaib et al. [128]	Malaysia	RO (*n* = 3), NS (*n* = 3), B (*n* = 3)	B (*n* = 9)	N (*n* = 9)
Pathak et al. [13]	India	B, B	CT, CT	N, N
Peng et al. [95]	Canada	*Dalea purpurea*	CT	N
Po et al. [129]	Australia	*Ilex paraguarensis*	CT	N
Priolo et al. [16]	Italy	*Corylus avellana*	B	N
Rajabi et al. [130]	Iran	*Punica granatum* (*n* = 3)	B, B, B	N, N, N
Rojas-Román et al. [15]	Mexico	B, B, B	B, B, B	E, E, E
Sanchez et al. [131]	Mexico	*Caesalpinia coriaria*	B	N
Sena et al. [132]	Brazil	*Passiflora edulis* (*n* = 3)	CT, CT, CT	N, N, N
Sharifi and Chaji [133]	Iran	*Punica granatum* (*n* = 3)	B, B, B	N, N, N
SoltaniNezhad et al. [134]	Iran	*Pistacia vera* (*n* = 3)	B, B, B	N, N, N
Sun et al. [135]	China	*Sorghum bicolor* (*n* = 3)	CT, CT, CT	N, N, N
Valenti et al. [17]	Italy	AM, QU, CS	CT, HT, HT	E, E, E
Wang et al. [22]	Ireland	*Corylus avellana* (*n* = 2)	B, B	N, N
Yisehak et al. [136]	Ethiopia	*Albizia gummifera*	CT	N
Zhao et al. [137]	China	No reported	B, B	E, E
Zhong et al. [19]	China	*Sorghum bicolor* (*n* = 3)	CT, CT, CT	N, N, N

B: blend; PA: *Pimpinela anisum*; ER: *Eucalyptus rudis*; AM: *Acacia mearnsii*; CS: *Cesalpinia spinosa*; CH: chestnut (Castanea *sativa*); QU: quebracho (*Schinopsis* spp.); VV: *Vitis vinifera*; CL: *Cistus ladanifer*; OP: *Orbignya phalerata*; Clep: *Combretum leprosum*; LC: *Lotus corniculatus*; OV: *Onobrychis viciifolia*; MI: *Mangifera indica*; VAC: *Vaccinium vitis-ideae*; sorghum (*Sorghum bicolor*); HT: hidrolisable tannin; CT: condensed tannin; E: extract; N: naturally present. *n*: number of comparisons.

References

1. Huang, Q.; Liu, X.; Zhao, G.; Hu, T.; Wang, Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Anim. Nutr.* **2018**, *4*, 137–150. [CrossRef]
2. Mund, M.D.; Khan, U.H.; Tahir, U.; Mustafa, B.E.; Fayyaz, A. Antimicrobial drug residues in poultry products and implications on public health: A review. *Int. J. Food Prop.* **2017**, *20*, 1433–1446. [CrossRef]
3. Wang, H.; Ren, L.; Yu, X.; Hu, J.; Chen, Y.; He, G.; Jiang, Q. Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. *Food Control* **2017**, *80*, 217–225. [CrossRef]
4. Callaway, T.R.; Lillehoj, H.; Chuanchuen, R.; Gay, C.G. Alternatives to Antibiotics: A Symposium on the Challenges and Solutions for Animal Health and Production. *Antibiotics* **2021**, *10*, 471. [CrossRef]
5. Valenzuela-Grijalva, N.V.; Pinelli-Saavedra, A.; Muhlia-Almazan, A.; Domínguez-Díaz, D.; González-Ríos, H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. *J. Anim. Sci. Technol.* **2017**, *59*, 8. [CrossRef] [PubMed]
6. Naumann, H.D.; Tedeschi, L.O.; Zeller, W.E.; Huntley, N.F. The role of condensed tannins in ruminant animal production: Advances, limitations and future directions. *Rev. Bras. Zootec.* **2017**, *46*, 929–949. [CrossRef]
7. Serra, V.; Salvatori, G.; Pastorelli, G. Dietary Polyphenol Supplementation in Food Producing Animals: Effects on the Quality of Derived Products. *Animals* **2021**, *11*, 401. [CrossRef]
8. Jerónimo, E.; Pinheiro, C.; Lamy, E.; Dentinho, M.T.; Sales-Baptista, E.; Lopes, O.; Capela e Silva, F. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. In *Tannins: Biochemistry, Food Sources and Nutritional Properties*; Combs, C.A., Ed.; Nova Science Publishers Inc.: New York, NY, USA, 2016; pp. 121–168.
9. Min, B.R.; Barry, T.N.; Attwood, G.T.; McNabb, W.C. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Anim. Feed Sci. Technol.* **2003**, *106*, 3–19. [CrossRef]
10. Frutos, P.; Hervás, G.; Giráldez, F.J.; Mantecón, A.R. Review. Tannins and ruminant nutrition. *Span. J. Agric. Res.* **2004**, *2*, 191–202. [CrossRef]
11. Patra, A.K.; Saxena, J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.* **2011**, *91*, 24–37. [CrossRef]
12. Pimentel, P.R.S.; Pelelgrini, C.B.; Lanna, D.P.D.; Brant, L.M.S.; Ribeiro, C.V.D.M.; Silva, T.M.; Barbosa, A.M.; da Silva, J.J.M.; Bezerra, L.R.; Oliveira, R.L. Effects of *Acacia mearnsii* extract as a condensed-tannin source on animal performance, carcass yield and meat quality in goats. *Anim. Feed Sci. Technol.* **2021**, *271*, 114733. [CrossRef]
13. Pathak, A.K.; Dutta, N.; Pattanaik, A.K.; Chaturvedi, V.B.; Sharma, K. Effect of condensed tannins from *Ficus infectoria* and Psidium guajava leaf meal mixture on nutrient metabolism, methane emission and performance of lambs. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 1702–1710. [CrossRef]
14. Fernandes, J.; Pereira, F.J.; Menezes, D.; Caldas, A.C.; Cavalcante, I.; Oliveira, J.; Silva, J.J.; Cézar, M.; Bezerra, L. Carcass and meat quality in lambs receiving natural tannins from *Mimosa tenuiflora* hay. *Small Rumin. Res.* **2021**, *198*, 106362. [CrossRef]
15. Rojas-Román, L.; Castro-Pérez, B.; Estrada-Angulo, A.; Angulo-Montoya, C.; Yocupicio-Rocha, J.; López-Soto, M.; Barreras, A.; Zinn, R.A.; Plascencia, A. Influence of long-term supplementation of tannins on growth performance, dietary net energy and carcass characteristics: Finishing lambs. *Small Rumin. Res.* **2017**, *153*, 137–141. [CrossRef]
16. Priolo, A.; Valenti, B.; Natalello, A.; Bella, M.; Luciano, G.; Pauselli, M. Fatty acid metabolism in lambs fed hazelnut skin as a partial replacer of maize. *Anim. Feed Sci. Technol.* **2021**, *272*, 114794. [CrossRef]
17. Valenti, B.; Natalello, A.; Vasta, V.; Campidonico, L.; Roscini, V.; Mattioli, S.; Pauselli, M.; Priolo, A.; Lanza, M.; Luciano, G. Effect of different dietary tannin extracts on lamb growth performances and meat oxidative stability: Comparison between mimosa, chestnut and tara. *Animal* **2019**, *13*, 435–443. [CrossRef]
18. Natalello, A.; Priolo, A.; Valenti, B.; Codini, M.; Mattioli, S.; Pauselli, M.; Puccio, M.; Lanza, M.; Stergiadis, S.; Luciano, G. Dietary pomegranate by-product improves oxidative stability of lamb meat. *Meat Sci.* **2020**, *162*, 108037. [CrossRef]
19. Zhong, R.Z.; Fang, Y.; Wang, Y.; Sun, H.; Zhou, D. Effects of substituting finely ground sorghum for finely ground corn on feed digestion and meat quality in lambs infected with *Haemonchus contortus*. *Anim. Feed Sci. Technol.* **2015**, *211*, 31–40. [CrossRef]
20. Dentinho, M.T.P.; Paulos, K.; Francisco, A.; Belo, A.T.; Jerónimo, E.; Almeida, J.; Bessa, R.J.B.; Santos-Silva, J. Effect of soybean meal treatment with *Cistus ladanifer* condensed tannins in growth performance, carcass and meat quality of lambs. *Livest. Sci.* **2020**, *236*, 104021. [CrossRef]
21. Liu, H.; Li, K.; Lv, M.; Zhao, J.; Xiong, B. Effects of chestnut tannins on the meat quality, welfare, and antioxidant status of heat-stressed lambs. *Meat Sci.* **2016**, *116*, 236–242. [CrossRef]
22. Wang, S.; Giller, K.; Hillmann, E.; Marquardt, S.; Schwarm, A. Effect of supplementation of pelleted hazel (*Corylus avellana*) leaves on blood antioxidant activity, cellular immune response and heart beat parameters in sheep. *J. Anim. Sci.* **2019**, *97*, 4496–4502. [CrossRef] [PubMed]
23. Caprarulo, V.; Giromini, C.; Rossi, L. Review: Chestnut and quebracho tannins in pig nutrition: A review of the effects on performance and intestinal health. *Animal* **2021**, *15*, 100064. [CrossRef] [PubMed]
24. Manessis, G.; Kalogianni, A.I.; Lazou, T.; Moschovas, M.; Bossis, I.; Gelasakis, A.I. Plant-Derived Natural Antioxidants in Meat and Meat Products. *Antioxidants* **2020**, *9*, 1215. [CrossRef]
25. Sauvant, D.; Schmidely, P.; Daudin, J.J.; St-Pierre, N.R. Meta-analyses of experimental data in animal nutrition. *Animal* **2008**, *2*, 1203–1214. [CrossRef] [PubMed]

26. Borenstein, M.; Hedges, L.V.; Higgins, J.P.T.; Rothstein, H.R. *Introduction to Meta-Analysis*, 1st ed.; John Wiley & Sons: Chichester, UK, 2009; p. 413.
27. Doré, T.; Makowski, D.; Malézieux, E.; Munier-Jolain, N.; Tchamitchian, M.; Tittonell, P. Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. *Eur. J. Agron.* **2011**, *34*, 197–210. [CrossRef]
28. Higgins, J.P.T.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analysis. *BMJ* **2003**, *327*, 557–560. [CrossRef]
29. Abhijith, A.; Dunshea, F.R.; Warner, R.D.; Leury, B.J.; Ha, M.; Chauhan, S.S. A Meta-Analysis of the Effectiveness of High, Medium, and Low Voltage Electrical Stimulation on the Meat Quality of Small Ruminants. *Foods* **2020**, *9*, 1587. [CrossRef]
30. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Prisma Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med.* **2009**, *6*, e1000097. [CrossRef] [PubMed]
31. Herremans, S.; Vanwindekens, F.; Decruyenaere, V.; Beckers, Y.; Froidmont, E. Effect of dietary tannins on milk yield and composition, nitrogen partitioning and nitrogen use efficiency of lactating dairy cows: A meta-analysis. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 1209–1218. [CrossRef]
32. Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Hernández-García, P.A. Effects of Dietary Tannins' Supplementation on Growth Performance, Rumen Fermentation, and Enteric Methane Emissions in Beef Cattle: A Meta-Analysis. *Sustainability* **2021**, *13*, 7410. [CrossRef]
33. Lean, I.J.; Thompson, J.M.; Dunshea, F.R. A Meta-Analysis of Zilpaterol and Ractopamine Effects on Feedlot Performance, Carcass Traits and Shear Strength of Meat in Cattle. *PLoS ONE* **2014**, *9*, e115904. [CrossRef] [PubMed]
34. Belanche, A.; Newbold, C.J.; Morgavi, D.P.; Bach, A.; Zweifel, B.; Yáñez-Ruiz, D.R. A Meta-analysis Describing the Effects of the Essential oils Blend Agolin Ruminant on Performance, Rumen Fermentation and Methane Emissions in Dairy Cows. *Animals* **2020**, *10*, 620. [CrossRef] [PubMed]
35. Higgins, J.; Thomas, J. *Cochrane Handbook for Systematic Reviews of Interventions*, 2nd ed.; John Wiley and Sons Ltd: Chichester, UK, 2019; pp. 143–176.
36. Wallace, B.C.; Lajeunesse, M.J.; Dietz, G.; Dahabreh, I.J.; Trikalinos, T.A.; Schmid, C.H.; Gurevitch, J. OpenMEE: Intuitive, open-source software for metaanalysis in ecology and evolutionary biology. *Methods Ecol. Evol.* **2016**, *8*, 941–947. [CrossRef]
37. Hedges, L.V. Distribution theory for Glass's estimator of effect size and related estimators. *J. Educ. Stat.* **1981**, *6*, 107–128. [CrossRef]
38. Der Simonian, R.; Laird, N. Meta-analysis in clinical trials. *Control. Clin. Trials* **1986**, *7*, 177–188. [CrossRef]
39. Lean, I.J.; Rabiee, A.R.; Duffield, T.F.; Dohoo, I.R. Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. *J. Dairy Sci.* **2009**, *92*, 3545–3565. [CrossRef] [PubMed]
40. SAS (Statistical Analysis System). *SAS/STAT User's Guide (Release 6.4)*; SAS Inst.: Cary, NC, USA, 2017.
41. Torres, R.N.S.; Moura, D.C.; Ghedini, C.P.; Ezequiel, J.M.B.; Almeida, M.T.C. Meta-analysis of the effects of essential oils on ruminal fermentation and performance of sheep. *Small Rumin. Res.* **2020**, *189*, 106148. [CrossRef]
42. Higgins, J.P.T.; Thompson, S.G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **2002**, *21*, 1539–1558. [CrossRef] [PubMed]
43. Egger, M.; Smith, G.D.; Altman, D.G. *Systematic Reviews in Health Care*, 2nd ed.; MBJ Publishing Group: London, UK, 2001; pp. 109–121.
44. Littell, J.H.; Corcoran, J.; Pillai, V. *Systematic Reviews and Meta-Analysis*, 1st ed.; Oxford University Press: Oxford, UK, 2008; pp. 111–132.
45. Sterne, J.A.C.; Harbord, R.M. Funnel plots in meta-analysis. *Stata J.* **2004**, *4*, 127–141. [CrossRef]
46. Begg, C.B.; Mazumdar, M. Operating Characteristics of a Rank Correlation Test for Publication Bias. *Biometrics* **1994**, *50*, 1088–1101. [CrossRef]
47. Egger, M.; Smith, G.D.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **1997**, *315*, 629–634. [CrossRef]
48. Ioannidis, J.P.A.; Trikalinos, T.A. The appropriateness of asymmetry tests for publication bias in meta-analyses: A large survey. *CMAJ* **2007**, *176*, 1091–1096. [CrossRef]
49. Duval, S.; Tweedie, R. A nonparametric "trim and fill" method of accounting for publication bias in meta-analysis. *J. Amer. Statist. Assoc.* **2000**, *95*, 89–98. [CrossRef]
50. Toldrá, F. *Lawrie's Meat Science*, 8th ed.; Woodhead Publishing Limited: Cambridge, UK, 2017; 713p.
51. Orlandi, T.; Kozloski, G.V.; Alves, T.P.; Mesquita, F.R.; Ávila, S.C. Digestibility, ruminal fermentation and duodenal flux of amino acids in steers fed grass forage plus concentrate containing increasing levels of *Acacia mearnsii* tannin extract. *Anim. Feed Sci. Technol.* **2015**, *210*, 37–45. [CrossRef]
52. Yang, K.; Wei, C.; Zhao, G.; Xu, Z.; Lin, S. Dietary supplementation of tannic acid modulates nitrogen excretion pattern and urinary nitrogenous constituents of beef cattle. *Livest. Sci.* **2016**, *191*, 148–152. [CrossRef]
53. Bhattacharyya, A.; Chattopadhyay, R.; Mitra, S.; Crowe, S.E. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. *Physiol. Rev.* **2014**, *94*, 329–354. [CrossRef] [PubMed]
54. Wang, Y.; Chen, Y.; Zhang, X.; Lu, Y.; Chen, H. New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: A review. *J. Funct. Foods* **2020**, *75*, 104248. [CrossRef]

55. Dong, S.; Li, H.; Gasco, L.; Xiong, Y.; Guo, K.J.; Zoccarato, I. Antioxidative activity of the polyphenols from the involucres of Castanea mollissima Blume and their mitigating effects on heat stress. *Poult. Sci.* **2015**, *94*, 1096–1104. [CrossRef] [PubMed]
56. Gessner, D.K.; Ringseis, R.; Eder, K. Potential of Plant Polyphenols to Combat Oxidative Stress and Inflammatory Processes in Farm Animals. *J. Anim. Physiol. Anim. Nutr.* **2017**, *101*, 605–628. [CrossRef] [PubMed]
57. Tedeschi, L.O.; Muir, J.P.; Naumann, H.D.; Norris, A.B.; Ramirez-Restrepo, C.A.; Mertens-Talcott, S.U. Nutritional Aspects of Ecologically Relevant Phytochemicals in Ruminant Production. *Front. Vet. Sci.* **2021**, *8*, 628445. [CrossRef]
58. Lesschaeve, I.; Noble, A.C. Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. *Am. J. Clin. Nutr.* **2005**, *81*, 330S–335S. [CrossRef]
59. Glendinning, J.I. Is the bitter rejection response always adaptive? *Physiol. Behav.* **1994**, *56*, 1217–1227. [CrossRef]
60. Lamy, E.; Da Costa, G.; Santos, R.; Capela e Silva, F.; Potes, J.; Pereira, A.; Coelho, A.V.; Baptista, E.S. Effect of condensed tannin ingestion in sheep and goat parotid saliva proteome. *J. Anim. Physiol. Anim. Nutr.* **2011**, *95*, 304–312. [CrossRef]
61. Lamy, E.; Rodrigues, L.; Guerreiro, O.; Soldado, D.; Francisco, A.; Lima, M.; Silva, F.C.E.; Lopes, O.; Santos-Silva, J.; Jerónimo, E. Changes in salivary protein composition of lambs supplemented with aerial parts and condensed tannins: Extract from *Cistus ladanifer* L.-A preliminary study. *Agrofor. Syst.* **2020**, *94*, 1501–1559. [CrossRef]
62. Austin, P.J.; Suchar, L.A.; Robbins, C.T.; Hagerman, A.E. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *J. Chem. Ecol.* **1989**, *15*, 1335–1347. [CrossRef]
63. McArthur, C.; Sanson, G.D.; Beal, A.M. Salivary proline-rich proteins in mammals—Roles in oral homeostasis and counteracting dietary tannin. *J. Chem. Ecol.* **1995**, *21*, 663–691. [CrossRef] [PubMed]
64. Lamy, E.; Rawel, H.; Schweigert, F.J.; Capela e Silva, F.; Ferreira, A.; Costa, A.R.; Antunes, C.; Almeida, A.M.; Coelho, A.V.; Sales-Baptista, E. The Effect of Tannins on Mediterranean Ruminant Ingestive Behavior: The Role of the Oral Cavity. *Molecules* **2011**, *16*, 2766–2784. [CrossRef] [PubMed]
65. Corazzin, M.; Del Bianco, S.; Bovolenta, S.; Piasentier, E. Carcass Characteristics and Meat Quality of Sheep and Goat. In *More than Beef, Pork and Chicken-The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet*; Lorenzo, J.M., Munekata, P.E.S., Barba, F., Toldrá, F., Eds.; Springer International Publishing: Cham, Switzerland, 2019; pp. 119–165. ISBN 978-3-030-05483-0.
66. Liang, H.; Xu, L.; Zhao, X.; Pan, K.; Yi, Z.; Bai, J.; Qi, X.; Xin, J.; Li, M.; Ouyang, K.; et al. RNA-Seq analysis reveals the potential molecular mechanisms of daidzein on adipogenesis in subcutaneous adipose tissue of finishing Xianan beef cattle. *J. Anim. Physiol. Anim. Nutr.* **2020**, *104*, 1–11. [CrossRef] [PubMed]
67. Qin, X.; Zhang, T.; Cao, Y.; Deng, B.; Zhang, J.; Zhao, J. Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. *Meat Sci.* **2020**, *166*, 108141. [CrossRef]
68. Biondi, L.; Rabdazzo, C.L.; Russo, N.; Pino, A.; Natalello, A.; Van Hoorde, K.; Caggia, C. Dietary Supplementation of Tannin-Extracts to Lambs: Effects on Meat Fatty Acids Composition and Stability and on Microbial Characteristics. *Foods* **2019**, *8*, 469. [CrossRef]
69. Cimmino, R.; Barone, C.M.A.; Claps, S.; Varricchio, E.; Rufrano, D.; Caroprese, M.; Albenzio, M.; De Palo, P.; Campanile, G.; Neglia, G. Effects of dietary supplementation with polyphenols on meat quality in Saanen goat kids. *BMC Vet. Res.* **2018**, *14*, 181. [CrossRef]
70. Calnan, H.; Jacob, R.; Pethick, D.; Gardner, G. Factors affecting the colour of lamb meat from the longissimus muscle during display: The influence of muscle weight and muscle oxidative capacity. *Meat Sci.* **2014**, *96*, 1049–1057. [CrossRef]
71. Komprda, T.; Kuchtík, J.; Jarošová, A.; Dračková, E.; Zemánek, L.; Filipčík, R. Meat quality characteristics of lambs of three organically raised breeds. *Meat Sci.* **2012**, *91*, 499–505. [CrossRef] [PubMed]
72. Teixeira, A.; Jimenez-Badillo, M.R.; Rodriguez, S. Effect of sex and carcass weight on carcass traits and meat quality in goat kids of cabrito transmontano. *Span. J. Agric. Res.* **2011**, *9*, 753–760. [CrossRef]
73. Fruet, A.P.B.; Giotto, F.M.; Fonseca, M.A.; Nörnberg, J.L.; de Mello, A.S. Effects of the incorporation of tannin extract from quebracho colorado wood on color parameters, lipid oxidation, and sensory attributes of beef patties. *Foods* **2020**, *9*, 667. [CrossRef] [PubMed]
74. Contini, C.; Alvarez, R.; O'Sullivan, M.; Dowling, D.P.; Gargan, S.O.; Monahan, F.J. Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. *Meat Sci.* **2014**, *96*, 1171–1176. [CrossRef]
75. Fraga-Corral, M.; Otero, P.; Echave, J.; GarciaOliveira, P.; Carpena, M.; Jarboui, A.; Nuñez-Estevez, B.; Simal-Gandara, J.; Prieto, M.A. By-Products of Agri-Food Industry as Tannin-Rich Sources: A Review of Tannins' Biological Activities and Their Potential for Valorization. *Foods* **2021**, *10*, 137. [CrossRef]
76. Malheiros, J.M.; Braga, C.P.; Grove, R.A.; Ribeiro, F.A.; Calkins, C.R.; Adamec, J.; Chardulo, L.A.L. Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Sci.* **2019**, *148*, 64–71. [CrossRef] [PubMed]
77. Zhong, R.Z.; Zhou, D.W.; Tan, C.Y.; Tan, Z.L.; Han, X.F.; Zhou, C.S.; Tang, S.X. Effect of tea catechins on regulation of antioxidant enzyme expression in H_2O_2-induced skeletal muscle cells of goat in vitro. *J. Agric. Food Chem.* **2011**, *59*, 11338–11343. [CrossRef]
78. Piccione, G.; Casella, S.; Giannetto, C.; Bazzano, M.; Giudice, E.; Fazio, F. Oxidative stress associated with road transportation in ewes. *Small Rumin. Res.* **2013**, *112*, 235–238. [CrossRef]
79. Ablikim, B.; Liu, Y.; Kerim, A.; Shen, P.; Abdurerim, P.; Zhou, G.H. Effects of breed, muscle type, and frozen storage on physico-chemical characteristics of lamb meat and its relationship with tenderness. *CyTA J. Food* **2016**, *14*, 109–116. [CrossRef]

80. Hocquette, J.F.; Gondret, F.; Baéza, E.; Médale, F.; Jurie, C.; Pethick, D.W. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal* **2010**, *4*, 303–319. [CrossRef] [PubMed]
81. Jin, Q.; Liu, G.; Tan, X.; Zhang, X.; Liu, X.; Wei, C. Gallic acid as a key substance to inhibit proliferation and adipogenesis in bovine subcutaneous adipocyte. *Anim. Biotechnol.* **2020**, 1–7. [CrossRef]
82. Lin, J.; Della-Fera, M.A.; Baile, C.A. Green tea polyphenol epigallocatechin gallate inhibits adipogenesis and induces apoptosis in 3T3-L1 adipocytes. *Obes. Res.* **2005**, *13*, 982–990. [CrossRef]
83. Liu, X.; Kim, J.K.; Li, Y.; Li, J.; Liu, F.; Chen, X. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. *J. Nutr.* **2005**, *135*, 165–171. [CrossRef]
84. Barlocco, N.; Vadell, A.; Ballesteros, F.; Galietta, G.; Cozzolino, D. Predicting intramuscular fat, moisture and Warner-Bratzler shear force in pork muscle using near infrared reflectance spectroscopy. *Anim. Sci.* **2006**, *82*, 111–116. [CrossRef]
85. Pateiro, M.; Barba, F.J.; Domínguez, R.; Sant'Ana, A.S.; Mousavi Khaneghah, A.; Gavahian, M.; Gómez, B.; Lorenzo, J.M. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food Res. Int.* **2018**, *113*, 156–166. [CrossRef] [PubMed]
86. Faustman, C.; Sun, Q.; Mancini, R.; Suman, S. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Sci.* **2010**, *86*, 86–94. [CrossRef]
87. Liu, H.; Zhou, D.; Li, K. Effects of chestnut tannins on performance and antioxidative status of transition dairy cows. *J. Dairy Sci.* **2013**, *96*, 5901–5907. [CrossRef]
88. Chivandi, E.; Dangarembizi, R.; Nyakudya, T.T.; Erlwanger, K.H. Use of Essential Oils as a Preservative of Meat. In *Essential Oils in Food Preservation, Flavor and Safety*; Elsevier: Amsterdam, The Netherlands, 2016. [CrossRef]
89. Jami, E.; Israel, A.; Kotser, A.; Mizrahi, I. Exploring the bovine rumen bacterial community from birth to adulthood. *ISME J.* **2013**, *7*, 1069–1079. [CrossRef]
90. Gladine, C.; Rock, E.; Morand, C.; Bauchart, D.; Durand, D. Bioavailability and Antioxidant Capacity of Plant Extracts Rich in Polyphenols, given as a Single Acute Dose, in Sheep Made Highly Susceptible to Lipoperoxidation. *Br. J. Nutr.* **2007**, *98*, 691–701. [CrossRef] [PubMed]
91. López-Andrés, P.; Luciano, G.; Vasta, V.; Gibson, T.M.; Biondi, L.; Priolo, A.; Mueller-Harvey, I. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. *Br. J. Nutr.* **2013**, *110*, 632–639. [CrossRef] [PubMed]
92. Ghiselli, A.; Serafini, M.; Natella, F.; Scaccini, C. Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radic. Biol. Med.* **2000**, *29*, 1106–1114. [CrossRef]
93. Celi, P. Oxidative stress in ruminants. In *Studies on Veterinary Medicine. Oxidative Stress in Applied Basic Research and Clinical Practice*; Mandelker, L., Vajdovich, P., Eds.; Humana Press: Totowa, NJ, USA; New York, NY, USA, 2011; pp. 191–231. ISBN 978-1-61779-070-6.
94. Cuervo, W.; Sordillo, L.M.; Abuelo, A. Oxidative Stress Compromises Lymphocyte Function in Neonatal Dairy Calves. *Antioxidants* **2021**, *10*, 255. [CrossRef] [PubMed]
95. Peng, K.; Shirley, D.C.; Xu, Z.; Huang, Q.; Mcallister, T.A.; Chaves, A.V.; Acharya, S.; Liu, C.L.; Wang, S.X.; Wang, Y.X. Effect of purple prairie clover (*Dalea purpurea* vent.) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. *Anim. Feed Sci. Technol.* **2016**, *222*, 100–110. [CrossRef]
96. Masella, R.; Di Benedetto, R.; Varì, R.; Filesi, C.; Giovannini, C. Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione-related enzymes. *J. Nutr. Biochem.* **2005**, *16*, 577–586. [CrossRef]
97. Celi, P. Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacol. Immunotoxicol.* **2011**, *33*, 233–240. [CrossRef]
98. Awawdeh, M.S.; Dager, H.K.; Obeidat, B.S. Effects of alternative feedstuffs on growth performance, carcass characteristics, and meat quality of growing Awassi lambs. *Ital. J. Anim. Sci.* **2019**, *18*, 777–785. [CrossRef]
99. Bandeira, P.A.V.; Pereira Filho, J.M.; Silva, A.M.A.; Cezar, M.F.; Bakke, A.O.; Silva, U.L.; Borburema, J.B.; Bezerra, L.R. Performance and carcass characteristics of lambs fed diets with increasing levels of *Mimosa tenuiflora* (Willd.) hay replacing Buffel grass hay. *Trop. Anim. Health Prod.* **2017**, *49*, 1001–1007. [CrossRef]
100. Bhatt, R.S.; Sahoo, A.; Sarkar, S.; Soni, L.; Sharma, P.; Gadekar, Y.P. Dietary supplementation of plant bioactive-enriched aniseed straw and eucalyptus leaves modulates tissue fatty acid profile and nuggets quality of lambs. *Animal* **2020**, *14*, 2642–2651. [CrossRef]
101. Bonanno, A.; Di Miceli, G.; Di Grigoli, A.; Frenda, A.S.; Tornambè, G.; Giambalvo, D.; Amato, G. Effects of feeding green forage of sulla (*Hedysarum coronarium* L.) on lamb growth, gastrointestinal nematode infection, and carcass and meat quality. *Animal* **2011**, *5*, 148–154. [CrossRef]
102. Buccioni, A.; Pauselli, M.; Minieri, S.; Roscini, V.; Mannelli, F.; Rapaccini, S.; Lupi, P.; Conte, G.; Serra, A.; Cappucci, A. Chestnut or quebracho tannins in the diet of grazing ewes supplemented with soybean oil: Effects on animal performances, blood parameters and fatty acid composition of plasma and milk lipids. *Small Rumin. Res.* **2017**, *153*, 23–30. [CrossRef]
103. Chikwanha, O.C.; Moelich, E.; Gouws, P.; Muchenje, V.; Nolte, J.V.E.; Dugan, M.E.R.; Mapiye, C. Effects of feeding increasing levels of grape (*Vitis vinifera* cv. Pinotage) pomace on lamb shelf-life and eating quality. *Meat Sci.* **2019**, *157*, 107887. [CrossRef] [PubMed]

104. Chikwanha, O.C.; Muchenje, V.; Nolte, J.E.; Dugan, M.E.R.; Mapiye, C. Grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality. *Meat Sci.* **2019**, *147*, 6–12. [CrossRef]
105. Costa, E.d.S.; Ribiero, C.; Silva, T.; Ribeiro, R.; Vieira, J.; Lima, A.d.O.; Barbosa, A.; da Silva, J.J.M.; Bezerra, L.R.; Oliveira, R.L. Intake, nutrient digestibility, nitrogen balance, serum metabolites and growth performance of lambs supplemented with *Acacia mearnsii* condensed tannin extract. *Anim. Feed Sci. Technol.* **2021**, *272*, 114744. [CrossRef]
106. Dey, A.; Dutta, N.; Pattanaik, A.K.; Sharma, K. Antioxidant status, metabolic profile and immune response of lambs supplemented with tannin rich *Ficus infectoria* leaf meal. *Jpn. J. Vet. Res.* **2015**, *63*, 15–24. [CrossRef]
107. Abdalla Filho, A.L.; Corrêa, P.S.; Lemos, L.N.; Dineshkumar, D.; Issakowicz, J.; Ieda, E.H.; Lima, P.M.T.; Barreal, M.; McManus, C.; Mui, T.S.; et al. Diets based on plants from Brazilian Caatinga altering ruminal parameters, microbial community and meat fatty acids of Santa Inês lambs. *Small Rumin. Res.* **2017**, *154*, 70–77. [CrossRef]
108. Francisco, A.; Alves, S.P.; Portugal, P.V.; Dentinho, M.T.; Jerónimo, E.; Sengo, S.; Almeida, J.; Bressan, M.C.; Pires, V.M.R.; Alfaia, C.M.; et al. Effects of dietary inclusion of citrus pulp and rockrose soft stems and leaves on meat quality and fatty acid composition. *Animal* **2017**, *12*, 872–881. [CrossRef]
109. Girard, M.; Dohme-Meier, F.; Silacci, P.; Ampuero Kragten, S.; Kreuzer, M.; Bee, G. Forage legumes rich in condensed tannins may increase n-3 fatty acid levels and sensory quality of lamb meat. *J. Sci. Food Agric.* **2016**, *96*, 1923–1933. [CrossRef]
110. Guerreiro, O.; Alves, S.P.; Soldado, D.; Cachucho, L.; Almeida, J.M.; Francisco, A.; Jerónimo, E. Inclusion of the aerial part and condensed tannin extract from Cistus ladanifer L. in lamb diets-Effects on growth performance, carcass and meat quality and fatty acid composition of intramuscular and subcutaneous fat. *Meat Sci.* **2020**, *160*, 107945. [CrossRef]
111. Gruffat, D.; Durand, D.; Rivaroli, D.; do Prado, I.N.; Prache, S. Comparison of muscle fatty acid composition and lipid stability in lambs stall-fed or pasture-fed alfalfa with or without sainfoin pellet supplementation. *Animal* **2020**, *14*, 1093–1101. [CrossRef]
112. Hart, K.J.; Sinclair, L.A.; Wilkinson, R.G.; Huntington, J.A. Effect of Whole-Crop Pea (*Pisum Sativum* L.) Silages Differing in Condensed Tannin Content as a Substitute for Grass Silage and Soybean Meal on the Performance, Metabolism, and Carcass Characteristics of Lambs. *J. Anim. Sci.* **2011**, *89*, 3663–3676. [CrossRef]
113. Hassan, T.M.M.; Ahmed-Farid, O.A.; Abdel-Fattah, F.A.I. Effects of different sources and levels of tannins on live performance and antioxidant response of Ossimi lambs. *J. Agric. Sci.* **2020**, *158*, 339–348. [CrossRef]
114. Hatami, A.; Alipour, D.; Hozhabri, F.; Tabatabaei, M. Effect of different levels of pomegranate marc with or without polyethylene glycol on performance, nutrients digestibility and protozoal population in growing lambs. *Anim. Feed Sci. Tech.* **2018**, *235*, 15–22. [CrossRef]
115. Jerónimo, E.; Alves, S.P.; Dentinho, M.T.P.; Martins, S.V.; Prates, J.A.M.; Vasta, V.; Santos-Silva, J.; Bessa, R.J.B. Effect of grape seed extract, *Cistus ladanifer* L. and vegetable oil supplementation on fatty acid composition of abomasal digesta and intramuscular fat of lambs. *J. Agric. Food Chem.* **2010**, *58*, 10710–10721. [CrossRef] [PubMed]
116. Jerónimo, E.; Alfaia, C.M.M.; Alves, S.P.; Dentinho, M.T.P.; Prates, J.A.M.; Vasta, V.; Santos-Silva, J.; Bessa, R.J.B. Effect of dietary grape seed extract and *Cistus ladanifer* L. in combination with vegetable oil supplementation on lamb meat quality. *Meat Sci.* **2012**, *92*, 841–847. [CrossRef] [PubMed]
117. Kamel, H.E.M.; Al-Dobaib, S.N.; Salem, A.Z.; Lopez, S.; Alaba, P.A. Influence of dietary supplementation with sunflower oil and quebracho tannins on growth performance and meat fatty acid profile of Awassi lambs. *Anim. Feed Sci. Technol.* **2018**, *235*, 97–104. [CrossRef]
118. Kazemi, M.; Mokhtarpour, A. In vitro and in vivo evaluation of some tree leaves as forage sources in the diet of Baluchi male lambs. *Small Rumin. Res.* **2021**, *201*, 106416. [CrossRef]
119. Leparmarai, P.T.; Sinz, S.; Kunz, C.; Liesegang, A.; Ortmann, S.; Kreuzer, M.; Marquardt, S. Transfer of total phenols from a grapeseed-supplemented diet to dairy sheep and goat milk, and effects on performance and milk quality. *J. Anim. Sci.* **2019**, *97*, 1840–1851. [CrossRef]
120. Lima, P.M.T.; Filho, A.L.A.; Issakowicz, J.; Ieda, E.H.; Corrêa, P.S.; Mattos, W.T.; Gerdes, L.; McManus, C.; Abdalla, A.L.; Louvandini, H. Methane emission, ruminal fermentation parameters and fatty acid profile of meat in Santa Inês lambs fed the legume macrotiloma. *Anim. Prod. Sci.* **2020**, *60*, 665–673. [CrossRef]
121. Majewska, M.P.; Kowalik, B. Growth Performance, Carcass Characteristics, Fatty Acid Composition, and Blood Biochemical Parameters of Lamb Fed Diet with the Addition of Lingonberry Leaves and Oak Bark. *Eur. J. Lipid Sci. Technol.* **2020**, *122*, 1900273. [CrossRef]
122. Flores, D.R.M.; da Fonseca, P.A.F.; Schmitt, J.; Tonetto, C.J.; Junior, A.G.R.; Hammerschmitt, R.K.; Facco, D.B.; Brunetto, G.; Nörnberg, J.L. Lambs fed with increasing levels of grape pomace silage: Effects on productive performance, carcass characteristics, and blood parameters. *Livest. Sci.* **2020**, *240*, 104169. [CrossRef]
123. Flores, D.R.M.; da Fonseca, P.A.F.; Schmitt, J.; Tonetto, C.J.; Junior, A.G.R.; Hammerschmitt, R.K.; Facco, D.B.; Brunetto, G.; Nörnberg, J.L. Lambs fed with increasing levels of grape pomace silage: Effects on meat quality. *Small Rumin. Res.* **2021**, *195*, 106234. [CrossRef]
124. Moghaddam, V.K.; Elahi, M.Y.; Nasri, M.H.F.; Elghandour, M.M.M.Y.; Monroy, J.C.; Salem, A.Z.M.; Karami, M.; Mlambo, V. Growth performance and carcass characteristics of finishing male lambs fed barberry pomace-containing diets. *Anim. Biotechnol.* **2019**, *32*, 178–184. [CrossRef]

125. Nobre, P.T.; Munekata, P.E.; Costa, R.G.; Carvalho, F.R.; Ribeiro, N.L.; Queiroga, R.C.; Sousa, S.; Silva, A.C.R.; Lorenzo, J.M. The impact of dietary supplementation with guava (*Psidium guajava* L.) agroindustrial waste on growth performance and meat quality of lambs. *Meat Sci.* **2020**, *164*, 108105. [CrossRef] [PubMed]
126. Norouzian, M.A.; Ghiasi, S.E. Carcass performance and mineral content in Balouchi lamb fed pistachio byproduct. *Meat Sci.* **2012**, *92*, 157–159. [CrossRef]
127. Obeidat, B.S.; Alrababah, M.A.; Abdullah, A.Y.; Alhamad, M.N.; Gharaibeh, M.A.; Rababah, T.M.; Ishmais, M.A. Growth performance and carcass characteristics of Awassi lambs fed diets containing carob pods (*Ceratonia siliqua* L.). *Small Rumin. Res.* **2011**, *96*, 149–154. [CrossRef]
128. Odhaib, K.J.; Adeyemi, K.D.; Sazili, A.Q. Carcass traits, fatty acid composition, gene expression, oxidative stability and quality attributes of different muscles in Dorper lambs fed Nigella sativa seeds, Rosmarinus officinalis leaves and their combination. *Asian-Australas. J. Anim. Sci.* **2018**, *31*, 1345–1357. [CrossRef]
129. Po, E.; Horsburgh, K.; Raadsma, H.W.; Celi, P. Yerba Mate (*Ilex paraguarensis*) as a novel feed supplement for growing lambs. *Small Rumin. Res.* **2012**, *106*, 131–136. [CrossRef]
130. Rajabi, M.; Rouzbehan, Y.; Rezaei, J. A strategy to improve nitrogen utilization, reduce environmental impact, and increase performance and antioxidant capacity of fattening lambs using pomegranate peel extract. *J. Anim. Sci.* **2017**, *95*, 499–510. [CrossRef]
131. Sánchez, N.; Mendoza, G.D.; Martínez, J.A.; Hernández, P.A.; Camacho, L.M.; Lee-Rangel, H.A.; Vázquez, A.; Flores, R. Effect of Caesalpinia coriaria fruits and soybean oil on finishing lamb performance and meat characteristics. *Biomed Res. Int.* **2018**, 1–6. [CrossRef] [PubMed]
132. Sena, J.A.B.; Villela, S.D.J.; Santos, R.A.; Pereira, I.G.; Castro, G.H.F.; Mourthe, M.H.F.; Bonfá, C.S.; Martins, P.G.M.A. Intake, digestibility, performance, and carcass traits of rams provided with dehydrated passion fruit (*Passiflora edulis* f. *flavicarpa*) peel, as a substitute of Tifton 85 (*Cynodon* spp.). *Small Rumin. Res.* **2015**, *129*, 18–24. [CrossRef]
133. Sharifi, A.; Chaji, M. Effects of processed recycled poultry bedding with tannins extracted from pomegranate peel on the nutrient digestibility and growth performance of lambs. *S. Afr. J. Anim. Sci.* **2019**, *49*, 290–300. [CrossRef]
134. SoltaniNezhad, B.; Dayani, O.; Khezri, A.; Tahmasbi, R. Performance and carcass characteristics in fattening lambs fed diets with different levels of pistachio byproducts silage with wasted date. *Small Rumin. Res.* **2016**, *137*, 177–182. [CrossRef]
135. Sun, H.X.; Gao, T.S.; Zhong, R.Z.; Fang, Y.; Di, G.L.; Zhou, D.W. Effects of corn replacement by sorghum in diets on performance, nutrient utilization, blood parameters, antioxidant status and meat color stability in lambs. *Can. J. Anim. Sci.* **2018**, *98*, 723–731. [CrossRef]
136. Yisehak, K.; Biruk, K.; Abegaze, B.; Janssens, G.P.J. Growth of sheep fed tanninrich Albizia gummifera with or without polyethylene glycol. *Trop. Anim. Health Prod.* **2014**, *46*, 1113–1118. [CrossRef]
137. Zhao, M.D.; Di, L.F.; Tang, Z.Y.; Jiang, W.; Li, C.Y. Effect of tannins and cellulase on growth performance, nutrients digestibility, blood profiles, intestinal morphology and carcass characteristics in Hu sheep. Asian-Australas. *J. Anim. Sci.* **2019**, *32*, 1540–1547. [CrossRef]

5. CONCLUSIONES GENERALES

Uno de los hallazgos más importantes que surgen de este estudio es que el impacto ambiental de los sistemas de producción de carne de res puede reducirse notablemente cuando se incluyen taninos en la dieta. Por lo tanto, la adición de taninos en la dieta de bovinos podría utilizarse como una alternativa natural sostenible para reducir el impacto ambiental de la producción de carne bovina, sin afectar la sostenibilidad económica. Sin embargo, es necesario abordar varias cuestiones antes de las recomendaciones específicas para el uso comercial de taninos para reducir el impacto ambiental.

Los resultados del presente trabajo también indican que la suplementación dietética con taninos no afecta el consumo de materia seca, pero mejora la ganancia diaria de peso, la tasa de conversión alimenticia, la capacidad antioxidante total y la actividad de las enzimas antioxidantes en el suero sanguíneo de ovinos.

Además, el fitogénico Peptasan® podría ser utilizado para mejorar la terneza de la carne de corderos, sin afectar el comportamiento productivo y las características de la canal. Sin embargo, la mayor pérdida por goteo observada en respuesta a la suplementación con Peptasan® podría ser un riesgo de deterioro microbiano durante el almacenamiento de la carne de cordero. Por lo tanto, es conveniente realizar más estudios de calidad de la carne a nivel muscular para evaluar el impacto de otras dosis de este producto en raciones para ovinos con diferente proporción de concentrado, en periodos experimentales y etapas fisiológicas diferentes.