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ADITIVOS FITOGÉNICOS PARA RUMIANTES: EFECTOS EN COMPORTAMIENTO PRODUCTIVO, FERMENTACIÓN RUMINAL, METABOLITOS SANGUÍNEOS Y CALIDAD DE CARNE

TESIS

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para obtener el grado de

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ADITIVOS FITOGÉNICOS PARA RUMIANTES: EFECTOS EN COMPORTAMIENTO PRODUCTIVO, FERMENTACIÓN RUMINAL, METABOLITOS SANGUÍNEOS Y CALIDAD DE CARNE

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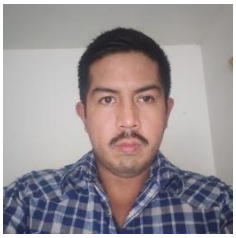

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RESUMEN GENERAL

ADITIVOS FITOGÉNICOS PARA RUMIANTES: EFECTOS EN COMPORTAMIENTO PRODUCTIVO, FERMENTACIÓN RUMINAL, METABOLITOS SANGUÍNEOS Y CALIDAD DE CARNE [1]

PHYTOGENIC ADDITIVES FOR RUMINANTS: EFFECTS ON PERFORMANCE, RUMINAL FERMENTATION, BLOOD METABOLITES, AND MEAT QUALITY [2]

Resumen

Los aditivos fitogénicos pueden mejorar la productividad, metabolismo, salud y calidad de la carne y leche en rumiantes. En un primer estudio, la suplementación dietética con aceites esenciales incrementó (p<0.05) la ingesta de materia seca, ganancia de peso, eficiencia alimenticia y concentración ruminal de propionato en bovinos para carne, pero disminuyó (p<0.05) la pérdida por cocción, fuerza de corte y contenido de malondialdehído en la carne. En un segundo estudio, la inclusión dietética de flavonoides en dietas para vacas lecheras y bovinos para carne mejoró (p<0.05) la ingesta de materia seca, la ganancia de peso, la concentración ruminal de propionato, la producción de leche y el contenido de proteína y grasa en leche. En un tercer estudio, la inclusión de ácidos hidroxicinámicos en dietas para corderos en finalización incrementó (p<0.05) la ingesta de materia seca, ganancia de peso y capacidad antioxidante total en suero sanguíneo. En un cuarto estudio, la suplementación dietética con algas marinas disminuyó (p<0.05) la producción (g d^{-1}) e intensidad (g kg^{-1} de leche producida) de metano entérico en vacas lecheras. En un quinto estudio, la inclusión de un fitogénico poliherbal en dietas para corderos en finalización mejoró (p<0.05) la ingesta de materia seca y la ganancia de peso. En conclusión, los aceites esenciales, flavonoides, ácidos hidroxicinámicos y el fitogénico poliherbal mejoran el comportamiento productivo en bovinos y ovinos. Asimismo, los aceites esenciales y flavonoides mejoran la concentración ruminal de propionato, así como la calidad de la carne y leche en bovinos, mientras que las algas marinas disminuyen las emisiones de metano entérico en vacas lecheras.

Palabras clave: aceites esenciales, flavonoides, ácidos hidroxicinámicos, algas marinas, fitogénico poliherbal.

Abstract

Phytogenic additives can enhance productivity, metabolism, health, and the quality of meat and milk in ruminants. In a first study, dietary supplementation with essential oils increased (p<0.05) dry matter intake, weight gain, feed efficiency, and ruminal propionate concentration in beef cattle, but decreased ($p < 0.05$) cooking loss, shear force, and malondialdehyde content in meat. In a second study, the dietary inclusion of flavonoids in the diets of dairy and beef cattle improved (p<0.05) dry matter intake, weight gain, ruminal propionate concentration, milk production, and the contents of milk protein and fat. In a third study, the inclusion of hydroxycinnamic acids in finishing lamb diets increased (p<0.05) dry matter intake, weight gain, and total antioxidant capacity in blood serum. In a fourth study, dietary supplementation with seaweed decreased (p<0.05) enteric methane production (g d^{-1}) and intensity (g kg^{-1} of milk produced) in dairy cows. In a fifth study, the inclusion of a polyherbal phytogenic in finishing lamb diets improved (p<0.05) dry matter intake and weight gain. In conclusion, essential oils, flavonoids, hydroxycinnamic acids, and the polyherbal phytogenic improve productive performance in cattle and sheep. Furthermore, essential oils and flavonoids improve ruminal propionate concentration, as well as the quality of meat and milk in cattle, while seaweed decreases enteric methane emissions in dairy cows.

Key words: essential oils, flavonoids, hydroxycinnamic acids, seaweed, phytogenic additive.

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1. INTRODUCCIÓN GENERAL

En años recientes, el interés en el uso de sistemas intensivos de producción de rumiantes ha incrementado por la creciente demanda de carne y leche a nivel mundial (Lucio-Ruíz et al., 2024). Desde la década de 1950, los antibióticos se han utilizado con éxito para prevenir, controlar y tratar enfermedades infecciosas en animales domésticos (Enshaie et al., 2025). Asimismo, una revisión reciente (Spilioti et al., 2025) muestra que la adición de dosis subterapéuticas de antibióticos en dietas para rumiantes mejora los parámetros productivos y la salud de los animales. Sin embargo, el uso irresponsable de antibióticos ha contribuido a la aparición de cepas bacterianas resistentes a sus efectos, lo cual ha provocado la prohibición de su uso como promotores del crecimiento en varios países (Enshaie et al., 2025). En consecuencia, en años recientes se ha incrementado el interés en la búsqueda de productos naturales que mejoren la salud y el comportamiento productivo de los rumiantes (Lucio-Ruíz et al., 2024). Entre los productos de origen natural disponibles actualmente a nivel comercial se encuentran los aditivos fitogénicos, los cuales son productos derivados de plantas y algas marinas con actividades biológicas que benefician el comportamiento productivo, los parámetros ruminales, la salud, y la reproducción de los rumiantes (Morais et al., 2020; Wang et al., 2024).

Entre los aditivos fitogénicos disponibles, los aceites esenciales, flavonoides, ácidos hidroxicinámicos, algas marinas y aditivos poliherbales son algunos de los más evaluados en rumiantes (Makkar et al., 2016; Valenzuela-Grijalva et al., 2017). Los aceites esenciales son mezclas de compuestos volátiles extraídos de plantas por destilación y químicamente compuestos por varias moléculas de bajo peso molecular, incluyendo terpenos, alcoholes, aldehídos y cetonas (Wang et al., 2024). De acuerdo con una revisión reciente (Caroprese et al., 2023), los aceites esenciales mejoran los parámetros productivos y ruminales en rumiantes, así como la calidad de la carne y leche. Por otro lado, los flavonoides constituyen el grupo más numeroso de polifenoles y, por lo general, cada uno contiene 15 átomos de carbono con dos anillos de benceno unidos por un puente de tres carbonos (Lucio-Ruíz et al., 2024). Los flavonoides se

encuentran en diversas plantas, frutas y semillas, y tienen efectos inmunoestimulantes, antimicrobianos, antiinflamatorios y antioxidantes (Serra et al., 2021). De acuerdo con Lucio-Ruíz et al. (2024), los flavonoides mejoran la productividad, fermentación ruminal, estado antioxidante en sangre y calidad de la carne de pequeños rumiantes. Sin embargo, el uso de flavonoides como aditivo dietético para vacas lecheras y bovinos para carne ha sido poco estudiado (Olagaray & Bradford, 2019).

Los ácidos hidroxicinámicos son compuestos fenólicos presentes en varias especies vegetales y tienen propiedades antioxidantes, inmunomoduladoras y antiinflamatorias (Peña-Torres et al., 2019). Los ácidos hidroxicinámicos más evaluados como aditivo para ganado son: ferúlico, clorogénico, cafeico y p-cumárico (Valenzuela-Grijalva et al., 2017). Sin embargo, el ácido ferúlico es el más estudiado como aditivo para rumiantes debido a su potencial como promotor natural del crecimiento y antioxidante en ovinos y bovinos (Valadez-García et al., 2021). Por otro lado, las algas marinas (macroalgas) son organismos eucariotas multicelulares fotosintéticos con diferentes tamaños y morfologías (Morais et al., 2020). De acuerdo con Makkar et al. (2016), las algas marinas crecen en el fondo del océano y se clasifican en algas rojas (Rhodophyta), algas marrones (Ochrophyta) y algas verdes (Chlorophyta). Aunque su perfil químico puede variar según el tipo de alga, generalmente contienen una amplia variedad de compuestos bioactivos, como polisacáridos, florotaninos y alcanos halogenados (Morais et al., 2020). El principal alcano halogenado de las algas marinas es el bromoformo, el cual inhibe la producción ruminal de metano, al bloquear la actividad de la enzima metil-coenzima M reductasa (Glasson et al., 2022). Finalmente, los aditivos poliherbales consisten en mezclas de plantas que contienen varios metabolitos bioactivos con propiedades biológicas (Velázquez-Cruz et al., 2025). De acuerdo con estudios recientes (Hashemzadeh et al., 2022; Razo et al., 2020), los aditivos poliherbales mejoran los parámetros productivos y ruminales de corderos en finalización, sin afectar los metabolitos sanguíneos ni la calidad de la carne.

1.1. Hipótesis

La suplementación dietética con aceites esenciales, flavonoides, ácidos hidroxicinámicos, algas marinas y un fitogénico poliherbal beneficiará el comportamiento productivo, la fermentación ruminal, los metabolitos séricos y la calidad de la carne de rumiantes.

1.2. Objetivo general

- Evaluar el efecto de diferentes aditivos fitogénicos en el comportamiento productivo, la fermentación ruminal, los metabolitos sanguíneos y la calidad de la carne de rumiantes.

1.3. Objetivos particulares

- Determinar el efecto de la suplementación dietética con aceites esenciales en el comportamiento productivo, fermentación ruminal, metabolitos sanguíneos y calidad de la carne de bovinos para carne, a través de un metaanálisis.

- Evaluar los efectos de la inclusión dietética de flavonoides en el comportamiento productivo, digestibilidad de nutrientes, estado antioxidante en suero sanguíneo, parámetros ruminales, calidad de la carne y composición de la leche de bovinos para carne y vacas lecheras, a través de un metaanálisis.

- Determinar los efectos de la suplementación dietética con ácidos hidroxicinámicos en el comportamiento productivo, estado antioxidante en suero sanguíneo y calidad de la carne de corderos en finalización, a través de un metaanálisis.

- Evaluar los efectos de la inclusión dietética de algas marinas en la producción y composición de la leche, digestibilidad de nutrientes, fermentación ruminal y emisiones de metano entérico de vacas lecheras, a través de un metaanálisis.

- Probar los efectos de la suplementación dietética con un aditivo fitogénico poliherbal en el comportamiento productivo, energética dietética, metabolitos sanguíneos, características de la canal, calidad de la carne, y expresión génica de corderos en finalización.

1.4. Organización de la tesis

El Capítulo 2 es un metaanálisis que sintetiza cuantitativamente la información publicada entre 2010 y 2022 sobre los efectos de la suplementación dietética con aceites esenciales en el comportamiento productivo, fermentación ruminal, metabolitos sanguíneos y calidad de la carne de bovinos para carne.

El Capítulo 3 es un metaanálisis de la información publicada desde 2010 hasta 2022 sobre los efectos de la suplementación dietética con flavonoides en el comportamiento productivo, digestibilidad de nutrientes, estado antioxidante en suero sanguíneo, parámetros ruminales, calidad de la carne y composición de la leche de bovinos para carne y vacas lecheras.

El Capítulo 4 es un metaanálisis de la información publicada entre 2010 y 2023 sobre los efectos de la suplementación dietética con ácidos hidroxicinámicos en el comportamiento productivo, estado antioxidante en suero sanguíneo y calidad de la carne de corderos en finalización.

El Capítulo 5 es un metaanálisis que comprende las publicaciones científicas desde 2014 hasta 2024 sobre los efectos de la suplementación dietética con algas marinas en la producción de leche, composición de leche, digestibilidad de nutrientes, fermentación ruminal y emisiones de metano entérico de vacas lecheras.

En el Capítulo 6 se muestra los resultados de un experimento donde se probaron los efectos de dosis crecientes de un aditivo fitogénico poliherbal conteniendo conjugados de colina, timoquinona, carvacrol, p-cimeno y otros compuestos bioactivos, en el comportamiento productivo, energética dietética,

metabolitos sanguíneos, características de la canal, calidad de la carne y expresión génica de corderos en finalización.

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2. A META-ANALYSIS OF ESSENTIAL OILS USE FOR BEEF CATTLE FEED: RUMEN FERMENTATION, BLOOD METABOLITES, MEAT QUALITY, PERFORMANCE AND, ENVIRONMENTAL AND ECONOMIC IMPACT

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Article

A Meta-Analysis of Essential Oils Use for Beef Cattle Feed: Rumen Fermentation, Blood Metabolites, Meat Quality, Performance and, Environmental and Economic Impact

José Felipe Orzuna-Orzuna [1], Griselda Dorantes-Iturbide [1], Alejandro Lara-Bueno [1,*], Luis Alberto Miranda-Romero [1], Germán David Mendoza-Martínez [2] and Itzel Santiago-Figueroa [1]

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Abstract: The objective of this study was to see how dietary supplementation with essential oils (EOs) affected rumen fermentation, blood metabolites, growth performance and meat quality of beef cattle through a meta-analysis. In addition, a simulation analysis was conducted to evaluate the effects of EOs on the economic and environmental impact of beef production. Data were extracted from 34 peer-reviewed studies and analyzed using random-effects statistical models to assess the weighted mean difference (WMD) between control and EOs treatments. Dietary supplementation of EOs increased ($p < 0.01$) dry matter intake (WMD = 0.209 kg/d), final body weight (WMD = 12.843 kg), daily weight gain (WMD = 0.087 kg/d), feed efficiency (WMD = 0.004 kg/kg), hot carcass weight (WMD = 5.45 kg), and *Longissimus dorsi* muscle area (WMD = 3.48 cm^2). Lower ($p < 0.05$) ruminal concentration of ammonia nitrogen (WMD = −1.18 mg/dL), acetate (WMD = −4.37 mol/100 mol) and total protozoa (WMD = -2.17×10^5/mL), and higher concentration of propionate (WMD = 0.878 mol/100 mol, $p < 0.001$) were observed in response to EOs supplementation. Serum urea concentration (WMD = −1.35 mg/dL, $p = 0.026$) and haptoglobin (WMD = −39.67 µg/mL, $p = 0.031$) were lower in cattle supplemented with EOs. In meat, EOs supplementation reduced ($p < 0.001$) cooking loss (WMD = −61.765 g/kg), shear force (WMD = −0.211 kgf/cm^2), and malondialdehyde content (WMD = −0.040 mg/kg), but did not affect pH, color (L* a* and b*), or chemical composition ($p > 0.05$). Simulation analysis showed that EOs increased economic income by 1.44% and reduced the environmental footprint by 0.83%. In conclusion, dietary supplementation of EOs improves productive performance and rumen fermentation, while increasing the economic profitability and reducing the environmental impact of beef cattle. In addition, supplementation with EOs improves beef tenderness and oxidative stability.

Keywords: beef cattle; essential oils; rumen fermentation; performance; economic impact; environmental impact; meta-analysis

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1. Introduction

Antibiotics are generally used for the treatment and prevention of diseases in livestock, but in some cases, they are also used as growth promoters for ruminants and non-ruminants [1]. However, according to Argudín et al. [2], the inappropriate use of antibiotics in food animals has contributed to the emergence of bacteria that are resistant to their effects. Consequently, the use of antibiotics as growth promoters has been banned in several countries, leading to research on natural products that may have similar effects to antibiotics [3]. Important alternatives to antibiotics reported in livestock are enzymes, organic acids, probiotics, prebiotics, yeasts, and phytogenics (i.e., tannins, and saponins, among others) [3,4]. Essential oils (EOs) are among the most studied and economically

relevant plant-derived products [4]. EOs are mixtures of volatile compounds extracted from plants by distillation [5] and chemically they comprise a wide diversity of low molecular weight molecules including terpenes, alcohols, aldehydes, and ketones [4].

The effects of purified EOs and plants with high EOs content have been investigated mainly in pigs and poultry [6–8], but information on the effects of EOs in ruminant diets is still limited. In ruminants, the effects of some bioactive metabolites of EOs (eugenol, cinnamaldehyde, and anethole) on ruminal fermentation change depending on rumen pH [9], which is influenced by the level of concentrate in the diet. Similarly, some studies [10,11] have reported better weight gain, feed efficiency, and digestibility in ruminants supplemented with EOs. In other studies, dietary supplementation of EOs had negative [12] or neutral [13] effects on nutrient digestibility and productive performance, respectively. In addition, some studies reported better color, higher tenderness, and lower lipid peroxidation in ruminant meat supplemented with EOs [14,15]. In contrast, in other studies, the dietary inclusion of EOs had no effect on the color, tenderness, and lipid peroxidation of sheep and goat meat [16,17].

Particularly in beef cattle, some studies have evaluated the effects of dietary inclusion of EOs on productive performance [18,19], ruminal fermentation [20,21], blood metabolites [22,23], and meat quality [24,25]. However, the results obtained to date are controversial and inconclusive. Therefore, to develop products containing EOs that can improve the productivity and health of beef cattle, it is necessary to identify the factors that contribute to this variability. Variations in the primary bioactive metabolite of EOs, the duration of the experimental period, the level of concentrate in the diet, and the dose of EOs appear to be factors contributing to the variability of the effects observed in beef cattle supplemented with EOs [4,26].

Several review articles [4,26–28] have mentioned that EOs can improve health, rumen fermentation, animal performance, and the characteristics of cattle-derived products. Furthermore, in a previously published meta-analytical study, Khiaosa-ard and Zebeli [29] reported that in ruminants (including beef cattle) the use of EOs improved some ruminal parameters. However, Khiaosa-ard and Zebeli [29] did not evaluate the variables related to productive performance, health, or meat quality of beef cattle. Meta-analysis (MA) is a statistical approach that allows combining and quantitatively synthesizing data previously published in different studies [30]. In addition, it is possible to use MA to identify sources of heterogeneity among the various studies [31]. The hypothesis of this study is that dietary supplementation of EOs will benefit productive performance and rumen parameters of beef cattle without affecting health and meat quality. The objective of this study was to perform a meta-analysis of the effects of dietary inclusion of essential oils on productive performance, ruminal parameters, blood metabolites, and meat quality of beef cattle. A second objective was to examine heterogeneity and publication bias among the studies. In addition, the results of the meta-analysis were used to evaluate the potential effect of EOs on the economic and environmental impact of beef production by simulation analysis.

2. Materials and Methods

2.1. Literature Search and Study Selection

To obtain a robust meta-analysis, PRISMA guidelines [32] were used during the processes of identification, selection, choice, and inclusion of references. The information search strategy used is shown in Figure A1. Studies that evaluated the effects of dietary supplementation of EOs on nutrient intake and digestibility, productive performance, carcass characteristics, ruminal parameters, blood metabolites, and meat quality of beef cattle were identified by systematically searching for information in the following databases: ScienceDirect, PubMed, Scopus, and Web of Science. During the search process, the following keywords were used in all databases: essential oils, beef cattle, finishing steer, finishing bull, growth performance, carcass traits, ruminal fermentation, blood metabolites, and meat quality. The results obtained during the search and selection processes were restricted to studies published between January 2010 and January 2022, and a total of

702 scientific publications were identified (Figure A1). Only articles published from 2010 to date were included because Khiaosa-ard and Zebeli [29] previously published a meta-analysis on ruminal parameters of beef cattle, and in that study, they included studies published before 2010.

All identified publications were subjected to a two-step screening process, as previously reported by Orzuna-Orzuna et al. [33,34]. First, titles and abstracts were reviewed, thereby excluding review articles, simulation articles, studies that did not use beef cattle, in vitro experiments, and studies that did not measure the variables of interest. Second, the articles analyzed had to meet some inclusion criteria, similar to those previously described by other authors [33–35]: (1) studies that used beef cattle housed in confinement, non-lactating, and non-pregnant conditions; (2) studies containing data on nutrient intake and digestibility, productive performance, carcass characteristics, rumen parameters, blood metabolites, and/or meat quality; (3) similarity between control and experimental treatments except for the presence of EOs; (4) present quantification or possible determination of the dose of EOs included in the diets; (5) articles written in the English language and published in peer-reviewed journals; and (6) reporting of least squares means of control and experimental treatments with at least one measure of variability (standard error or standard deviation).

2.2. Data Extraction

After going through the screening process, 54 full-text articles were evaluated, of which only 34 articles met all the inclusion criteria used (Table A1). The quantitative data used for the meta-analysis were extracted from these articles. Moreover, variables had to be reported in at least three different studies to be included [33,34,36]. Therefore, the response variables included in the present meta-analysis were: dry matter and nutrient intake and digestibility (crude protein, neutral detergent fiber, among others), daily weight gain, feed efficiency, hot and cold carcass weight and yield, backfat thickness, *Longissimus dorsi* muscle area, ruminal parameters (pH, ammonia nitrogen, volatile fatty acids, protozoa, among others), blood metabolites (urea, glucose, triglycerides, hemoglobin, white blood cells, and acute-phase proteins), and characteristics related to meat quality (pH, shear force, malondialdehyde content, color, and chemical composition). In addition to the response variables, the following information was extracted from the 34 selected articles: publication reference, the nutritional composition of the diet, amount of concentrate in the diet (g/kg DM), country, the dose of EOs in the diet (g/kg DM), number of replicates, duration of supplementation with EOs (days), and primary bioactive metabolite of the EOs.

Table A1 shows the complete list of articles that were included in the final database. For each of these articles, the number of replicates, treatment means, and standard deviations (SDs) were obtained. When articles did not report SD, it was calculated using the equation [37]: SD = SEM $\times \sqrt{n}$, where SEM = standard error of treatment means, and n = number of replicates.

2.3. Calculations and Statistical Analysis

All meta-analysis, meta-regression, heterogeneity analysis, publication bias, and subgroup analysis data were analyzed with the "metafor" package [38] of the R statistical software (v. 4.1.2, R Core Team, Vienna, Austria). The effects of dietary inclusion of EOs in beef cattle were evaluated through weighted mean differences (WMDs) between diets without EOs (control treatments) and diets with the inclusion of EOs (experimental treatments). To calculate WMDs, treatment means were weighted by the inverse of the variance, using the methods for random effects models proposed by DerSimonian and Laird [39]. The WMD allows the interpretation of the results in the original units of measurement [40].

Descriptive statistical values of the nutritional composition of the diets were obtained using the MEANS procedure of SAS statistical software [41]. Similarly, the MIXED procedure of SAS was used to evaluate the differences in the chemical composition of the diets of

the control and EO-supplemented treatments. For this, the studies were used as a random effect and Tukey's test was applied to detect differences between treatments [33,34,42].

2.4. Heterogeneity

To evaluate variations among WMD at the trial level, the chi-square (Q) test for heterogeneity and the I^2 statistic, which measures the percentage of variation due to heterogeneity, were used [31]. For the Q test, an α level of 0.10 was used because of its relatively low power [43]. On the other hand, when I^2 values were negative, they were assigned a value of zero; therefore, I^2 values are between 0 and 100%. I^2 values below 25% indicate low heterogeneity, values between 25 and 50% indicate moderate heterogeneity, while values above 50% indicate high and significant heterogeneity [30,31,44].

2.5. Publication Bias

The evaluation of publication bias was performed with Egger's regression asymmetry test [45]. When $p \leq 0.05$ in Egger's test was obtained, publication bias was considered to be present. The "trim-and-fill" method of Duval and Tweedie [46] was applied to variables that had statistical evidence of publication bias, to estimate the number of possible missing observations.

2.6. Meta-Regression and Subgroup Analysis

Meta-regression analysis was used to evaluate sources of heterogeneity for variables that had Q with $\alpha \leq 0.10$ [43] or I^2 greater than 50% [30]. It is not appropriate to use meta-regression when there are fewer than 10 studies reporting the variable of interest [47], so it was only applied to parameters that met this criterion. The method of moments of DerSimonian and Laird [39] was used to estimate the meta-regression because it is well established for estimating between-study variance. Categorical and continuous covariates were used in the meta-regression. The primary bioactive compound (blend, cinnamaldehyde, eugenol, thymol, capsaicin, and anethole) was used as a categorical covariate. The continuous covariates were the doses of EOs (mg/kg DM), the duration of the experimental phase (days), and the level of concentrate in the diet (g/kg DM). When the categorical covariate (primary bioactive metabolite) was significant at a level $\alpha \leq 0.05$, subgroup analysis was used to assess the WMD [33,42]. Similarly, when the meta-regression was significant ($p \leq 0.05$) for EOs dose, experimental period, and concentrate level, these covariates were evaluated using the following subgroups: EOs dose ($\leq$400 and >400 mg/kg DM), experimental period ($\leq$90 and >90 days), and concentrate level ($\leq$700 and >700 g/kg DM).

2.7. Simulation Analysis

Based on the results of dietary supplementation of EOs on the productive performance of beef cattle, a simulation analysis was conducted to evaluate how dietary supplementation of EOs influences the economic and environmental impact of beef production. The simulation was based on the impact of supplementing EOs in the diet of 1000 cattle to gain 200 kg of body weight each. Table A2 shows the data used for the simulation inputs. The variables included in the simulation input were previously used by Salami et al. [48]: the number of animals, daily weight gain (DWG), dry matter intake (DMI), feed efficiency (FE), target body weight, protein production, and lean meat yield. Regrading indicators of the economic impact of EOs supplementation on beef production, total feed cost (USD/1000 animals) and feeding days to slaughter were calculated. Moreover, as indicators of the environmental impact of beef production, the emission intensity attributed to the use of feed (EIAFU) was calculated, in addition to the intensity of total emissions. For this, the equations reported by Salami et al. [48] were applied:

$$\text{FR to gain 200 kg BW} = \text{TLWG}/\text{FE} \quad (1)$$

$$\text{DFS} = \text{TLWG}\ /\text{DWG} \quad (2)$$

$$\text{TFU} = \text{FR to gain 200 kg BW} \times (1/\text{DM content of diet}) \quad (3)$$

$$\text{FC (USD)} = \text{TFU} \times \text{ration cost (US\$)} \quad (4)$$

$$\text{Total FC (USD/1000 cattle)} = \text{FC} \times 1000 \quad (5)$$

$$\text{EIAFU} = \text{BPO} \times \text{AEIAFU} \quad (6)$$

$$\text{EI} = \text{EIAFU} + (\text{AEINAFU} \times \text{BPO}) \quad (7)$$

$$\text{Total EI} = \text{EI} \times 1000 \quad (8)$$

where: FR: feed required; BW: body weight; TLWG: target live weight gain; FE: feed efficiency; DFS: days on feed to the slaughter; DWG: daily live weight gain; TFU: total feed use; DM: dry matter; FC: feed cost; EI: emission intensity; EIAFU: the EI attributed to feeding use; BPO: beef protein output; AEIAFU: the global average EI attributed to feeding use; AEINAFU: the global average EI not attributed to feeding use.

To the previously described equations, we applied the assumptions used by Salami et al. [48]:

(1) Cost of the basal diet = 0.25 USD/kg DM. A cost of 0.0021 USD/kg DM was added to the cost of the diet supplemented with EOs, considering the average dose of EOs used in the present study (Table 1) and the average cost of 7.45 USD/kg reported in the literature for some of the most commonly used EOs [49–51].

(2) AEIAFU = 108 kg CO_2-eq/kg of protein, based on data previously reported by Gerber et al. [52], which indicated that the global average emission intensity (GAEI) of beef is 300 kg CO_2-eq/ kg of protein and that on average 36% of beef emissions (BEs) were attributed to animal feed use. In addition, data on beef GAEI were used because worldwide there is wide variation in the environmental footprint of beef production systems [48].

(3) Moreover, considering the above assumption (2), the remaining component of BEs was allocated to non-food use [48]: AEINAFU = 192 kg CO_2-eq/kg of protein.

Table 1. Descriptive statistics of the complete dataset for the effect of EO supplementation on beef cattle diets.

Parameter		Mean		Median		Minimum		Maximum		SD	
Dietary Features	**NC**	**Control**	**EOs**	**Control**	**EOs**	**Control**	**EOs**	**Control**	**EOs**	**Control**	**EOs**
Concentrate, g/kg DM	84	704.1	704.1	850.0	850.0	0.0	0.0	930.0	930.0	261.6	261.6
DM, g/kg DM	74	752.4	753.8	719.0	724.0	403.4	403.4	931	931	141.7	140.3
OM, g/kg DM	50	944.6	944.5	971.0	968.0	807	807	977	977	38.2	38.0
CP, g/kg DM	84	134.3	134.3	131.0	131.0	88.7	88.7	191.5	191.5	17.9	18.13
EE, g/kg DM	59	33.01	33.11	35.0	35.0	8.1	8.1	80.3	80.3	12.9	12.9
NDF, g/kg DM	87	299.8	299.3	237.0	241.0	143.0	151.0	688.0	688.0	135.8	134.8
ADF, g/kg DM	75	158.8	158.7	123.0	123.0	39.0	44.0	428.8	428.0	94.6	94.5
Starch, g/kg DM	20	416.7	415.9	440.0	440.0	199.6	199.6	545.0	545.0	106.9	107.8
Ca, g/kg DM	33	6.8	6.8	6.2	6.2	3.9	4.0	14.8	14.8	2.47	2.46
P, g/kg DM	33	3.7	3.7	3.6	3.6	2.7	2.6	4.7	4.7	0.45	0.46
EOs, mg/kg DM	89	-	295.0	-	175.0	-	5.0	-	1200	-	288
Duration, days	89	111.0		84.0		21.0		390.0		67.1	

NC = number of comparisons; EOs = essential oils; 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. In the same column, means followed by different letters differ significantly by the Tukey test ($p \leq 0.05$).

3. Results

3.1. Study Attributes and Excluded Studies

Table 1 shows the descriptive statistics and comparison of means for the chemical composition of the diets. No significant differences were observed between the control treatment and the EOs treatment for any of the diet components ($p > 0.05$). Therefore, it is

possible to exclude the effects of diet components on the response of beef cattle to dietary supplementation of EOs in our dataset.

In the present meta-analysis, the included studies were conducted in 10 countries, mainly Brazil (44.2%), Canada (17.6%), and the United States of America (14.7%). The experimental doses of EOs ranged from 5 to 1200 mg/kg DM, and the duration of the experimental periods ranged from 21 to 390 days (Table 1). Based on the primary bioactive metabolite, the EOs used were grouped as follows: mixture, eugenol, thymol, cinnamaldehyde, capsaicin, and anethole. In most of the treatments (66.4%), EOs with mixtures of bioactive metabolites were used in a similar proportion, 12.3% of the treatments used EOs with cinnamaldehyde as the primary bioactive metabolite and, in 7.8% of the treatments, the primary bioactive metabolite was capsaicin. In the remaining treatments (13.5%), EOs with thymol, eugenol and anethole were used as primary bioactive metabolites. In addition, 61.7% of the treatments used diets high in concentrate (>700 g/kg DM), and 38.3% used diets with 0–700 g/kg DM of concentrate. Moreover, in most of the treatments (57.3%) the experimental phase lasted up to 90 days, and 42.7% of the treatments used experimental periods longer than 90 days.

3.2. Nutrient Intake and Digestibility

Dietary inclusion of EOs increased ($p < 0.01$) dry matter intake (DMI), crude protein intake (CPI), neutral detergent fiber intake (NDFI), and acid detergent fiber intake (ADFI) (Table 2). However, dietary supplementation of EOs did not affect ($p > 0.05$) organic matter intake (OMI).

Table 2. Nutrient intake and digestibility of beef cattle supplemented with essential oils.

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
Intake, kg/d							
DMI	26 (68)	8.40 (1.79)	0.209 (0.129; 0.288)	<0.001	<0.001	98.93	0.645
OMI	5 (13)	8.5 (1.95)	−0.078 (−0.274; 0.119)	0.437	0.436	0.98	0.136
CPI	5 (13)	1.10 (0.16)	0.067 (0.019; 0.115)	0.006	<0.001	77.21	0.806
NDFI	8 (21)	2.71 (0.86)	0.129 (0.036; 0.222)	0.007	<0.001	86.40	0.497
ADFI	3 (9)	1.21 (0.57)	0.122 (0.094; 0.149)	<0.001	0.835	0.00	NA
Digestibility, g/kg of DM							
DMD	8 (21)	675.5 (73.4)	1.121 (−17.557; 19.799)	0.906	<0.001	99.64	0.207
OMD	6 (15)	721.4 (77.0)	−11.260 (−38.157; 15.638)	0.412	<0.001	99.79	0.116
CPD	7 (20)	700.1 (67.9)	−8.954 (−24.050; 6.143)	0.245	<0.001	99.35	0.126
NDFD	10 (27)	547.3 (97.3)	−2.752 (−26.906; 21.402)	0.823	<0.001	99.74	0.489
ADFD	3 (8)	536.2 (18.9)	−22.771 (−37.735; −7.807)	0.003	<0.001	99.39	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments; CI: confidence interval of WMD; SE: standard error; P-value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; NA: variables with $n < 10$ observations, the test does not apply; DMI: dry matter (DM) intake; OMI: organic matter (OM) intake; CPI: crude protein (CP) intake; NDFI: neutral detergent fiber (NDF) intake; ADFI: acid detergent fiber (ADF) intake; DMD: DM digestibility; OMI: OM digestibility; CPD: CP digestibility; NDFD: NDF digestibility; ADFD: ADF digestibility.

By comparison, there was no significant impact ($p > 0.05$) of dietary inclusion of EOs on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and neutral detergent fiber digestibility (NDFD). However, acid detergent fiber digestibility (ADFD) decreased in response to dietary supplementation with EOs ($p < 0.05$).

3.3. Growth Performance and Carcass Characteristics

Dietary inclusion of EOs increased ($p < 0.05$) final body weight (FBW), daily weight gain (DWG), feed efficiency (FE), hot carcass weight (HCW), cold carcass weight (CCW), and *Longissimus dorsi* muscle area (LMA) (Table 3). However, dietary supplementation of

EOs did not affect ($p > 0.05$) hot carcass yield (HCY), cold carcass yield (CCY), and backfat thickness (BFT).

Table 3. Growth performance and carcass characteristics of beef cattle supplemented with essential oils.

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
FBW, kg	17 (38)	440.6 (160.6)	12.843 (7.352; 18.321)	<0.001	<0.001	77.67	0.084
DWG, kg/d	16 (36)	1.213 (0.463)	0.087 (0.053; 0.120)	<0.001	<0.001	75.15	0.062
FE, kg/kg	13 (29)	0.173 (0.034)	0.004 (0.000; 0.008)	0.039	0.902	0.00	0.235
Carcass traits							
HCW, kg	9 (22)	295.1 (81.30)	5.455 (1.860; 9.050)	0.003	<0.001	57.24	0.360
HCY, %	8 (19)	56.31 (3.54)	−0.159 (−0.416; 0.099)	0.227	0.014	46.38	0.287
CCW, kg	4 (13)	229.7 (43.70)	9.975 (3.757; 16.194)	0.002	<0.001	82.34	0.369
CCY, %	4 (13)	53.80 (1.07)	−0.016 (−0.741; 0.710)	0.966	<0.001	99.32	0.847
BFT, mm	11 (24)	8.64 (6.55)	0.074 (−0.245; 0.393)	0.648	<0.001	78.92	0.903
LMA, cm^2	10 (22)	74.66 (15.52)	3.480 (1.597; 5.364)	<0.001	<0.001	73.54	0.435

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments; CI: confidence interval of WMD; SE: standard error; P-value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; FBW: final body weight; DWG: daily weight gain; FE: feed efficiency; HCW: hot carcass weight; HCY: hot carcass yield; CCW: cold carcass weight; CCY: cold carcass yield; BFT: backfat thickness; LMA: Longissimus dorsi muscle area.

3.4. Ruminal Parameters and Nitrogen Balance

No significant effects ($p > 0.05$) of dietary inclusion of EOs on pH and ruminal butyrate concentration were observed (Table 4). Supplementation of EOs in the diet decreased ($p < 0.05$) ruminal concentration of ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA), and acetate. However, higher ($p < 0.001$) ruminal propionate concentration was observed in response to dietary EOs supplementation (Table 4).

Table 4 shows that dietary inclusion of EOs decreased total ($p = 0.041$), *Isotricha* ($p = 0.004$), and *Dasytricha* ($p < 0.001$) protozoan populations but did not affect *Entodinium* populations ($p > 0.05$). In addition, dietary supplementation of EOs reduced urinary nitrogen (N) excretion ($p = 0.005$) and increased N retention ($p = 0.011$). However, no significant impact ($p > 0.05$) of dietary inclusion of EOs on N intake, fecal N excretion, microbial ruminal nitrogen (RNM), or microbial protein supply efficiency (MPSE) was observed.

Table 4. Ruminal parameters and nitrogen metabolism of beef cattle supplemented with essential oils.

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
pH	12 (36)	6.21 (0.45)	0.001 (−0.01; 0.02)	0.943	0.275	11.45	0.475
NH_3-N, mg/dL	10 (30)	13.71 (6.64)	−1.18 (−1.63; −0.74)	<0.001	0.121	37.73	0.190
TVFA, mM	13 (37)	103.3 (26.07)	−2.44 (−4.76; −0.13)	0.039	<0.001	84.71	0.799
SCFA, mol/100 mol							
Acetate	14 (40)	61.74 (15.65)	−4.37 (−7.72; −1.02)	0.011	<0.001	98.84	0.554
Propionate	14 (40)	23.44 (7.36)	0.878 (0.48; 1.27)	<0.001	0.152	35.29	0.120
Butyrate	14 (40)	11.31 (3.96)	−0.01 (−0.27; 0.25)	0.922	<0.001	56.81	0.337
Protozoa, $\times 10^5$/mL							
Total	5 (12)	12.47 (4.68)	−2.17 (−4.26; −0.09)	0.041	<0.001	78.63	0.610
Entodinium	3 (7)	9.55 (1.88)	0.08 (−0.36; 0.52)	0.726	<0.001	83.97	NA
Isotricha	3 (7)	1.49 (0.47)	−0.65 (−1.09; −0.21)	0.004	0.350	45.63	NA
Dasytricha	3 (7)	5.37 (1.54)	−1.26 (−1.61; −0.91)	<0.001	0.617	0.00	NA
CH_4, g/kg DM	3 (7)	22.11 (7.02)	−0.08 (−1.29; 1.13)	0.893	<0.001	78.79	NA
Nitrogen balance, g/d							
N intake	3 (8)	182.1 (52.7)	2.89 (−3.41; 9.19)	0.368	0.642	0.00	NA

Table 4. *Cont.*

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
N urine	3 (8)	55.2 (34.3)	−6.4 (−10.93; −1.96)	0.005	<0.001	90.48	NA
N fecal	3 (8)	57.1 (12.2)	0.23 (−3.56; 4.03)	0.904	0.857	0.00	NA
N retained	3 (8)	69.7 (10.6)	7.44 (1.73; 13.14)	0.011	0.167	46.95	NA
RNM	4 (9)	96.28 (26.73)	−1.00 (−6.61; 4.61)	0.726	0.056	47.21	NA
EMPS	3 (7)	31.57 (14.31)	0.87 (−1.94; 3.67)	0.544	0.936	0.00	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments; CI: confidence interval of WMD; SE: standard error; P-value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; NA: variables with $n < 10$ observations, the test does not apply; NH_3-N: nitrogen ammonia; TVFA: total volatile fatty acids; SCFA: short-chain fatty acids; CH_4: methane; N: nitrogen; RNM: rumen nitrogen microbial; EMPS: efficiency of microbial protein supply (g nitrogen/kg digestible organic matter apparently fermented in the rumen).

3.5. Blood Metabolites

Dietary supplementation with EOs did not affect the serum concentration of triglycerides and glucose ($p > 0.05$) but decreased the serum concentration of urea ($p = 0.026$) and non-esterified fatty acids (NEFA; $p = 0.004$) (Table 5).

Table 5. Blood metabolites of beef cattle supplemented with essential oils.

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
Blood metabolites, mg/dL							
Urea	7 (18)	18.42 (5.78)	−1.35 (−2.553; −0.162)	0.026	0.128	30.22	0.071
Glucose	7 (18)	77.49 (26.51)	1.44 (−0.107; 2.998)	0.068	0.945	0.00	0.895
Triglycerides	4 (12)	12.12 (3.29)	0.53 (−0.295; 1.356)	0.208	0.980	0.00	0.693
NEFA, μM	3 (9)	102.2 (44.1)	−9.05 (−15.20; −2.89)	0.004	0.509	0.00	NA
Hb, g/dL	3 (6)	14.537 (1.45)	−1.78 (−6.059; 2.505)	0.416	<0.001	99.35	NA
WBC, $10^3/\mu L$	3 (7)	9.907 (0.96)	−0.19 (−0.943; 0.564)	0.622	0.904	0.00	NA
White blood cells (WBC), % of total							
Lymphocytes	3 (7)	58.56 (4.01)	2.67 (−0.299; 5.642)	0.078	0.564	0.00	NA
Neutrophils	3 (7)	28.17 (3.35)	−2.89 (−5.551; −0.230)	0.033	0.716	0.00	NA
Monocytes	3 (7)	8.823 (1.66)	−0.32 (−1.885; 1.241)	0.686	0.995	0.00	NA
Eosinophils	3 (7)	4.37 (1.78)	0.24 (−1.016; 1.495)	0.709	0.707	0.00	NA
Basophils	3 (7)	1.89 (1.15)	−1.02 (−1.790; −0.249)	0.010	<0.001	79.56	NA
Acute phase proteins μg/mL							
Haptoglobin	4 (10)	265.3 (107.2)	−39.67 (−75.74; −3.59)	0.031	0.791	0.00	0.123
SAA	3 (9)	0.317 (0.054)	0.02 (−0.053; 0.036)	0.603	0.820	0.00	NA
LBP	3 (9)	1.405 (0.268)	0.06 (−0.135; 0.251)	0.556	0.404	3.72	NA

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments; CI: confidence interval of WMD; SE: standard error; P-value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; NA: variables with $n < 10$ observations, the test does not apply; NEFA: non-esterified fatty acids; Hb: hemoglobin; WBC: white blood cells; SAA: serum amyloid A; LBP: lipopolysaccharide-binding protein.

By comparison, there was no significant impact ($p > 0.05$) of EOs dietary inclusion on the blood concentration of hemoglobin, white blood cells (WBC), lymphocytes, monocytes, and eosinophils. However, neutrophils and basophils decreased ($p < 0.05$) in response to dietary EOs supplementation (Table 5). In addition, plasma haptoglobin concentration decreased ($p = 0.031$) with dietary EOs supplementation, but no significant changes ($p > 0.05$) were observed in serum amyloid A (SAA) and lipopolysaccharide-binding protein (LBP) concentration.

3.6. Meat Quality

Dietary supplementation of EOs did not affect meat pH ($p > 0.05$), but decreased cooking loss ($p = 0.009$), shear force (ShF; $p = 0.029$), and malondialdehyde content (MDA; $p = 0.008$) (Table 6). In addition, there was no significant impact ($p > 0.05$) of EOs dietary inclusion on the lightness (L*), redness (a), yellowness (b*), and moisture, protein, fat, ash, and collagen content of meat.

Table 6. Meat quality of beef cattle supplemented with essential oils.

Item	N (NC)	Control Means (SD)	WMD (95 % CI)	*p*-Value	Heterogeneity		Egger Test [1]
					p-Value	I^2 (%)	*p*-Value
Meat pH 24 h	7 (21)	5.637 (0.11)	−0.002 (−0.025; 0.021)	0.865	<0.001	66.13	0.099
Cook loss, g/kg	6 (17)	270.80 (45.90)	−61.765 (−107.9; −15.59)	0.009	<0.001	99.09	0.062
ShF, kgf/cm^2	8 (19)	5.53 (1.98)	−0.211 (−0.400; −0.022)	0.029	<0.001	62.36	0.124
MDA, mg/kg	7 (18)	0.346 (0.19)	−0.040 (−0.070; −0.010)	0.008	<0.001	88.38	0.942
Meat color							
Lightness (L*)	8 (21)	38.76 (1.92)	−0.382 (−0.817; 0.053)	0.085	0.001	55.22	0.808
Redness (a*)	8 (21)	15.58 (3.43)	0.018 (−0.160; 0.196)	0.841	0.445	1.02	0.078
Yellowness (b*)	8 (21)	10.62 (2.46)	−0.042 (−0.210; 0.127)	0.627	0.067	36.86	0.243
Chemical composition, g/kg of DM							
Moisture	6 (14)	728.49 (16.10)	1.307 (−2.923; 5.537)	0.545	<0.001	62.53	0.301
Protein	5 (12)	227.48 (5.19)	0.935 (−0.600; 2.574)	0.233	0.507	0.00	0.682
Fat	5 (12)	19.54 (7.50)	−0.736 (−2.275; 0.804)	0.349	0.942	0.00	0.206
Ash	5 (12)	12.66 (1.65)	−0.033 (−0.256; 0.191)	0.773	0.475	0.00	0.725
Collagen	3 (10)	18.32 (6.71)	0.271 (−0.083; 0.626)	0.133	0.534	0.00	0.061

N: number of studies; NC: number of comparisons; SD: standard deviation; WMD: weighted mean differences between control and treatments; CI: confidence interval of WMD; SE: standard error; P-value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; ShF: shear force; MDA: malondialdehyde in meat.

3.7. Publication Bias and Meta-Regression

Tables 2–6 show that the presence of publication bias from Egger's regression asymmetry test was not significant for any variable ($p > 0.05$).

By comparison, significant heterogeneity (Q; $p \leq 0.10$) was observed for DMI, CPI, NDFI, DMD, OMD, CPD, NDFD (Table 2), ADFD, FBW, DWG, HCW, HCY, CCW, CCY, BFT, LMA (Table 3), TVFA, acetate, butyrate, total protozoa, *Entodinium*, CH_4, urinary N, RNM (Table 4), Hb, basophils (Table 5), meat pH, cooking loss, MDA, lightness (L*), and meat moisture (Table 6). However, it is not recommended to use meta-regression when the response variable of interest is reported in less than 10 studies [47]. Therefore, this analysis was only performed for the variables DMI, NDFD, FBW, DWG, BFT, LMA, TVFA, acetate, and butyrate.

Table 7 shows that the dose of EOs explained ($p < 0.05$) 33.59, 20.17, 55.43 and 12.50% of the heterogeneity observed for DMI, BFT, TVFA, and acetate, respectively. In addition, the period of EOs supplementation in the diet had a significant relationship ($p < 0.05$) with DMI, FBW, DWG, and BFT, which explained between 3.08 and 65.50% of the observed heterogeneity. The primary bioactive metabolite did not significantly ($p > 0.05$) influence the variability of the evaluated response parameters (DMI, NDFD, FBW, DWG, BFT, LMA, TVFA, acetate, and butyrate). The level of concentrate in the diet explained ($p < 0.05$) 40.07, 33.61 and 27.56% of the observed heterogeneity for DMI, FBW, and DWG, respectively. There was no significant relationship ($p > 0.05$) between the covariates used (EOs dose, supplementation period, primary bioactive metabolite, and concentrate level) and the response variables NDFD, LMA, and butyrate (Table 7).

Table 7. Meta-regression comparing the associations between covariates and measured outcomes.

Parameter	Covariates	QM	df	*p*-Value	R^2 (%)
Dry matter intake (DMI)	Essential oils dose	9.84	1	0.002	33.59
	Supplementation period	8.96	1	0.003	26.21
	Primary bioactive compound	2.09	5	0.835	0.00
	Concentrate level	44.07	1	<0.001	40.07
Neutral detergent fiber digestibility (NDFD)	Essential oils dose	0.01	1	0.999	0.00
	Supplementation period	2.12	1	0.145	23.69
	Primary bioactive compound	1.26	4	0.868	0.00
	Concentrate level	0.94	1	0.332	18.90
Final body weight (FBW)	Essential oils dose	0.30	1	0.584	0.00
	Supplementation period	45.12	1	<0.001	65.50
	Primary bioactive compound	3.13	3	0.372	0.00
	Concentrate level	14.88	1	<0.001	33.61
Daily weight gain (DWG)	Essential oils dose	0.57	1	0.451	0.00
	Supplementation period	3.74	1	0.053	14.45
	Primary bioactive compound	1.97	3	0.578	0.00
	Concentrate level	7.91	1	0.005	27.56
Backfat thickness (BFT)	Essential oils dose	8.96	1	0.003	20.17
	Supplementation period	3.81	1	0.051	3.08
	Primary bioactive compound	2.67	3	0.445	0.00
	Concentrate level	0.74	1	0.388	0.00
Longissimus dorsi muscle area (LMA)	Essential oils dose	0.05	1	0.823	0.00
	Supplementation period	0.79	1	0.372	0.00
	Primary bioactive compound	1.43	3	0.699	0.00
	Concentrate level	1.21	1	0.271	2.32
Total volatile fatty acids (TVFA)	Essential oils dose	8.88	1	0.003	55.43
	Supplementation period	0.15	1	0.698	0.00
	Primary bioactive compound	1.21	5	0.944	0.00
	Concentrate level	2.70	1	0.100	5.08
Acetate	Essential oils dose	5.89	1	0.015	12.50
	Supplementation period	0.01	1	0.935	0.00
	Primary bioactive compound	1.76	5	0.881	6.14
	Concentrate level	2.24	1	0.134	3.56
Butyrate	Essential oils dose	0.70	1	0.402	0.00
	Supplementation period	2.97	1	0.184	9.12
	Primary bioactive compound	5.38	5	0.372	0.00
	Concentrate level	0.40	1	0.526	0.00

QM: coefficient of moderators; QM is considered significant at $p \leq 0.05$; R^2: the amount of heterogeneity accounted for; df: degree of freedom.

3.8. Subgroup Analysis

DMI increased regardless of the dose of EOs used ($p < 0.05$); however, the effect was greater when doses greater than 400 mg/kg DM (WMD = 0.324 kg) were used in comparison to doses of ≤400 mg/kg DM (WMD = 0.110 kg/d) (Figure 1a). BFT increased when doses of EOs greater than 400 mg/kg DM were used (WMD = 0.368 mm; $p = 0.033$), but doses of ≤400 mg/kg DM did not affect BFT (Figure 1b; $p = 0.089$). Figure 1c shows that the ruminal concentration of TVFA was not affected by the dose of EOs in the diet ($p > 0.05$). Ruminal acetate concentration decreased (WMD = −3.504 mol/100 mol; $p = 0.017$) when low doses of EOs (≤400 mg/kg DM) were used, but doses of EOs higher than 400 mg/kg DM did not affect acetate concentration (Figure 1d; $p > 0.05$).

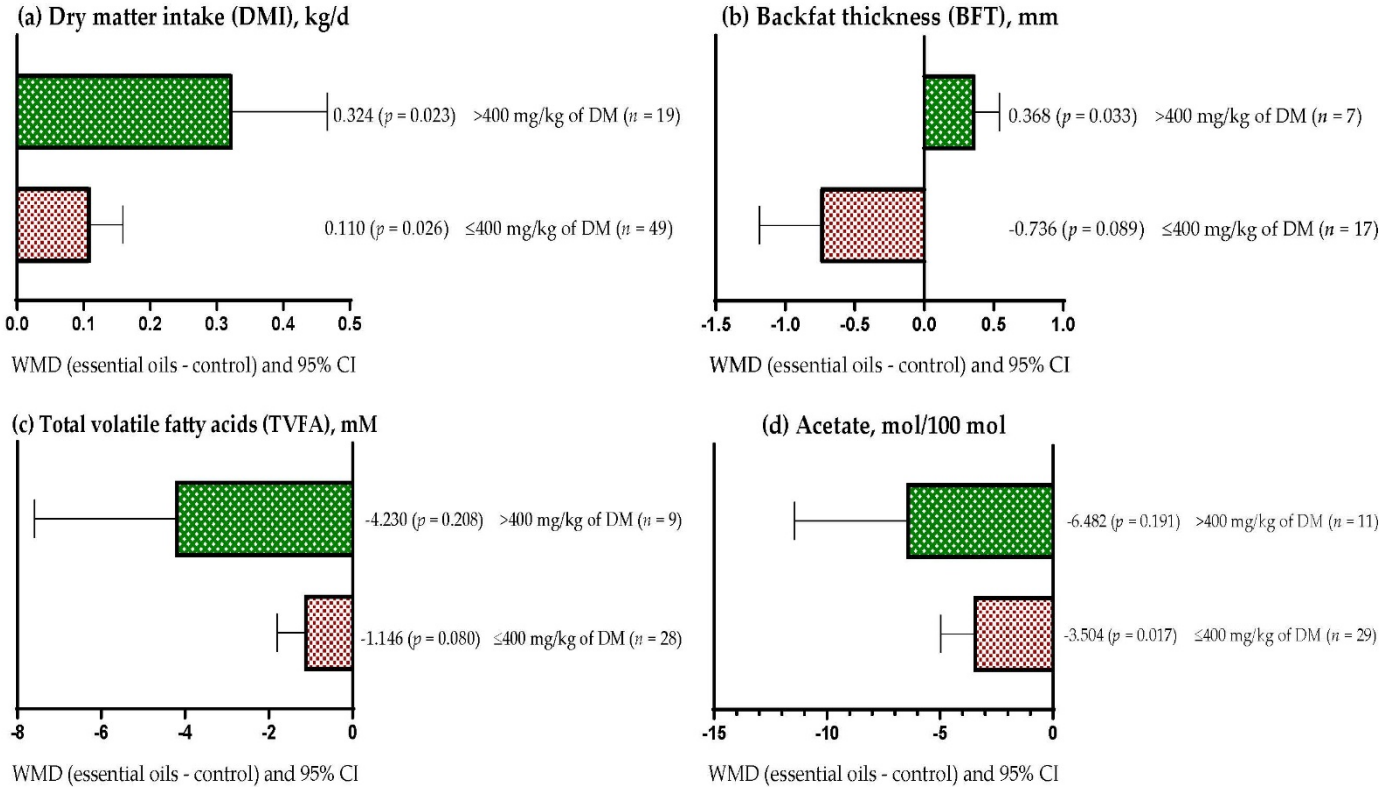

Figure 1. Subgroup analysis (subgroup = essential oils dose (mg/kg DM)) of the effect of essential oils in diet of the beef cattle, WMDs = weighted mean differences between essential oil treatments and control.

DMI increased when dietary supplementation with EOs lasted longer than 90 days (WMD = 0.191 kg/d; $p < 0.001$) but was not affected when EOs were offered for up to 90 days (WMD = 0.007 kg; $p > 0.05$) (Figure 2a). FBW increased regardless of the period of EO supplementation used (Figure 2b); however, the effect was greater when EOs were offered for more than 90 days (WMD = 20.16 kg; $p < 0.001$) compared to periods of up to 90 days (WMD = 5.38 kg; $p = 0.008$). Similarly, DWG increased regardless of the period of EO supplementation used ($p < 0.05$); however, the effect was greater when EOs were offered for more than 90 days (WMD = 0.110 kg/d) compared to periods up to 90 days (Figure 2c; WMD = 0.069 kg/d). Figure 2d shows that BFT was not affected by the period of supplementation with EOs in the diet ($p > 0.05$).

DMI increased when EOs were supplemented in diets with more than 700 g/kg DM of concentrate (WMD = 0.194 kg/d; $p < 0.001$), but DMI was not affected when EOs were offered in diets with up to 700 g/kg DM of concentrate (WMD = −0.033 kg/d; $p > 0.05$) (Figure 3a). FBW increased (WMD = 21.02 kg; $p < 0.001$) when EOs were included in diets high in concentrate (>700 g/kg DM); however, it was not affected when EOs were fed in diets with less than 700 g/kg DM of concentrate (WMD = 2.48 kg; $p = 0.115$) (Figure 3b). DWG increased regardless of the level of concentrate used in the diets ($p < 0.01$; Figure 3c); however, the effect was greater when EOs were included in diets with more than 700 g/kg DM of concentrate (WMD = 0.115 kg/d) compared to diets having up to 700 g/kg DM of concentrate (WMD = 0.041 kg/d).

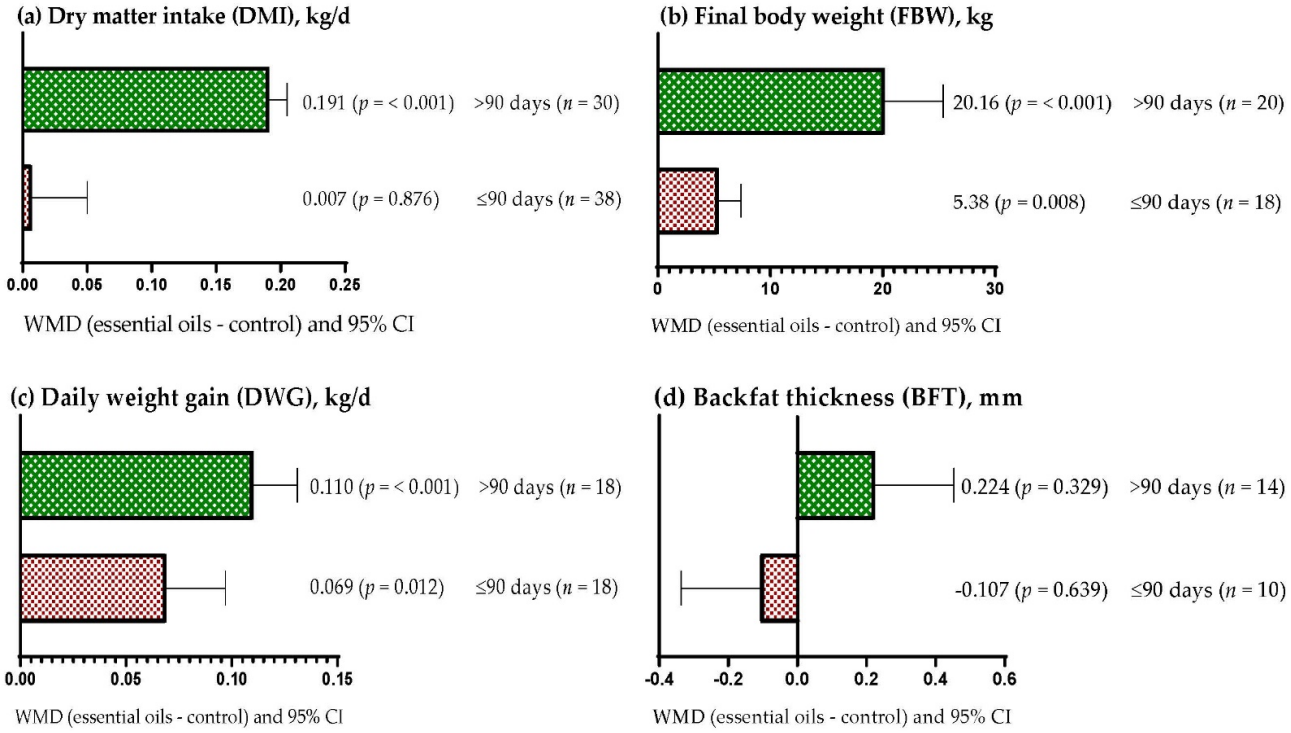

Figure 2. Subgroup analysis (subgroup = supplementation period (days)) of the effect of essential oils in diet of the beef cattle, WMD = weighted mean differences between essential oil treatments and control.

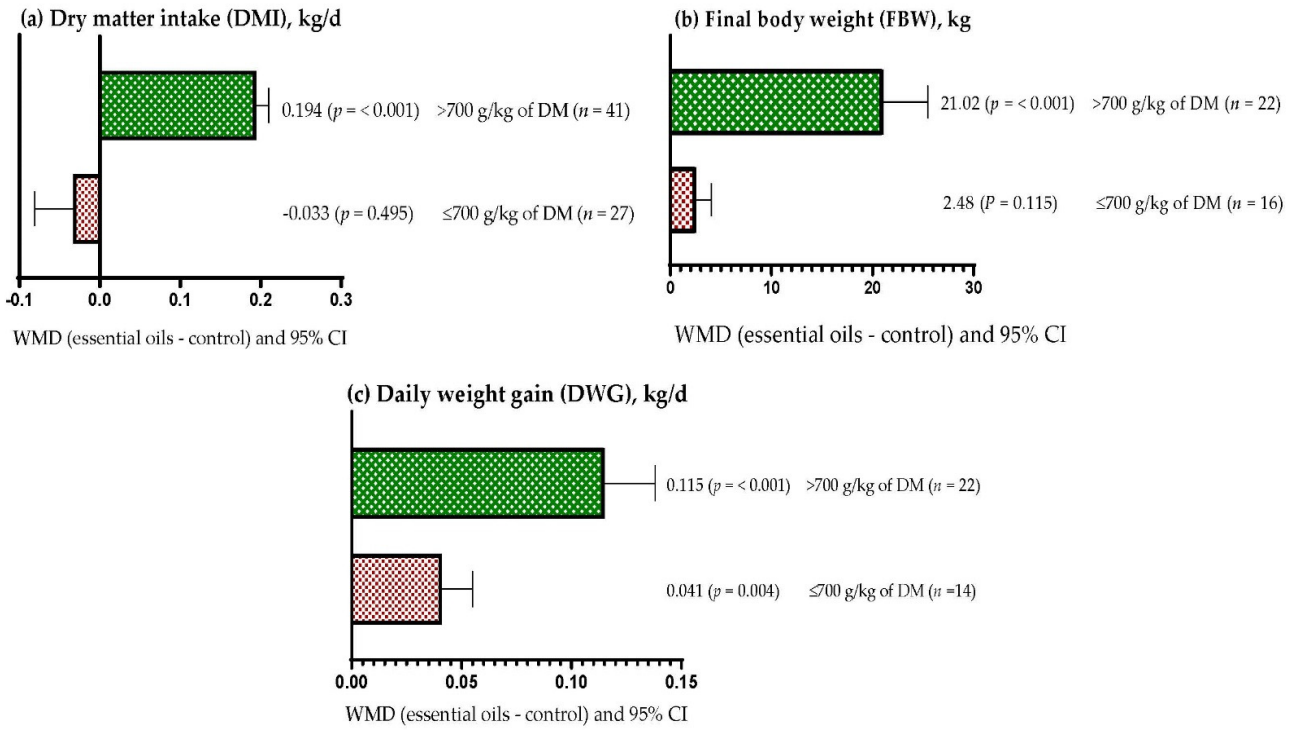

Figure 3. Subgroup analysis (subgroup = concentrate in diet (g/kg of DM)) of the effect of essential oils in the diet of the beef cattle, WMD = weighted mean differences between essential oil treatments and control.

3.9. Environmental and Economic Impacts of Dietary Supplementation with Essential Oils in Beef Cattle Production

Simulation analysis indicated that the positive effects of dietary supplementation of EOs on DWG and FE of beef cattle improved the economic and environmental parameters of beef production. Table 8 shows that supplementation of EOs to 1000 cattle required to gain 200 kg BW, decreased 11 days of feeding days until slaughter. Moreover, dietary supplementation of EOs increased economic income (USD 4170/1000 animals) through a reduction in feed cost. In addition, dietary supplementation of EOs reduced emissions from feed used for beef production by 43 tons of CO_2-eq, which contributed to a 0.83% reduction in the carbon footprint of beef production.

Table 8. Economic and environmental impacts of essential oils (EOs) supplementation in beef cattle.

Item	Control	EOs	Difference	% Change
Economic impact analysis [1]				
FR to gain 200 kg BW, kg DM/animal	1156.07	1129.94	−26.13	−2.26
DFS, days	165	154	−11	−6.66
FC, USD/animal	289.02	284.85	−4.17	−1.44
Total FC, USD/1000 animals	289,020	284,850	−4.170	−1.44
Environmental impact analysis				
EI attributed to FU, kg CO_2-eq per BPO/animal [2]	1857	1815	−42	−2.26
EI, kg CO_2-eq per BPO/animal	5160	5117	−43	−0.83
Total EI, tonnes CO_2-eq per BPO/1000 animals	5160	5117	−43	−0.83

FR: feed required; BW: body weight; DM: dry matter; DFS: days on feed to the slaughter; FC: feed cost; EI: emission intensity; FU: feed use; BPO: beef protein output; [1]: this analysis considers only the economic benefit derived from a reduction in FC due to the positive effect of EOs on beef cattle performance. [2]: EI is calculated relative to the BPO. As previously reported by other authors [48,52], data on the global average EI of beef was reported as 300 kg CO_2-eq/kg of protein. In addition, an average of 36% of beef emissions was attributed to FU. This data was used to calculate the global average EI of beef attributed to FU as 108 kg CO_2-eq per kg of protein. Thus, EI attributed to FU = BPO × 108. For the EOs diet, the EI attributed to FU was corrected by the −2.26% reduction in simulated FU.

4. Discussion

4.1. Nutrient Intake and Digestibility

Some EOs have positive effects on rumen fungal and fibrolytic bacteria populations [53], which can result in increased DMD and NDFD. This can increase the rate of particle passage in the rumen and consequently increase DMI. It has been reported that EOs can improve the taste and palatability of livestock feeds [54]. In addition, dietary inclusion of EOs can increase DMI by improving feed quality, because EOs contain bioactive compounds (e.g., terpenes and terpenoids) with antioxidant and antimicrobial properties [55]. Similar effects of EOs consumption in the present study would partially explain the higher DMI observed in response to dietary supplementation of EOs. By comparison, Mucha and Witkowska [54] mentioned that EOs doses included in the diet of animals should be carefully established because the intense aroma of EOs may negatively affect the DMI of livestock. However, in the present study, a subgroup analysis revealed that DMI increased regardless of the dose of EOs used. Moreover, another subgroup analysis revealed that DMI increased significantly only when EOs were administered for more than 90 days. This suggests that beef cattle can adapt to consuming EOs, but this adaptation may require the use of EOs for prolonged periods of time.

With respect to digestibility, Zhou et al. [53] observed that dietary supplementation of EOs enhanced fungal growth in sheep rumen. In addition, in vitro [56] and in vivo [57] studies reported that EOs increase the relative abundance of fiber-degrading bacteria (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *R. flavefaciens*) in sheep rumen fluid. Simlarly, in beef cattle, EOs have been reported to increase the relative abundance of ruminal microorganisms (Bacteroidetes, Fibrobacteres, and Firmicutes) that are positively correlated with NDFD [58], and with the concentration of β-glucosidase and cellulase in the rumen [59]. This can result in higher DMD and NDFD; however, in the present meta-analysis dietary supplementation of EOs did not affect DMD, CPD, OMD, or NDFD,

but reduced ADFD. It has been mentioned that rumen protozoa play an important role in ruminal fiber degradation [60]. Therefore, the lower ADFD could be related to the reduction (−17.4%) observed in the population of rumen protozoa in cattle supplemented with EOs.

4.2. Growth Performance and Carcass Characteristics

Dietary supplementation of EOs increased DM and nutrient intake, which partially explains the higher DWG observed in the present study. Similarly, in beef cattle, Zhang et al. [59] reported that supplementation with EOs increased the relative abundance of Bacteroidetes and Firmicutes in the rumen microbiome, which have been reported to be positively correlated with higher DWG in goats [61]. Similar effects of EO consumption observed in our meta-analysis partially explain the observed increase in DWG for beef cattle supplemented with EOs. By comparison, it has been reported that low doses (260 mg/day) of EOs increase up to 30% of the length of ruminal papillae in beef cattle [59]. This can increase the area of volatile fatty acid absorption through the ruminal wall and lead to higher DWG and FE. Furthermore, in the present meta-analysis protozoan populations decreased in response to EOs supplementation. Removal of protozoa from the rumen increases the duodenal flux of microbial protein by up to 30% [62]. This can increase the metabolic availability of amino acids, which, in our case partially explains the higher DWG and FE observed in cattle supplemented with EOs.

In ruminants, the definition of the optimal supplementation period that improves animal performance is one of the main limitations of the use of EOs and other phytochemicals [28]. For example, in sheep, it has been reported that the metabolic benefits of dietary inclusion of EOs decrease over the supplementation period [42]. However, in the present meta-analysis, it was observed that FBW and DWG increased regardless of the supplementation period used, but the increase was greater when long supplementation periods (>90 days) were used. This is congruent with the higher DMI observed in the present study with supplementation of EOs for more than 90 days.

Dietary supplementation of EOs increased HCW and CCW. This may be related to the observed increase in FBW and LMA because HCW and CCW are positively correlated with FBW and LMA [63]. In addition, the increase observed for CCW may be beneficial because there is a positive correlation (r between 0.64 and 0.78) between CCW and the yield of primal cuts (ribs, sirloin, among others) in beef cattle [64].

Information on the mechanisms of action of EOs on muscle development and lipogenesis in beef cattle is still limited. However, in rats, it has been reported that some monoterpene-rich EOs can reduce skeletal muscle atrophy [65], which is characterized by reduced cross-sectional area and protein content of muscle fibers [66]. Similar effects of EO consumption observed in our meta-analysis partially explain the observed increase in LMA for cattle supplemented with EOs.

It has been mentioned that the size and number of adipocytes are related to the fat deposition process in beef cattle [67]. Ngamdokmai et al. [68] evaluated the effects of various EOs (*Zingiber officinale*, *Piper nigrum*, among others) and their primary bioactive metabolites (camphor, citral, limonene, and β-pinene, among others) on adipogenesis. In their study, they observed that all EOs and bioactive metabolites inhibited preadipocyte differentiation, and decreased lipid accumulation in maturing preadipocytes. However, in the present meta-analysis dietary supplementation of EOs did not affect BFT.

The main precursors of lipogenesis in ruminants are acetate and butyrate [69]. In the present study, subgroup analyses showed that high doses of EOs (>400 mg/kg DM) increased BFT without altering ruminal acetate concentration. This suggests that EOs can stimulate lipogenesis in ruminants through mechanisms unrelated to rumen acetate and butyrate concentration.

4.3. Ruminal Parameters and Nitrogen Balance

Rumen pH is an important parameter that can be used as an indicator of the internal homeostasis of the rumen environment [70]. In the present study, dietary inclusion of

EOs did not affect rumen pH, suggesting that ruminal functions were performed under stable conditions of the rumen environment. By comparison, the lower rumen NH_3-N concentration in cattle supplemented with EOs can be associated with the reduction (−17.4%) of total rumen protozoa, because Newbold et al. [62] reported that complete elimination of rumen protozoa decreases the rumen NH_3-N concentration up to 26%.

In ruminants, it has been mentioned that increased rumen concentration of TVFA can be associated with increased metabolic availability of energy because rumen concentration of TVFA serves as the main source of energy for ruminants [71,72]. In the present meta-analysis, dietary supplementation of EOs reduced rumen TVFA concentration. This suggests that EOs reduce energy availability in beef cattle; however, DWG, FBW, and FE were higher in cattle supplemented with EOs. Moreover, the lower rumen TVFA concentration appears to be associated only with the observed reduction in acetate concentration, because EOs increased ruminal propionate concentration and did not affect butyrate concentration.

In beef cattle, Zhang et al. [59] observed that EOs supplementation increases the presence of ruminal microorganisms (*Proteiniphilum acetatigenes* and *Parabacteroides distasonis*) that are positively and negatively correlated with ruminal propionate and acetate concentration, respectively. Consequently, similar effects of EOs consumption in the present study would partially explain the higher rumen propionate concentration and lower acetate concentration observed.

Guyader et al. [73] reported that rumen protozoa are associated with CH_4 emissions through the equation: CH_4 (dry matter intake in g/kg) = $-30.7 + 8.14 \times$ protozoa ($\log_{10}$ cells/mL). Although in the present study dietary supplementation of EOs reduced rumen protozoan populations, no significant changes in CH_4 emissions were observed. However, these results should be interpreted carefully due to the low number of studies reporting this response variable.

It has been mentioned that enteric CH_4 emissions represent around 43% of greenhouse effect gases emitted in beef production worldwide [52]; therefore, considerable efforts have been made to reduce CH_4 emissions. In this regard, in recent years there has been increased interest in evaluating the potential of some natural additives that could be used to reduce methanogenesis [18,29]. In the present meta-analysis, dietary inclusion of EOs did not decrease CH_4 emissions. However, a meta-analysis by Belanche et al. [36] reported that the use of a commercial blend of EOs (Agolin) significantly decreased CH_4 emissions in dairy cows. Therefore, EOs can be considered as a possible CH_4 mitigation strategy, but more in vivo research is needed on the effects of EOs on enteric methane emissions and rumen methanogenic microorganisms.

It has been reported that N plays an important role in the growth and productivity of ruminants [74]. N balance can be used to evaluate the efficiency of utilization of ingested protein in ruminants [72]. In the present study, retained N was higher in cattle supplemented with EOs. This suggests that beef cattle can better utilize ingested protein in the presence of EOs in the diet. Moreover, the higher level of retained N may be related to lower urinary N excretion observed in response to EOs supplementation. By comparison, it has been mentioned that N excreted by ruminants can pollute the environment through ammonia volatilization emissions and nitrous oxide emissions [75]. In the present study, the reduction observed for urinary N excretion suggests that EOs can be useful in reducing environmental N pollution. This is because, compared to fecal N excretion, urinary N causes more nitrous oxide emissions [76]. However, these results should be interpreted with caution because of the low number of studies that reported these response variables.

4.4. Blood Metabolites

When there is an excess of NH_3-N in the rumen, it is absorbed through the rumen wall, then passes to the liver where it is converted to urea and, finally, the urea is transferred to the bloodstream [77]. Similarly, Paengkoum et al. [78] reported a positive correlation (r = 0.55) between serum urea concentration and ruminal NH_3-N concentration. In the

present meta-analysis, the lower serum urea concentration was observed in response to dietary supplementation of EOs, which may be associated with lower rumen NH_3-N concentration observed in animals supplemented with EOs. In addition, serum glucose and NEFA concentration are considered important indicators of energy status in cattle [79]. In the present study, the serum glucose concentration was similar between treatments; however, the inclusion of EOs in the diet decreased the serum NEFA concentration. This indicates better energy balance in beef cattle supplemented with EOs compared to control treatment cattle. In addition, the dietary inclusion of EOs increased DMI; this provides more energy to the animal and partially explains the lower serum NEFA concentration observed.

Dietary supplementation of EOs reduced the percentage of neutrophils and basophils; however, these results should be interpreted with caution due to the low number of studies reporting these response variables. Blood neutrophil depletion usually occurs in the presence of bacterial disease [80]. However, it has been mentioned that neutrophils are not always related to the presence of bacterial infections because the number of neutrophils also decreases in response to other factors [81]. It has also been reported that basophils increase in response to bacterial, viral, and parasitic infections [82]. Therefore, the lower percentage of basophils in beef cattle supplemented with EOs suggests that these animals had a better health status.

According to Ceciliani et al. [83], activation of the innate immune system under conditions of inflammation, infection, and tissue injury can result in the release of acute-phase proteins (e.g., haptoglobin, SAA, and LBP) into the bloodstream. For example, plasma haptoglobin concentration increases in cattle with subacute ruminal acidosis [84]. In the present meta-analysis, dietary inclusion of EOs reduced haptoglobin concentration, suggesting that EOs can reduce the incidence of SARA. On the other hand, SAA and LBP are involved in endotoxin clearance during an acute phase response [85], and their blood concentration increases when there is endotoxin transfer to the bloodstream [83]. Therefore, in the present study, the absence of changes in the blood concentration of SAA and LBP suggests that EO supplementation does not significantly affect the transfer of endotoxins into the blood.

4.5. Meat Quality

It has been mentioned that meat pH is related to water holding capacity (WHC) and tenderness [86]. Similarly, in beef, Węglarz [87] reported negative correlations (r between −0.24 and −0.29) between pH and color parameters (L* a* and b*). In the present study, EOs supplementation did not affect meat pH, suggesting that EOs can be used in beef diets without deteriorating meat color, tenderness, and WHC. The cooking loss can also be used as an indicator of WHC in meat because there is a negative correlation (r = −0.89) between these parameters [88]. Therefore, the values observed for cooking loss in the present meta-analysis suggest that the dietary inclusion of EOs improves WHC.

Dietary supplementation of EOs reduced ShF, suggesting that EOs increase beef tenderness. Collagen content is related to ShF of beef [89]. However, in the present study, collagen content was similar between treatments. By comparison, Rowe et al. [90] observed that the presence of post-mortem oxidative conditions inactivates calpain activity and decreases myofibrillar proteolysis in beef, which decreases meat tenderness. In the present meta-analysis, lipid oxidation was lower in beef supplemented with EOs, which partially explains the lower ShF observed. In addition, tenderness has been identified as one of the main attributes that determine meat quality [91], and some consumers are willing to pay more for meat if it is guaranteed to have higher tenderness [92]. Therefore, the dietary inclusion of EOs can be used as a nutritional strategy to improve the quality, acceptability, and price of beef.

According to Falowo et al. [93], the main non-microbial cause of quality loss in meat and meat products is lipid oxidation. MDA content is used to determine the presence of lipid peroxidation in meat [94]. In the present meta-analysis, lower MDA content was observed in meat from beef cattle supplemented with EOs, indicating that dietary

inclusion of EOs reduces lipid peroxidation and improves beef quality. There is limited information on the mechanisms of action of EOs on the activity of antioxidant enzymes in ruminants. However, in pigs and poultry, dietary supplementation of EOs has been reported to increase mRNA abundance and activity of superoxide dismutase, catalase, and glutathione peroxidase in smooth and skeletal muscle [95,96]. Therefore, similar effects of EOs consumption in the present study would partially explain the lower MDA content observed in the meat.

The L* in beef makes this product more attractive to consumers because the brightness of red meat is associated with fresh products [97]. In the present study, dietary supplementation of EOs did not affect the L* of meat, suggesting that dietary inclusion of EOs does not affect the appearance of beef. By comparison, a* values in meat decrease because of metmyoglobin formation [98]. Therefore, the absence of observed changes in a* in meat suggests that EOs do not affect metmyoglobin formation. Furthermore, it has been reported that in meat b* values are related to its fat content [99] and pH [100]. In the present meta-analysis, EOs supplementation did not affect the fat content and pH of meat, which would partially explain the lack of changes observed in b*.

Dietary supplementation of EOs did not affect the protein, fat, ash, and moisture content of meat. It has been mentioned that the nutritional value of meat is related to its protein, fat, vitamin, and mineral content [101]. This suggests that the dietary inclusion of EOs does not affect the nutritional value of beef. In the present meta-analysis, the lack of observed differences in the chemical composition of the meat was expected, because the nutritional composition of the diets was similar between the control and EOs-supplemented treatments.

4.6. Simulation Analysis

The environmental impact of beef cattle production systems has become a global concern [102] because beef production contributes about 41% of greenhouse gas emissions from the global livestock sector [52]. According to Capper and Hayes [103], in beef cattle, the improvement of productivity is positively related to economic and environmental sustainability. In the present study, the simulation analysis used showed that the positive effects of EOs on DWG and FE increased economic income through a 1.44% reduction in feed cost. Moreover, the benefits of dietary inclusion of EOs in DWG and FE decreased the environmental footprint by 0.83%, as a consequence of the reduction in emissions associated with bovine feed use. This suggests that dietary inclusion of EOs can improve the economic and environmental sustainability of beef production.

5. Conclusions

The results of the present meta-analysis indicate that the inclusion of EOs in beef cattle diets does not affect digestibility but improves dry matter intake, final body weight and daily weight gain, feed efficiency, carcass weight, and *Longissimus dorsi* muscle area. The best results for dry matter intake are obtained with EOs doses higher than 400 mg/kg DM, with supplementation periods longer than 90 days, and using diets high in concentrate (>700 g/kg DM). Similarly, the best results for final body weight and daily weight gain are achieved with EOs supplementation periods longer than 90 days and using high concentrate diets (>700 g/kg DM). Therefore, EOs can be used as natural growth promoters in beef cattle; however, more in vivo research is needed to confirm the effects of EOs on the performance and feed efficiency of beef cattle.

Additionally, EOs improve fermentation by increasing ruminal propionate concentration and reducing the concentration of ammonia nitrogen, acetate, and rumen protozoa. Moreover, the results of blood metabolites indicate that EOs do not negatively affect the health of beef cattle. Similarly, EOs do not affect the color and chemical composition of meat but reduce lipid oxidation and improve water holding capacity and tenderness. Finally, the results of the simulation analysis indicate that the addition of EOs in beef cattle diets

can be used as a natural alternative to reduce the environmental impact and increase the economic profitability of beef production.

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Appendix A

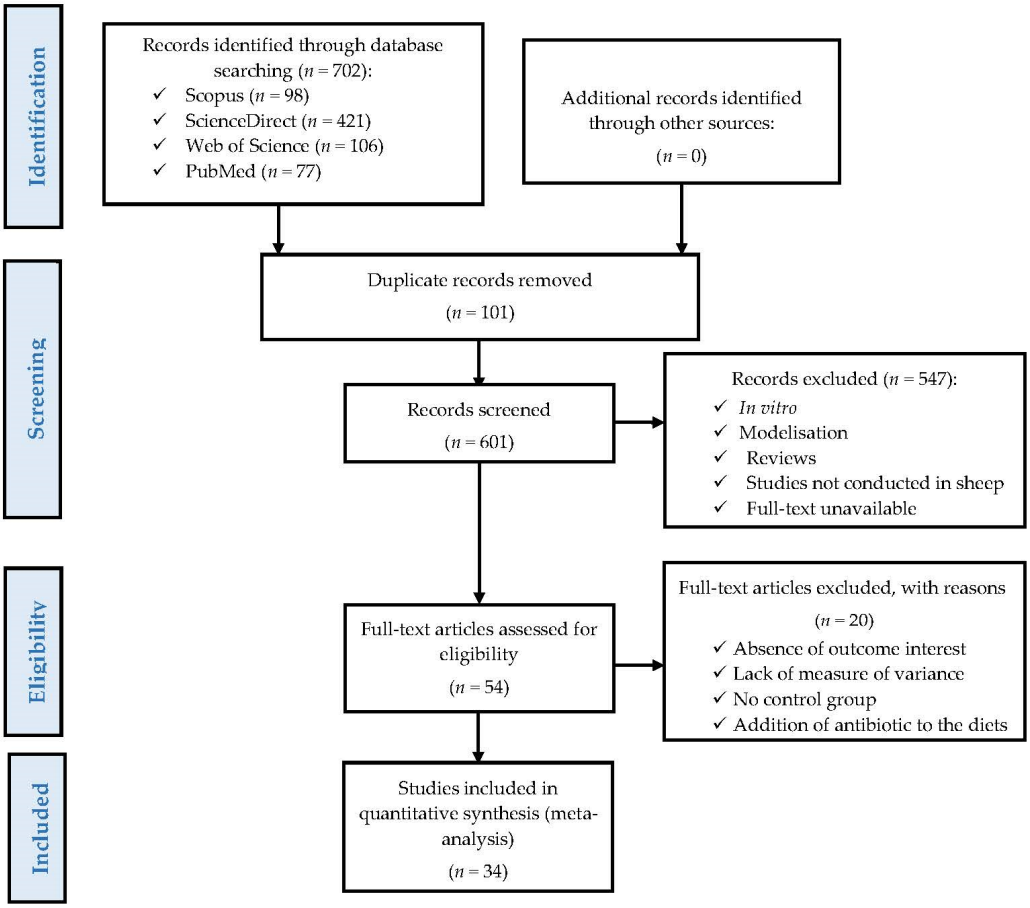

Figure A1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

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

Author	Country	Primary bioactive compound	Dose, mg/kg DM	C in Diet, g/kg DM	Duration, d
Afzalani et al. [104]	Indonesia	Blend (n = 4)	200, 400, 800, 1200	400 (n = 4)	90 (n = 4)
Alemu et al. [9]	Canada	Blend (n = 2)	150 (n = 2)	200 (n = 2)	84 (n = 2)
Almeida et al. [105]	Brazil	Blend (n = 1)	500 (n = 1)	700 (n = 1)	105 (n = 1)
Brand et al. [106]	Canada	Blend (n = 2)	50, 100	900 (n = 4)	112 (n = 4)
Carvalho et al. [107]	Brazil	Blend (n = 3)	191, 398, 601	700 (n = 3)	74 (n = 3)
Chapman et al. [108]	United States	Cinnamaldehyde (n = 2)	37, 76	470 (n = 2)	70 (n = 2)
Dorleku et al. [16]	Canada	Blend (n = 2)	85, 337	850 (n = 2)	100 (n = 2)
Fandiño et al. [11]	Spain	Capsaicin, blend, anethole (n = 3)	12, 31, 62 (n = 3)	900 (n = 9)	96 (n = 9)
Filho et al. [109]	Brazil	Thymol (n = 3)	232, 469, 965	500 (n = 3)	84 (n = 3)
Gouvêa et al. [10]	United States	Blend (n = 1)	120 (n = 1)	930 (n = 1)	154 (n = 1)
Guerrero et al. [110]	Brazil	Blend (n = 2)	500, 1000	900 (n = 2)	120 (n = 2)
Khorrami et al. [111]	Iran	Thymol, cinnamaldehyde	500, 500	700 (n = 2)	84 (n = 2)
Kim et al. [112]	Korea	Blend (n = 3)	39, 79, 113	900 (n = 3)	390 (n = 3)
Latack et al. [113]	United States	Blend (n = 2)	110 (n = 2)	880 (n = 2)	216, 84
Monteschio et al. [15]	Brazil	Blend (n = 4)	500 (n = 4)	Not reported	73 (n = 4)
Monteschio et al. [114]	Brazil	Blend (n = 4)	500 (n = 4)	Not reported	73 (n = 4)
Ornaghi et al. [115]	Brazil	Blend (n = 4)	444, 865, 450, 890	900 (n = 4)	187 (n = 4)
Ornaghi et al. [97]	Brazil	Blend (n = 4)	153, 305, 444, 594	700 (n = 4)	62 (n = 4)
Prado et al. [116]	Brazil	Blend (n = 1)	442 (n = 1)	500 (n = 1)	115 (n = 1)
Pukrop et al. [117]	United States	Blend (n = 1)	104 (n = 1)	860 (n = 1)	167 (n = 1)
Rivaroli et al. [118]	Brazil	Blend (n = 2)	500, 1000	900 (n = 2)	120 (n = 2)
Souza et al. [119]	Brazil	Blend (n = 4)	789, 640, 678, 644	750 (n = 2)	73 (n = 2)
Teobaldo et al. [12]	Brazil	Blend (n = 2)	150, 300	280 (n = 2)	76 (n = 2)
Tomkins et al. [120]	Australia	Blend (n = 2)	185, 370	0 (n = 2)	200 (n = 2)
Torrecilhas et al. [121]	Brazil	Eugenol (n = 2), cinnamaldehyde (n = 2)	450, 880, 450, 880	900 (n = 4)	187 (n = 4)
Vakili et al. [122]	Iran	Thymol, cinnamaldehyde	617, 641	850 (n = 2)	45 (n = 2)
Valero et al. [123]	Brazil	Blend (n = 1)	550 (n = 1)	550 (n = 1)	55 (n = 1)
Wanapat et al. [124]	Thailand	Blend (n = 3)	17, 25, 40	220 (n = 3)	84 (n = 3)
Westphalen et al. [14]	United States	Capsaicin (n = 4)	15, 5, 10, 15	900 (n = 4)	84 (n = 3), 80
Wu et al. [125]	China	Blend (n = 1)	26 (n = 1)	400 (n = 1)	240 (n = 1)
Yang et al. [126]	Canada	Cinnamaldehyde (n = 3)	37, 79, 184	850 (n = 3)	84 (n = 3)
Yang et al. [85]	Canada	Eugenol (n = 3)	42, 81, 166	850 (n = 3)	84 (n = 3)
Yang et al. [13]	Canada	Cinnamaldehyde (n = 3)	47, 98, 208	900 (n = 3)	112 (n = 3)
Zotti et al. [12]	Brazil	Blend (n = 2)	400 (n = 2)	923 (n = 2)	21 (n = 2)

DM: dry matter; C: concentrate; d: days; n: number of treatments.

Table A2. Simulation inputs were used for the economic and environmental impacts of essential oils (EOs) supplementation in beef cattle.

Item	Control	EOs	Difference	% Change
Number of animals	1000	1000		
DMI (kg DM/d/animal)	8.40	8.40		
DWG (kg/d/animal) [1]	1.213	1.300	+0.087	+7.2
FE (kg DWG/kg DMI/animal)	0.173	0.177	+0.004	+2.3
TLWG (kg/animal)	200	200		
LMY (kg/animal) [2]	82	82		
BPO (kg/animal) [3]	17.2	17.2		

DMI: dry matter intake; DWG: daily weight gain; FE: feed efficiency; TLWG; target live weight gain; LMY: lean meat yield; BPO: beef protein output; [1]: DWG of beef cattle fed EOs diets was corrected based on the current meta-analysis results which showed an average increase of +87 g/d/head in the DWG of beef cattle fed EOs diets. [2]: meat yield was calculated as a proportion of the TLWG (200 kg). According to Holland et al. [127], the average LMY of beef cattle was assumed to be 41% of body weight. [3]: BPO was calculated as a proportion of the LMY. Previous studies [128,129] have reported that beef contains an average of 21% protein.

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3. META-ANALYSIS OF FLAVONOIDS USE INTO BEEF AND DAIRY CATTLE DIET: PERFORMANCE, ANTIOXIDANT STATUS, RUMINAL FERMENTATION, MEAT QUALITY, AND MILK COMPOSITION

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Meta-analysis of flavonoids use into beef and dairy cattle diet: Performance, antioxidant status, ruminal fermentation, meat quality, and milk composition

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The objective of this study was to evaluate the effects of dietary supplementation with flavonoids (FLAs) on animal performance, diet digestibility, antioxidant status in blood serum, rumen parameters, meat quality, and milk composition in beef and dairy cattle through a meta-analysis. Thirty-six peer-reviewed publications were included in the data set. The weighted mean differences (WMD) between the FLAs treatments and the control treatment were used to assess the effect size. Dietary supplementation with FLAs decreased feed conversion ratio (WMD = −0.340 kg/kg; $p = 0.050$) and increased ($p < 0.05$) dry matter intake (WMD = 0.191 kg/d), dry matter digestibility (WMD = 15.283 g/kg of DM), and daily weight gain (WMD = 0.061 kg/d). In blood serum, FLAs supplementation decreased the serum concentration of malondialdehyde (WMD = −0.779 nmol/mL; $p < 0.001$) and increased ($p < 0.01$) the serum concentration of superoxide dismutase (WMD = 8.516 U/mL), glutathione peroxidase (WMD = 12.400 U/mL) and total antioxidant capacity (WMD = 0.771 U/mL). A higher ruminal propionate concentration (WMD = 0.926 mol/100 mol; $p = 008$) was observed in response to FLAs supplementation. In meat, the dietary inclusion of FLAs decreased ($p < 0.05$) shear force (WMD = −1.018 kgf/cm^2), malondialdehyde content (WMD = −0.080 mg/kg of meat), and yellowness (WMD = −0.460). Supplementation with FLAs decreased milk somatic cell count (WMD = −0.251 ×103 cells/mL; $p < 0.001$) and increased ($p < 0.01$) milk production (WMD = 1.348 kg/d), milk protein content (WMD = 0.080/100 g) and milk fat content (WMD = 0.142/100 g). In conclusion, dietary supplementation with FLAs improves animal performance and nutrient digestibility in cattle. In addition, FLAs improve the antioxidant status in blood serum and the quality of meat and milk.

KEYWORDS

oxidative stress, natural antioxidants, natural phytochemicals, immunity, digestibility

1. Introduction

As part of the strategies to satisfy the growing demand for meat and dairy products, it is necessary to increase effectiveness and productivity in bovine production systems (1). Dairy cows and beef cattle are frequently exposed to a wide variety of stressors, such as environmental (heat or cold stress), physiological (for example, rapid growth rate), and nutritional (presence of mycotoxins or oxidized fat in diets) (2, 3). All these factors promote the overproduction of reactive oxygen species, alter the redox balance and cause oxidative stress in animals (4). Oxidative stress is associated with a higher incidence of diseases (5, 6) and leads to diminished cattle productive and reproductive performance (3). According to Abuelo et al. (7), dietary supplementation with exogenous antioxidants such as vitamins and trace elements can reduce oxidative stress and improve cattle's health status and productive performance. However, in recent years, interest in using natural antioxidants (not from chemical synthesis) as alternatives to the synthetic antioxidants commonly used in animal feed has increased (8). Potential natural antioxidants include flavonoids (FLAs). The FLAs consist of two benzene rings joined by three carbon atoms to form an oxygenated heterocycle (9) and are present in a wide variety of plants (10).

It has been documented that FLAs possess diverse biological properties, such as antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial (10). The effects of dietary inclusion of FLAs have been investigated mainly in broilers and laying hens (11–13). However, in ruminants, there is limited information on the effects of dietary supplementation with FLAs. In growing ruminants, dietary supplementation with FLAs results in the reduction of diarrhea's occurrence and severity. However, it is ineffective in improving animal metabolism and productive performance (14). On the other hand, in adult ruminants, there is evidence that dietary supplementation with FLAs increases the serum concentration of antioxidant enzymes, reduces lipid peroxidation, and improves total antioxidant capacity in blood serum (15). In cattle and goats, some parts of plants containing FLAs have been used to increase the productive performance and digestibility of consumed nutrients (16, 17). Previous studies (18, 19) have shown that, in adult cattle, FLAs supplementation reduces agonistic interactions and modifies the differential expression of genes involved in inflammation, regulation of feeding behavior, and animal behavior. Specifically, in the ruminal epithelium of beef cattle, Paniagua et al. (19) detected greater gene expression of two genes (free fatty acid receptor 3 and free fatty acid receptor 2) that improve the feeding pattern in beef cattle by increasing the time the animals spend consuming forage and concentrate (18). Likewise, in adult sheep and cattle, it has been reported that the dietary inclusion of FLAs has a positive impact on the composition of the rumen microbiome and the production of volatile fatty acids in the rumen (20, 21).

Particularly in beef cattle and dairy cows, some studies have evaluated the effects of dietary supplementation with FLAs on animal performance (19, 22), serum antioxidant status (23, 24), rumen fermentation and nutrient digestibility (16, 20), meat physicochemical characteristics (25, 26) and milk production and composition (15, 27). However, the results obtained so far are still inconsistent and controversial, probably due to the wide variability among these studies regarding the experimental periods, the doses, and the type of FLAs used (14). Therefore, it is necessary to identify and control this variability to develop products containing FLAs that can improve the antioxidant status, animal performance, and quality of beef and dairy cattle products.

In recent years, some review articles have been published (9, 14), mentioning that it is possible to use FLAs for the improvement of the antioxidant status in blood serum, health status, animal performance, and quality of food products derived from ruminants. However, these review articles neither focus only on beef cattle or dairy cows nor used a meta-analytic approach. Meta-analysis (MA) is a method that allows previously published results of a series of individual studies to be collected, combined, and statistically analyzed (28). Likewise, the MA helps identify sources of heterogeneity between studies (29). Therefore, there is a growing interest in the application of MA in the field of animal nutrition (30). However, the use of MA in research related to the inclusion of natural feed additives in ruminant diets is still limited (31). The hypothesis of this meta-analysis states that adding FLAs in beef and dairy cattle diets will benefit animal performance, antioxidant status, and rumen parameters without affecting the quality of products derived from these animals. Therefore, the objective of this study was to evaluate the effects of dietary supplementation with flavonoids FLAs on animal performance, diet digestibility, serum antioxidant status, rumen parameters, meat quality, and milk composition derived from beef and dairy cattle through a meta-analysis.

2. Materials and methods

2.1. Literature search and study selection

To reduce publication bias and ensure the quality of the meta-analysis, the present study was conducted following PRISMA guidelines (32), as shown in Figure 1. A systematic search for information was conducted using Web of Science, Scopus, PubMed, and ScienceDirect databases to identify previous studies evaluating the effects of dietary supplementation with FLAs on nutrient digestibility, animal performance, carcass characteristics, antioxidant status in blood serum, ruminal fermentation, as well as meat and milk quality in beef (Holstein, Simmental, Angus × Nellore, Jinjiang, Xianan, and native) and dairy cattle (Holstein). The keywords that were used in all the databases were the following: "flavonoids, beef cattle, growth performance, finishing steer, finishing bull, carcass, meat quality, dairy cattle, milk production, milk quality, digestibility, ruminal fermentation, antioxidant status", and the main representatives of FLAs (33), such as "daidzein, naringin, puerarin, anthocyanin, and quercetin". In all searches performed, results were restricted to studies published between January 2010 and November 2022. In total, 1,010 scientific publications were identified (Figure 1); however, duplicate publications found in more than one of the databases were excluded. After this, the remaining publications underwent a two-step selection process, as previously reported by other authors (34–36). First, based on the titles and abstracts of each publication, we excluded studies that were not conducted in beef cattle or dairy cows, studies that did not measure any of the variables of interest, *in vitro* experiments, studies that used animals experimentally infected, as well as simulation and review articles.

Secondly, the articles analyzed had to meet some previously defined inclusion criteria to be included in the final database. In the present meta-analysis, the inclusion criteria used were similar to those previously reported by Dorantes-Iturbide et al.

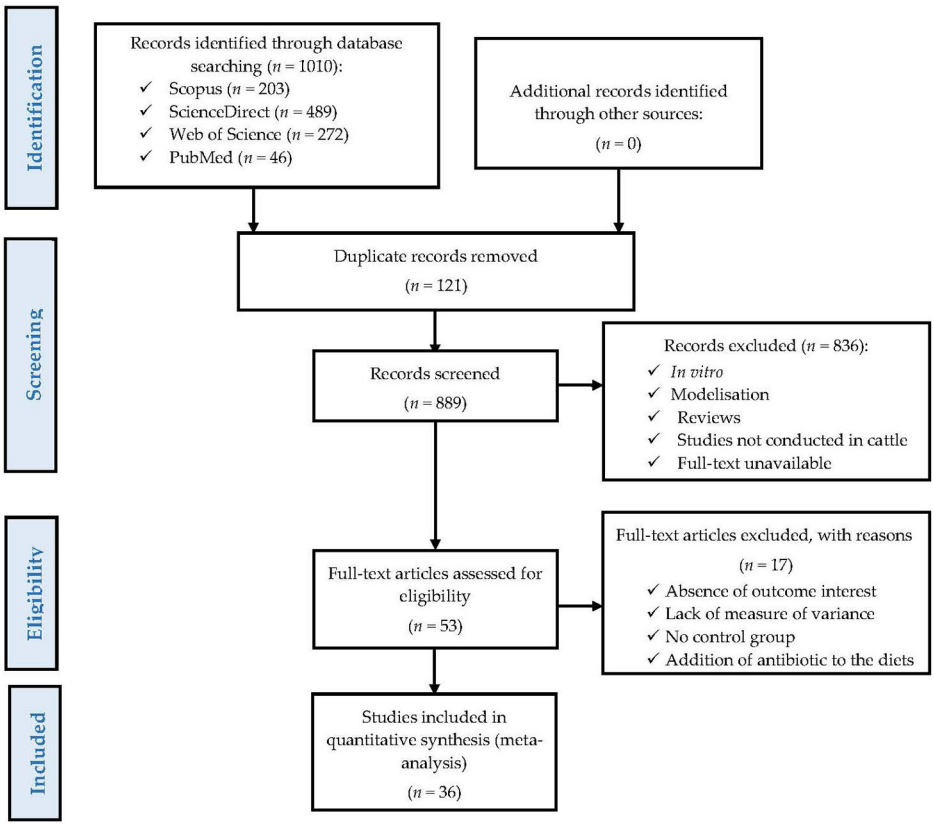

FIGURE 1
A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

(35) and Orzuna-Orzuna et al. (36): (1) studies with beef cattle or dairy cows housed in confined conditions; (2) data on animal performance, nutrient digestibility, antioxidant status in blood serum, carcass characteristics, ruminal fermentation or quality of the derived products (meat or milk); (3) studies that had control and experimental treatments with similar diets, except for the presence of FLAs in the diets; (4) studies that reported the doses of FLAs used or had sufficient information to estimate the amount of FLAs included in the diets; (5) studies written and published in English and in peer-reviewed scientific journals; and (6) studies that reported the means of the control and FLA-supplemented treatments, the standard error or standard deviation, and the number of replicates.

2.2. Data extraction

After applying the inclusion criteria, only 36 peer-reviewed articles were included in the final database (Supplementary Table S1). Likewise, we only extracted quantitative data for response variables that were reported in at least three individual studies (31, 35, 36). Among the response variables included in the final database of this meta-analysis are the following: dry matter intake and nutrient digestibility (neutral detergent fiber, crude protein), daily weight gain, feed conversion ratio, carcass characteristics (carcass yield, backfat thickness), serum concentration of malondialdehyde and antioxidant enzymes (for example, superoxide dismutase), serum immunoglobulins (IgA, IgM, and IgG), rumen parameters (pH, ammonia nitrogen), physicochemical characteristics of the meat (pH, shear force, color), milk production, and milk composition (lactose, protein, and fat content).

Additionally, when available, the following complementary information was obtained from the selected publications: (1) author and year of publication; (2) period of supplementation with FLAs (days); (3) type of FLAs (for example, anthocyanin, daidzein); (4) method of inclusion of the FLAs (extract or naturally present in the diet); (5) amount of concentrate included in the diets (g/kg DM); (6) days in milk from dairy cows; (7) type of cattle (beef cattle or dairy cow); (8) nutritional composition of the diets used; and (9) country where the study was conducted.

Supplementary Table S1 shows the complete list of publications included in the final database of the present meta-analysis. The number of replicates, means, and standard deviations (SD) for the control and experimental treatments (supplemented with FLAs) were extracted from each of these publications. In all the publications in which the SD was not reported, SD was determined using the standard errors of the treatment means (SEM), by using the following equation (37): $SD = SEM \times \sqrt{n}$, where n = number of repetitions.

2.3. Calculations and statistical analysis

Meta-analysis and meta-regression, as well as analyzes of subgroups, heterogeneity, and publication bias, were performed using the "metaphor" package (38), which is available in the statistical

TABLE 1 Dry matter intake and nutrient digestibility of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	WMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
DMI, kg/d	24 (43)	11.83 (5.08)	0.191 (0.047; 0.334)	0.009	<0.001	77.63	0.184
Digestibility, g/kg of DM							
DMD	11 (22)	687.84 (34.53)	15.283 (7.306; 23.259)	<0.001	0.348	0	0.112
OMD	10 (20)	712.10 (58.30)	7.204 (0.353; 14.055)	0.039	0.361	7.65	0.643
CPD	12 (25)	670.00 (90.10)	19.785 (13.099; 26.471)	<0.001	0.116	27.97	0.090
NDFD	12 (25)	523.30 (69.60)	15.563 (9.215; 21.911)	<0.001	0.723	0	0.377
ADFD	10 (20)	446.70 (104.80)	6.894 (0.524; 13.265)	0.034	0.417	3.19	0.883
EED	5 (8)	772.60 (87.80)	24.945 (8.962; 40.927)	0.002	0.498	0	NA

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; p-value to χ2 (Q) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
NA, variables with n < 10 observations, the test does not apply; DMI, dy matter intake; DMD, dry matter digestibility; OMI, organic matter digestibility; CPD, crude protein digestibility; EE, ether extract digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility; EED, ether extract digestibility.

TABLE 2 Growth performance and carcass characteristics of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	WMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
ADG, kg/d	12 (22)	0.926 (0.41)	0.061 (0.026; 0.097)	<0.001	<0.001	73.47	0.657
FCR, kg/kg	9 (16)	7.90 (2.41)	−0.340 (−0.686; 0.005)	0.050	<0.001	81.77	0.122
Carcass traits							
HCW, kg	5 (5)	277.7 (68.8)	0.101 (−3.145; 3.347)	0.951	0.656	0	NA
HCY, %	6 (7)	54.56 (3.03)	−0.059 (−0.662; 0.544)	0.847	0.016	61.41	NA
BFT, mm	6 (8)	13.42 (3.63)	2.178 (0.829; 3.528)	0.002	<0.001	96.69	NA
LDMA, cm^2	5 (7)	90.92 (19.98)	0.535 (−1.954; 3.025)	0.673	0.146	37.03	NA

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; *p*-value to χ2 (Q) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
NA, variables with $n < 10$ observations, the test does not apply; ADG, average daily gain; FCR, feed conversion ratio; HCW, hot carcass weight; HCY, hot carcass yield; BFT, backfat thickness; LDMA, *Longissimus dorsi* muscle area.

software R (version 4.1.2, R Core Team, Vienna, Austria). The effects of including FLAs in diets of beef cattle and dairy cows were evaluated using the weighted mean differences (WMD) between treatments supplemented with FLAs (diets with FLAs) and control treatments (diets without FLAs). For this, the means of the treatments were weighted by the inverse of the variance, according to the method for random effects models previously proposed by DerSimonian and Laird (39). In the present meta-analysis, the WMD was used because it allows interpretation of the results obtained in the original units of measurement (40). Additionally, with the PROC MEANS procedure of the statistical software SAS (41), descriptive statistics values were obtained for the continuous covariates level of concentrate in the diet, dose of FLAs, experimental period, and days in milk.

2.4. Heterogeneity and publication bias

The presence of heterogeneity between studies was identified with the chi-square (Q) test, in which a significance level of $p \leq 0.10$ was used since this test has relatively low power (42). Additionally, to quantify the proportion of observed heterogeneity, we used the I^2 statistic (29). For this test, I^2 values <25% indicate that the degree of heterogeneity is low, I^2 values between 25 and 50% indicate moderate heterogeneity, while I^2 values >50% indicate high and significant heterogeneity (29, 43). On the other hand, to detect the presence of publication bias, the Egger regression asymmetry test (44) was applied, in which a significance level of $p \leq 0.05$ was used. When publication bias was detected ($p \leq 0.05$ in Egger's test), the "trim and fill" method of Duval and Tweedie (45) was applied to estimate the possible number of missing observations.

2.5. Meta-regression and subgroup analysis

Meta-regression analyses were performed on some of the variables evaluated to identify the presence of possible sources of heterogeneity. The criteria considered to apply meta-regression analysis were: (1) presence of significant heterogeneity (i.e., $p \leq 0.10$ for Q or $I^2 > 50\%$); (2) *p*-value > 0.05 for Egger's test (44); and (3) response variables reported in 10 or more individual studies (46). For

TABLE 3 Oxidative status and immune response of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	RMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
SOD, U/mL	10 (27)	72.39 (39.23)	8.516 (5.095; 11.937)	<0.001	<0.001	91.50	0.125
CAT, U/mL	3 (10)	39.26 (15.32)	3.762 (1.691; 5.833)	<0.001	<0.001	92.69	0.481
GPx, U/mL	7 (22)	63.73 (25.51)	12.400 (8.481; 16.319)	<0.001	<0.001	80.42	0.063
TAC, U/mL	5 (13)	7.86 (3.52)	0.771 (0.274; 1.267)	0.002	0.118	35.53	0.067
MDA, nmol/mL	7 (24)	5.51 (2.82)	−0.779 (−1.220; −0.339)	<0.001	<0.001	85.26	0.203
Immunoglobulins, g/L							
IgA	4 (14)	0.792 (0.14)	0.063 (0.018; 0.108)	0.006	0.001	61.32	0.492
IgG	5 (15)	9.137 (2.13)	1.150 (0.633; 1.667)	<0.001	<0.001	76.47	0.063
IgM	4 (14)	2.183 (0.59)	0.215 (0.139; 0.292)	<0.001	<0.001	73.86	0.400

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; *p*-value to $\chi 2$ (*Q*) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; MDA, malondialdehyde; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.

TABLE 4 Ruminal fermentation of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	RMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
Ruminal pH	11 (20)	6.43 (0.46)	0.029 (−0.059; 0.117)	0.517	<0.001	83.29	0.129
NH_3-N, mg/dL	9 (18)	15.03 (6.23)	0.030 (−0.559; 0.618)	0.921	<0.001	85.95	0.061
SCFA, mol/100 mol							
Acetate	12 (22)	62.12 (8.22)	0.188 (−0.794; 1.170)	0.708	<0.001	79.63	0.104
Propionate	12 (22)	22.34 (4.51)	0.926 (0.240; 1.611)	0.008	<0.001	89.01	0.261
Butyrate	12 (22)	11.72 (3.62)	0.138 (−0.105; 0.381)	0.265	0.237	17.09	0.086
Total protozoa, $\times 10^5$/mL	3 (8)	6.50 (2.40)	−0.301 (−0.561; −0.042)	0.023	0.054	49.37	NA

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; *p*-value to $\chi 2$ (*Q*) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
NA, variables with $n < 10$ observations, the test does not apply; NH_3-N, nitrogen ammonia; SCFA, short chain fatty acids.

all meta-regression analyses, the method of moments of DerSimonian and Laird (39) was used, as it is well-established for estimating between-study variance. Subsequently, for the covariates analyzed that were significant with $p \leq 0.05$, the WMD was evaluated through subgroup analysis. A subgroup assessment was not performed when an individual stratum has less than two effect sizes in the meta-analysis (35, 36). The type of FLAs (daidzein, anthocyanin, puerarin, naringin, quercetin, catechin, and blend), the method of supplementation with FLAs (extract or naturally present in an ingredient in the diet), and the type of cattle (beef cattle or dairy cow) were used as categorical covariates. On the other hand, the duration of the experimental period (days), the days in milk of the dairy cows, the content of concentrate in the diet (g/kg of DM), and the doses of FLAs were used as continuous covariates. When any categorical covariate (type of FLAs, type of bovine, and method of supplementation with FLAs) was found to be statistically significant ($p \leq 0.05$), subgroup analysis was used to assess WMD (34, 35). Likewise, when the meta-regression was significant ($p \leq 0.05$) for the continuous covariates, these were analyzed using the following subgroups: dietary dose of FLAs (≤600 and >600 mg/kg of DM), level of concentrate in the diet (≤400, 401–700, and >700 g/kg of DM), days in milk (≤100 and >100 days) and period of supplementation with FLAs (≤75 and >75 days).

3. Results

3.1. Study attributes

The studies included in this meta-analysis were conducted in eight different countries, mainly in China (44.4%), Spain (16.7%), Brazil (13.9%), and Japan (5.5%). Regarding the animal species (bovine), in 66.7% of the studies, beef cattle were used, and in the remaining studies (33.3%), dairy cows were used (Supplementary Table S1). Supplementary Table S2 shows that the doses of FLAs used were between 12 and 3,104 mg/kg DM. Dairy cows had between 7 and 164 days in milk and the experimental periods ranged between 24 and 168 days (Supplementary Table S2).

TABLE 5 Meat quality of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	WMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
pH 24 h	6 (10)	5.47 (0.21)	0.063 (−0.032; 0.158)	0.194	<0.001	86.34	0.769
CL, g/100 g	4 (8)	23.54 (4.98)	0.628 (−1.433; 2.690)	0.550	0.028	55.52	NA
ShF, kgf/cm^2	5 (8)	5.98 (2.54)	−1.018 (−1.470; −0.566)	<0.001	<0.001	88.17	NA
MDA, mg/kg	4 (8)	0.44 (0.16)	−0.080 (−0.101; −0.059)	<0.001	0.608	0	NA
Meat color							
Lightness (L*)	6 (13)	44.52 (10.32)	−2.174 (−5.117; 0.769)	0.148	<0.001	93.47	0.337
Redness (a*)	6 (13)	24.05 (6.62)	−0.065 (−0.734; 0.605)	0.850	0.177	26.42	0.669
Yellowness (b*)	6 (13)	10.65 (2.33)	−0.460 (−0.892; −0.028)	0.037	0.272	17.50	0.681
Chemical composition, g/100 g of DM							
Protein	6 (10)	20.61 (1.90)	0.390 (−0.622; 1.401)	0.450	<0.001	87.37	0.897
IMF	6 (10)	5.90 (2.52)	0.703 (0.070; 1.336)	0.029	<0.001	69.02	0.079
Moisture	6 (10)	70.28 (1.80)	−0.601 (−1.304; 0.101)	0.093	0.073	42.76	0.734
Ash	3 (5)	2.63 (1.17)	0.013 (−0.012; 0.039)	0.304	0.905	0	NA

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; *p*-value to χ2 (*Q*) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
NA, variables with $n < 10$ observations, the test does not apply; WHC, water holding capacity; CL, cook loss; ShF, shear force; MDA, malondialdehyde; IMF, intramuscular fat.

TABLE 6 Milk production and quality of cattle supplemented with flavonoids.

Item	*N* (NC)	Control means (SD)	WMD (95% CI)	*P*-value	Heterogeneity		Egger test[a]
					P-value	I^2 (%)	*P*-value
Milk production, kg/d	11 (24)	21.29 (6.87)	1.348 (0.517; 2.179)	0.001	<0.001	75.86	0.193
Milk composition, g/100 g							
Protein	11 (24)	3.71 (0.67)	0.080 (0.045; 0.116)	<0.001	0.004	53.81	0.347
Fat	11 (24)	3.65 (0.88)	0.142 (0.073; 0.211)	<0.001	<0.001	69.98	0.060
Lactose	10 (18)	5.10 (0.95)	0.016 (−0.033; 0.066)	0.517	0.118	26.76	0.132
SCC, $\times 10^3$ cell/mL	6 (12)	2.52 (0.72)	−0.251 (−0.364; −0.138)	<0.001	<0.001	74.29	0.344

N, number of studies; NC, number of comparisons between flavonoids treatment and control treatment; SD, standard deviation; WMD, weighted mean differences between control and treatments with flavonoids; CI, confidence interval of WMD; *p*-value to χ2 (*Q*) test of heterogeneity; I^2, proportion of total variation of size effect estimates that is due to heterogeneity.
[a]Egger's regression asymmetry test.
SCC, somatic cell count.

Supplementary Table S1 shows seven different types of FLAs used in the present meta-analysis. Most of the studies used mixtures of FLAs (36.1%), daidzein (16.7%), anthocyanin (16.7%), and naringin (16.7%). Three other different types of FLAs were used in the remaining studies (13.8%). In addition, 61.1% of the studies used FLAs extracts, and 38.9% used plants or by-products naturally high in FLAs.

3.2. Dry matter intake and nutrient digestibility

Dry matter intake (DMI) increased in response to FLAs supplementation (Table 1). Likewise, dietary supplementation with FLAs increased ($p < 0.05$) dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), acid detergent fiber digestibility (ADFD), and ether extract digestibility (EED).

3.3. Growth performance and carcass traits

Table 2 shows that daily weight gain (ADG) and backfat thickness (BFT) increased in response to dietary supplementation with FLAs ($p < 0.05$). In contrast, the dietary inclusion of FLAs decreased the feed conversion ratio (FCR; $p = 0.050$). However, hot carcass weight (HCW), hot carcass yield (HCY), and *Longissimus dorsi* muscle area (LDMA) were not affected by FLAs supplementation ($p > 0.05$; Table 2).

3.4. Antioxidant status and immune response

Table 3 shows that dietary supplementation with FLAs increased ($p < 0.01$) the serum concentration of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and total antioxidant capacity (TAC). In contrast, a lower ($p < 0.001$) serum concentration of malondialdehyde (MDA) was observed in

TABLE 7 Meta-regression comparing the associations between covariates and measured outcomes.

Parameter	Covariates	QM	Df	*P*-value	R^2 (%)
Dry matter intake (DMI)	Flavonoids dose	1.62	1	0.202	0.00
	Supplementation period	12.97	1	<0.001	5.90
	Concentrate level	0.22	1	0.634	0.00
	Flavonoid type	25.68	5	<0.001	29.55
	Method of inclusión	0.85	1	0.654	0.00
	Beef cattle/dairy cattle	0.38	1	0.536	0.00
Average daily gain (ADG)	Flavonoids dose	0.28	1	0.596	0.00
	Supplementation period	5.62	1	0.018	28.28
	Concentrate level	3.89	1	0.049	40.10
	Flavonoid type	6.81	4	0.146	33.47
	Method of inclusion	0.93	1	0.628	0.00
Ruminal pH	Flavonoids dose	1.38	1	0.240	9.18
	Supplementation period	0.08	1	0.772	0.00
	Concentrate level	4.50	1	0.034	37.92
	Flavonoid type	8.49	3	0.037	20.69
	Method of inclusion	0.02	1	0.883	0.00
	Beef cattle/dairy cattle	0.258	1	0.611	0.00
Acetate	Flavonoids dose	0.25	1	0.615	0.00
	Supplementation period	6.98	1	0.108	0.00
	Flavonoid type	2.71	3	0.100	0.00
	Concentrate level	13.45	3	0.104	0.00
	Method of inclusion	1.73	1	0.188	0.00
	Beef cattle/dairy cattle	1.78	1	0.182	0.00
Propionate	Flavonoids dose	1.18	1	0.277	0.00
	Supplementation period	6.77	1	0.009	6.87
	Concentrate level	0.58	1	0.445	0.00
	Flavonoid type	0.99	3	0.803	0.00
	Method of inclusion	1.69	1	0.193	0.00
	Beef cattle/dairy cattle	1.59	1	0.207	0.00
Superoxide dismutase (SOD)	Flavonoids dose	2.11	1	0.146	0.00
	Supplementation period	0.01	1	0.977	3.26
	Concentrate level	0.06	1	0.940	2.55
	Flavonoid type	25.18	4	<0.001	41.84
	Method of inclusion	8.39	1	0.004	15.12
	Beef cattle/dairy cattle	19.66	1	<0.001	40.60
Milk production	Flavonoids dose	4.72	1	0.030	23.80
	Supplementation period	1.40	1	0.236	0.00
	Concentrate level	0.49	1	0.484	0.00
	Flavonoid type	80.93	3	<0.001	35.49
	Method of inclusion	19.19	1	<0.001	40.45
	Days in milk	1.26	1	0.261	0.00

(Continued)

TABLE 7 (Continued)

Parameter	Covariates	QM	Df	*P*-value	R^2 (%)
Milk protein	Flavonoids dose	3.21	1	0.073	06.87
	Supplementation period	11.44	1	<0.001	39.61
	Concentrate level	10.84	1	<0.001	10.18
	Flavonoid type	14.03	3	0.003	48.21
	Method of inclusion	1.44	1	0.229	12.92
	Days in milk	0.56	1	0.455	0.00
Milk fat	Flavonoids dose	1.28	1	0.257	0.00
	Supplementation period	0.21	1	0.648	11.24
	Concentrate level	2.97	1	0.085	0.00
	Flavonoid type	18.54	3	<0.001	56.70
	Method of inclusion	0.53	1	0.464	0.00
	Days in milk	17.75	1	<0.001	41.75

QM, coefficient of moderators; QM is considered significant at $p \leq 0.05$; R^2, the amount of heterogeneity accounted for; df, degree of freedom.

animals supplemented with FLAs. On the other hand, the serum concentration of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) increased in response to dietary supplementation with FLAs ($p < 0.01$).

3.5. Rumen fermentation and protozoal count

Table 4 shows that the pH and the ruminal concentration of ammonia nitrogen (NH3-N), acetate, and butyrate were not affected by dietary supplementation with FLAs ($p > 0.05$). However, a higher ($p = 0.008$) rumen concentration of propionate and a lower ($p = 0.023$) concentration of total protozoa were observed in response to supplementation with FLAs.

3.6. Meat quality

Dietary supplementation with FLAs did not affect ($p > 0.05$) pH, cooking loss (CL), lightness (L*), redness (a*), or meat protein, moisture, and ash content (Table 5). On the other hand, FLAs supplementation decreased ($p < 0.05$) the shear force (ShF), the malondialdehyde content (MDA), and the yellowness (b*) of the meat. However, meat's intramuscular fat content (IMF) increased in response to FLAs supplementation ($p = 0.029$).

3.7. Milk production and composition

Dietary supplementation with FLAs increased ($p < 0.01$) milk production and milk protein and fat content (Table 6). However, the lactose content in milk was not affected by FLAs supplementation ($p > 0.05$). In addition, lower milk somatic cell (SCC) counts were observed in response to dietary supplementation with FLAs ($p < 0.001$).

3.8. Meta-regression and publication bias

Tables 1–6 show no publication bias since the Egger regression asymmetry test was not significant ($p > 0.05$) for any of the variables evaluated. On the other hand, Tables 1–6 show that there was significant ($p \leq 0.10$) heterogeneity (Q) for DMI, ADG, FCR, HCY, BFT, SOD, CAT, GPx, MDA in blood serum, IgA, IgG, IgM, rumen pH, NH3-N, acetate, propionate, total protozoa, meat pH, CL, ShF, L*, protein content, meat IMF and moisture, milk yield, and protein, fat, and SCC content in milk. However, to obtain reliable results, meta-regression analyses are only recommended when the variable of interest is reported in 10 or more studies (46). Consequently, meta-regression analyses were only performed for the following variables: DMI, ADG, rumen pH, acetate, propionate, SOD, milk yield, and milk protein and fat content.

Table 7 shows that the FLAs dose explained ($p < 0.05$) 23.80% of the observed heterogeneity for milk production. The supplementation period explained ($p < 0.05$) 5.90, 28.28, 6.87, and 39.61% of the heterogeneity observed for DMI, ADG, rumen propionate concentration, and milk protein content, respectively. On the other hand, the level of concentrate in the diet explained ($p < 0.05$) 40.10, 37.92, and 10.18% of the heterogeneity observed for ADG, ruminal pH, and milk protein content, respectively. The type of FLAs used explained ($p < 0.05$) between 20.69 and 56.70% of the observed heterogeneity for DMI, rumen pH, SOD, milk yield, milk protein, and milk fat content. Likewise, the FLAs inclusion method explained ($p < 0.05$) 15.12 and 40.45% of the observed heterogeneity for SOD and milk production, respectively. Bovine type explained ($p < 0.001$) 40.60% of the observed heterogeneity for SOD, and days in milk explained ($p < 0.001$) 41.75% of the observed heterogeneity for milk fat content. There was no significant relationship ($p > 0.05$) between the covariates used and the ruminal acetate concentration.

3.9. Subgroup analysis

DMI increased (WMD = 0.532 kg/d; $p = 0.038$) when dietary supplementation with FLAs lasted up to 75 days (Figure 2A).

However, supplementation with FLAs for more than 75 days did not affect DMI (WMD = 0.015 kg/d; $p = 0.805$). Higher ADG (WMD = 0.093 kg/d; $p < 0.001$) was observed when cattle were supplemented with FLAs for periods up to 75 days (Figure 2B). However, when supplementation with FLAs lasted more than 75 days ADG was not affected (WMD = 0.017 kg/d; $p = 0.206$). Ruminal propionate concentration was increased (WMD = 1.962 mol/100 mol; $p = 0.043$) in animals supplemented with FLAs for up to 75 days (Figure 2C). However, ruminal propionate concentration was not affected when FLAs were offered for more than 75 days (WMD = 0.306 mol/100 mol; $p = 0.486$). The protein content in milk increased ($p < 0.05$) regardless of the period of supplementation with FLAs used (Figure 2D). However, the effect was greater when FLAs supplementation lasted longer than 75 days (WMD = 0.113/100 g) than when it lasted up to 75 days (WMD = 0.097/100 g).

Figure 3A shows that ADG increased (WMD = 0.048 kg/d; $p < 0.001$) only when FLAs were included in high-concentrate diets (>700 g/kg DM). However, the inclusion of FLAs in diets with low (≤400 g/kg DM) or moderate (401–700 g/kg DM) concentrate levels did not affect ADG ($p > 0.05$). Rumen pH increased (WMD = 0.336; $p < 0.001$) when FLAs were supplemented in diets with more than 700 g/kg DM of concentrate (Figure 3B). However, the inclusion of FLAs in diets with low (≤400 g/kg DM) or moderate (401–700 g/kg DM) concentrate levels did not affect rumen pH. Figure 3C shows that the inclusion of FLAs in diets with 401–700 g/kg DM of concentrate increased the protein content in milk (WMD = 0.089/100 g; $p < 0.001$). However, milk protein content was not affected when FLAs were fed in low-concentrate diets (≤400 g/kg DM).

Figure 4A shows that DMI increased ($p < 0.05$) when the type of FLAs used was daidzein (WMD = 0.500 kg/d), puerarin (WMD = 0.700 kg/d), and anthocyanin (WMD = 0.535 kg/d). However, DMI decreased when the type of FLAs used was naringin (WMD = −0.129 kg/d; $p = 0.021$) and was not affected when mixtures of FLAs were used ($p > 0.05$). Rumen pH increased (WMD = 0.071; $p = 0.030$) when FLAs mixtures were used (Figure 4B); however, it decreased when the FLAs used were daidzein (WMD = −0.350; $p = 0.001$) and anthocyanin (WMD = −0.100; $p = 0.041$). Likewise, when the type of FLAs used was naringin, the rumen pH was not affected ($p > 0.05$). Figure 4C shows that the serum concentration of SOD increased ($p < 0.001$) only when the FLAs used were daidzein (WMD = 9.373 U/mL) and puerarin (WMD = 19.733 U/mL). However, the serum SOD concentration was not affected when anthocyanin or FLA mixtures were used ($p > 0.05$). On the other hand, milk production increased ($p < 0.001$; Figure 4D) when mixtures of FLAs (WMD = 0.701 kg/d) and daidzein (WMD = 3.923 kg/d) were used; however, it decreased when the FLAs used were anthocyanins (WMD = −1.612 kg/d; $p < 0.001$). Figure 4E shows that milk protein content increased when mixtures of FLAs (WMD = 0.113/100 g; $p < 0.001$) and daidzein (WMD = 0.174/100 g; $p = 0.044$) were used. However, the protein content in milk was not affected when the FLAs used were anthocyanins ($p > 0.05$). Milk fat content increased ($p < 0.01$; Figure 4F) in response to supplementation with mixtures of FLAs (WMD = 0.106/100 g) and daidzein (WMD = 0.373/100 g); however, it was not affected by anthocyanin supplementation ($p > 0.05$).

Serum SOD concentration increased (WMD = 11.016 U/mL; $p < 0.001$) when FLAs extracts were added to diets (Figure 5A). However, when FLAs were supplied as part of the diet ingredients, serum SOD concentration was not affected ($p > 0.05$). Milk production increased (WMD = 2.748 kg/d; $p < 0.001$) in response to supplementation with FLAs extracts (Figure 5B). However, milk production was not affected ($p > 0.05$) when FLAs were supplied as part of the diet ingredients.

Figure 6A shows that milk production increased when FLAs doses ≤600 mg/kg DM were used (WMD = 1.774 kg/d; $p < 0.001$). However, milk production decreased (WMD = −1.209 kg/d; $p = 0.002$) when the FLAs doses used were >600 mg/kg DM. In addition, serum SOD concentration increased regardless of the type of bovine used ($p < 0.001$; Figure 6B). However, the effect was greater when FLAs were offered to beef cattle (WMD = 14.712 U/mL) than dairy cows (WMD = 3.615 U/mL). Likewise, milk fat content increased (WMD = 0.217/100 g; $p < 0.001$) when FLAs were offered to cattle that were longer than 100 days in milk (Figure 6C). However, in cattle that were up to 100 days in milk, FLA supplementation did not affect milk fat content ($p > 0.05$).

4. Discussion

4.1. Dry matter intake and nutrient digestibility

It has been reported that the dietary inclusion of FLAs increases the relative abundance of ruminal bacteria involved in fiber degradation in adult sheep and cattle (20, 47). This effect could increase the rate of passage of feed particles in the rumen and result in higher DMI. In addition, in ruminants (yaks and sheep) dietary supplementation with FLAs increases the relative rumen abundance of the bacterial family *Rikenelleceae* (48, 49), which have a positive correlation with DMI in beef cattle (50). Therefore, similar effects of FLAs supplementation in the present meta-analysis partially explain the observed increase in DMI. On the other hand, in beef cattle, it has been reported that FLAs supplementation reduces the gene expression of bitter taste receptors (TAS2R, such as TAS2R7, TAS2R16, TAS2R38, and TAS2R39) in the rumen (18) and duodenal epithelium (51). This effect could decrease the release of anorexigenic molecules and increase DMI, since the activation of TAS2R triggers the release of anorexigenic molecules, such as cholecystokinin and peptide YY (52, 53). However, a subgroup analysis revealed that naringin supplementation decreased DMI. Naringin is part of the flavanones (a particular class of FLAs), which are abundant in citrus and impart a bitter taste (33). This effect could reduce the food's palatability and explain the lower DMI observed in response to naringin supplementation.

Previous studies (20, 47) have reported that, in adult ruminants (sheep and cattle), dietary supplementation with FLAs increases the relative abundance of ruminal bacteria of the genus *Ruminococcus*. Within this genus are the species *Ruminococcus albus* and *R. flavefaciens*, which play an important role in fiber degradation in the rumen (54). Kim et al. (55) observed that, under *in vitro* conditions, FLAs (catechins) increase the relative abundance of *Fibrobacter succinogenes* bacteria, which are also involved in fiber degradation in the rumen. Low doses (60 mg/kg body weight) of FLAs (mixtures of various types not reported) have been documented to increase the relative abundance of fungi in the rumen of dairy cows by up to 79% (56). According to Akin and Borneman (57), rumen fungi can completely penetrate the cell wall and produce large amounts of cellulases, hemicelluloses, and xylanases, which can increase cellulose degradation. In beef cattle, Niu et al. (58)

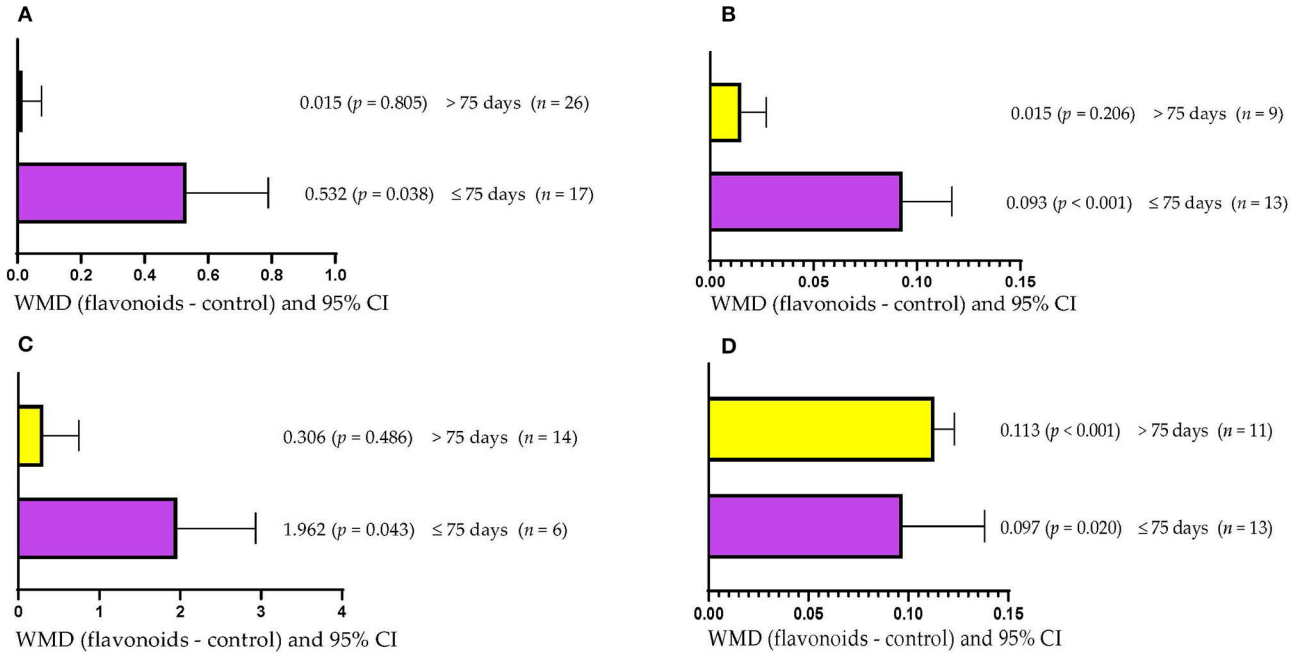

FIGURE 2
Subgroup analysis [subgroup = supplementation period (days)] of the effect of flavonoids on the diet of the cattle; WMDs, weighted mean differences between flavonoid treatments and control. **(A)** Dry matter intake (DMI), kg/d. **(B)** Average daily gain (ADG), kg/d. **(C)** Propionate, mol/100 mol. **(D)** Milk protein, g/100 g.

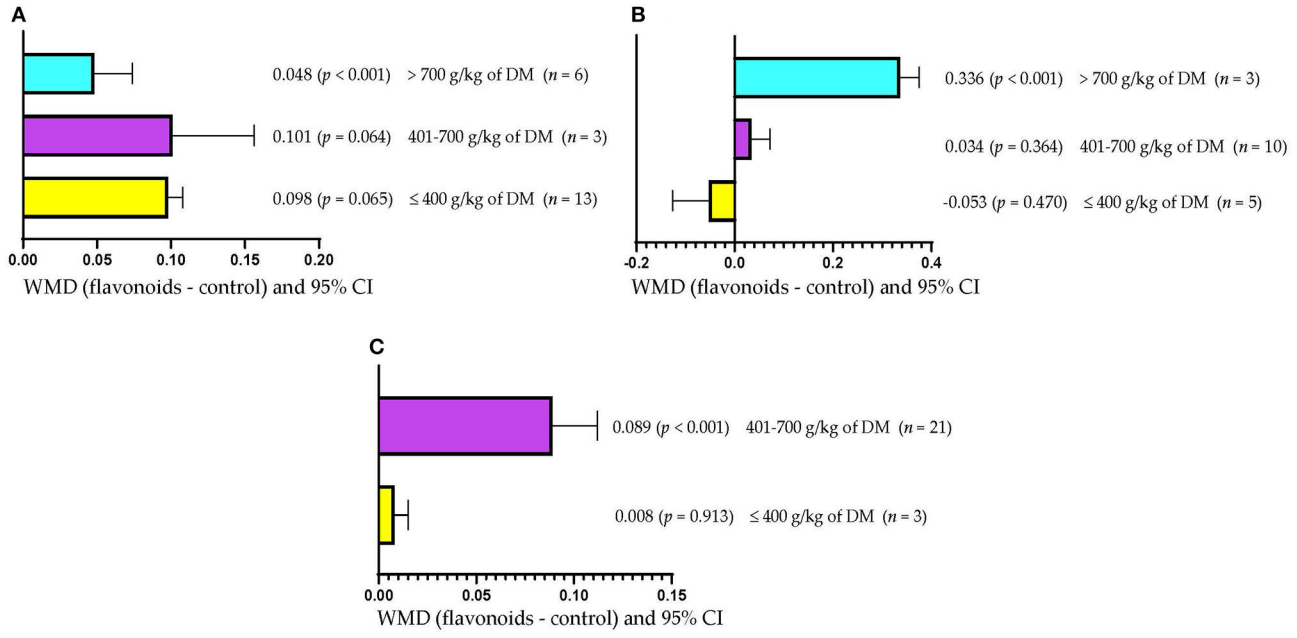

FIGURE 3
Subgroup analysis [subgroup = concentrate in diet (g/kg of DM)] of the effect of flavonoids on the diet of the cattle; WMDs, weighted mean differences between flavonoid treatments and control. **(A)** Average daily gain (ADG), kg/d. **(B)** Ruminal pH. **(C)** Milk protein, g/100 g.

observed that the dietary inclusion of plants with FLAs increased the relative abundance of rumen bacteria of the genus *Succinivibrio*, which have been positively correlated with NDFD, ADFD, and DMD in cattle (58). Furthermore, Zhao et al. (47) reported that, in growing lambs, supplementation with FLAs (anthocyanins) extracts decreases the relative abundance of ruminal microorganisms of the genus *Prevotella*, which have been negatively correlated with CPD in dairy cows (59). Thus, similar effects of FLAs supplementation in the present study partially explain the observed increases in CPD, NDFD, ADFD, DMD, and OMD.

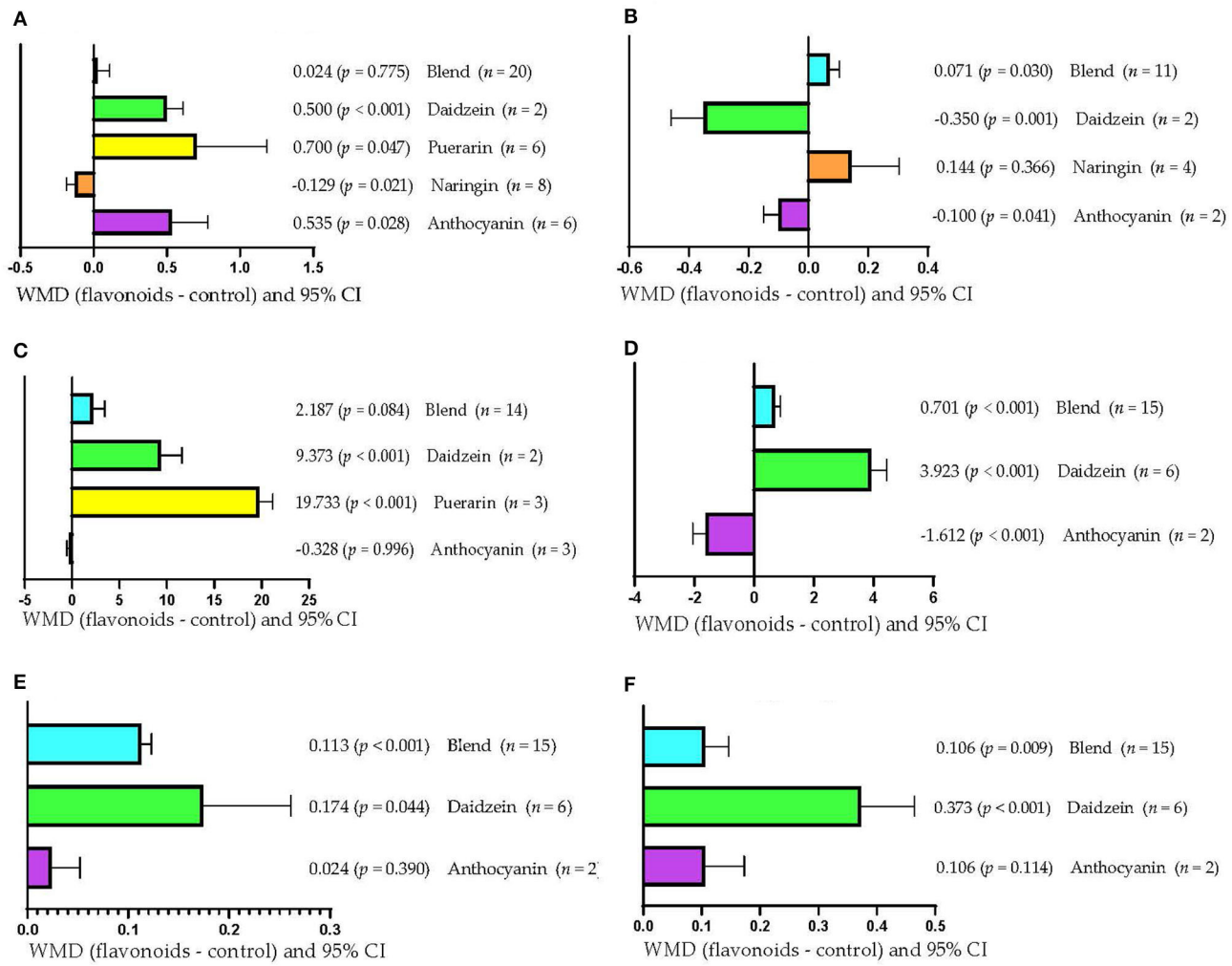

FIGURE 4
Subgroup analysis (subgroup = flavonoid type) of the effect of flavonoids on the diet of the cattle; WMDs, weighted mean differences between flavonoid treatments and control. **(A)** Dry matter intake (DMI), kg/d. **(B)** Ruminal pH. **(C)** Superoxide dismutase (SOD), U/mL. **(D)** Milk production, kg/d. **(E)** Milk protein, g/100 g. **(F)** Milk fat, g/100 g.

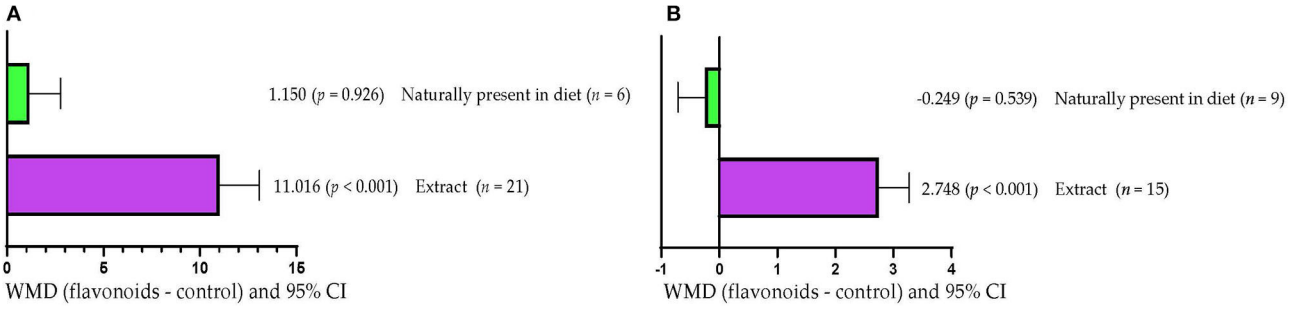

FIGURE 5
Subgroup analysis [subgroup = method of FLA's inclusion (extract or naturally present in the diet)] of the effect of flavonoids on the diet of the cattle; WMDs, weighted mean differences between flavonoid treatments and control. **(A)** Superoxide dismutase (SOD), U/mL. **(B)** Milk production, kg/d.

4.2. Growth performance and carcass traits

In the present study, supplementation with FLAs increased DMI, CPD, NDFD, ADFD, EED, OMD, and DMD, which partially explains the higher ADG and lower FCR observed. In dairy cows, Zhan et al. (56) reported that dietary supplementation with FLAs increases the relative abundance of *Tenericutes* and *Mollicutes* rumen microorganisms, which have been positively correlated with ADG

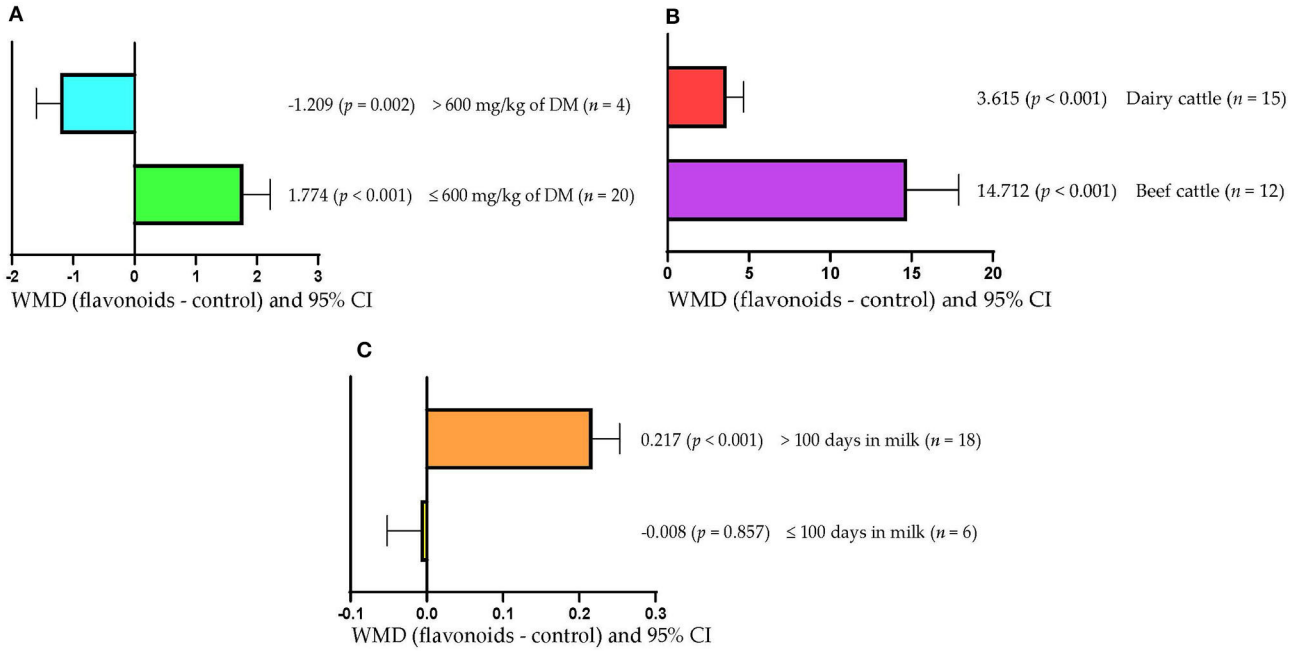

FIGURE 6
Subgroup analysis [subgroup = flavonoid dose (mg/kg of DM), type of cattle (beef cattle or dairy cattle), and days in milk] of the effect of flavonoids on the diet of the cattle; WMDs, weighted mean differences between flavonoid treatments and control. **(A)** Milk production, kg/d. **(B)** Superoxide dismutase (SOD), U/mL. **(C)** Mil fat, g/100 g.

in finishing lambs (48). In growing lambs, FLAs supplementation reduces the relative ruminal abundance of the bacterial family *Veillonellaceae* (47), which has a negative correlation with ADG in sheep (60). Du et al. (48) reported that the dietary inclusion of plants containing FLAs increases the relative abundance of the *Rikenellaceae* microbial family in rumen fluid, which has a positive and negative correlation with ADG and FCR in beef cattle, respectively (50). Dorantes-Iturbide et al. (61) reported that, in finishing lambs, supplementation with low doses (1 g/kg DM) of polyherbal additives with FLAs increases up to 23% the efficiency of utilization of dietary energy for weight gain. Furthermore, supplementation with FLAs-rich plants increases muscle protein synthesis in lambs (62). Thus, similar effects of FLAs supplementation in the present study partially explain the observed increase and decrease for ADG and FCR, respectively.

In beef cattle, supplementation with FLAs (200 and 400 mg/kg DM) increases serum levels of IGF-1 (insulin-like growth factor 1) (24), which have a positive correlation (r within 0.61 and 0.67) with ADG in ruminants (63). In addition, in the present meta-analysis, higher serum concentrations of antioxidant enzymes (SOD, CAT, and GPx) and immunoglobulins (IgA, IgG, and IgM) were observed in response to FLAs supplementation. These effects could reduce oxidative stress and improve the health status of the animals, which could result in improved animal performance. On the other hand, a subgroup analysis revealed that ADG was significantly increased when FLAs were offered with high-concentrate diets (>700 g/kg DM). In beef cattle fed high-concentrate diets, FLAs supplementation increases the duodenal flux of microbial protein (64), which may increase metabolic amino acid availability and lead to higher ADG. In addition, previous studies (51, 65) have shown that the dietary inclusion of FLAs (400 mg/kg DM) improves the health of the rumen epithelium in beef cattle fed diets high in concentrate. This effect could result in increased absorption of volatile fatty acids and lead to increased ADG since the rumen epithelium contains papillae that serve as absorptive structures (66).

It has been documented that FLAs supplementation increases the number and diameter of muscle fibers in the ruminant *Longissimus dorsi* muscle (62, 67), which may result in increased LDMA. However, in the present study, FLAs supplementation did not affect LDMA. On the other hand, Liang et al. (25) reported that, in beef cattle, supplementation with FLAs (500 mg/kg DM) increases serum leptin concentration, which has been positively correlated with BFT in beef cattle (68). Consequently, similar effects of FLAs supplementation in the present study partially explain the higher BFT observed. In addition, FLAs promote adipogenesis in the subcutaneous adipose tissue of beef cattle through changes in the expression of several genes (delta like non-canonical notch ligand, insulin like growth factor binding protein 2, wnt family member 6, enhancer binding protein beta, DNA-binding protein inhibitor ID-3, sonic hedgehog protein, and family zinc finger 1) involved in adipogenesis differentiation of subcutaneous adipocytes (69).

4.3. Antioxidant status and immune response

According to Celi (5), the excessive accumulation of reactive oxygen species (ROS) causes oxidative stress in ruminants. Shi et al. (70) mentioned that FLAs can be used as natural antioxidants for cattle since they stimulate antioxidant enzymes and eliminate ROS. In the present study, supplementation with FLAs increased the serum levels of SOD, CAT, and GPx. These results suggest that FLAs reduce the oxidative stress caused by ROS in bovines since SOD, CAT, and

GPx play an important role in converting ROS into other compounds that are less damaging to the tissues and cells of organisms (10). Furthermore, FLAs have been reported to induce activation of the transcription factor Nrf2 (71), which activates several antioxidant enzymes (10). Consequently, similar effects of FLAs consumption in the present meta-analysis partially explain the observed increases in SOD, CAT, and GPx.

In the present meta-analysis, FLAs supplementation increased TAC in beef and dairy cattle blood serum. This result suggests that the consumption of FLAs improves the total antioxidant status of bovines since TAC considers the total antioxidants present in the blood serum (5). Furthermore, Ghiselli et al. (72) mentions that serum TAC levels obtained after consuming products with antioxidants serve as indicators of the absorption and bioavailability of ingested antioxidants. Consequently, the higher TAC observed in the present study suggests that FLAs consumed by bovines may be absorbed and transferred to the bloodstream to act as blood antioxidants. Furthermore, it has been documented that TAC and ROS serum levels are negatively correlated (73). Therefore, the observed reduction of TAC in the present study suggests that FLAs supplementation decreases ROS in bovine blood serum. On the other hand, supplementation with FLAs decreased the serum concentration of MDA. This result suggests that the consumption of FLAs decreases lipid peroxidation in cattle blood because when lipid peroxidation is low, serum levels of MDA decrease (74).

According to Zhan et al. (75), immunoglobulins are a type of protein with chemical structures similar to antibodies, which participate in the regulation of immune responses. Therefore, obtaining information related to serum immunoglobulin concentrations in ruminants is important since it is an indicator of immunity against pathogenic microorganisms (76). Wolf et al. (77) mention that IgA inhibits the release of inflammatory cytokines, phagocytosis, and antibody-dependent cellular cytotoxicity. In addition, IgM and IgG act against infection since they participate in the phagocytic system and activate the complement system (24). In the present meta-analysis, FLAs supplementation increased serum IgA, IgG, and IgM concentrations, suggesting that FLAs improve immune competence in cattle. The mechanism of action of FLAs on serum immunoglobulin concentrations has not been studied in ruminants. However, FLAs have been documented to increase the expression of genes encoding IgA in mice (78). Likewise, various FLAs increase the number and activity of B1 and B2 lymphocytes (79), which secrete IgG and IgM (80, 81). Therefore, similar effects of FLAs consumption in the present meta-analysis would explain the observed increases in IgA, IgG, and IgM.

4.4. Ruminal fermentation

In the present meta-analysis, FLAs supplementation did not affect rumen pH. This result suggests that FLAs do not affect the stability of rumen functions in bovines since rumen pH is an important indicator of internal rumen homeostasis (36, 82). However, a subgroup analysis revealed that rumen pH increased when FLAs were offered in high-concentrate diets (>700 g/kg DM). Under *in vitro* conditions, FLAs decrease the concentration of lactate-producing bacteria (*Streptococcus bovis*) (83). In addition, in beef cattle fed high-concentrate diets, FLAs supplementation increases the abundance of lactate-consuming bacteria (*Megasphera elsdenii* and *Selenomonas rumiantium*) (64, 84). Similar effects of FLAs consumption in the present study could result in a lower rumen lactate concentration, which partially explains the increased rumen pH. On the other hand, the ruminal concentration of NH_3-N is the primary nitrogenous substrate used by rumen bacteria for microbial protein synthesis (85). Therefore, the absence of changes observed in the present study for the ruminal concentration of NH_3-N suggests that, in cattle, FLAs supplementation does not affect the synthesis of microbial protein in the rumen. Likewise, the absence of changes observed for NH_3-N suggests that FLAs supplementation does not affect the balance between rumen ammonia release and uptake.

Balcells et al. (64) mentioned that FLAs supplementation improves rumen fermentation in cattle. In the present study, supplementation with FLAs increased the rumen concentration of propionate with no effect on the concentration of acetate and butyrate. It has been reported that FLAs supplementation decreases the relative abundance of the microbial families *Succiniclasticum* and *Christensenellaceae* (47), which negatively correlates with the rumen concentration of propionate in sheep (67). In addition, under *in vitro* conditions, FLAs increase the relative abundance of the microbial family *Succinivibrionaceae* (86), which positively correlates with the rumen concentration of propionate in beef cattle (87). Therefore, similar effects of FLAs consumption in the present meta-analysis partially explain the increased ruminal propionate concentration. Furthermore, the observed increase in propionate suggests that FLAs increase energy availability for growth and production in cattle, since ruminal propionate is the main precursor of gluconeogenesis in ruminants (88).

FLAs supplementation decreased the ruminal concentration of total protozoa. This effect could improve the utilization efficiency of the protein and energy consumed by bovines since the reduction of rumen protozoa leads to less rumen protein degradation (89) and decreases enteric methane emissions (90).

4.5. Meat quality

The supplementation with FLAs did not affect the meat's pH or CL. These results indicate that the FLAs do not affect the quality or the water-holding capacity (WHC) of beef since the pH and CL serve as indicators to evaluate the quality (91) and WHC of the meat (92), respectively. On the other hand, lower ShF and MDA were observed in beef cattle meat in response to FLAs supplementation. These results indicate that FLAs improve beef's tenderness and oxidative stability, as ShF and MDA are indicators of meat tenderness (93) and lipid peroxidation (94), respectively. The lower ShF could be related to the reduction in IMF observed in beef from bovines supplemented with FLAs, since there is a negative correlation ($r = -0.54$) between ShF and IMF in beef (95). In beef cattle, low doses (400 mg/kg DM) of FLAs have been reported to decrease skeletal muscle fiber diameter (67), which is positively correlated with ShF in beef (96). The reduction observed in the present study for lipid peroxidation of meat partially explains the lower ShF, as oxidation decreases post-mortem calpain activity and myofibrillar proteolysis, leading to higher ShF (97).

The reduction observed for MDA in meat indicates that FLAs supplementation improves beef's quality and shelf life because when

oxidation reactions in meat increase, the quality, and shelf-life decrease (98). Previous studies (62, 99) have reported that FLAs supplementation increases the activity of SOD, CAT, and GPx in the *Longissimus dorsi* muscle of small ruminants. Therefore, similar effects of FLAs consumption in the present meta-analysis partially explain the lower MDA content observed. On the other hand, it is widely documented that meat color is a crucial factor that consumers consider when choosing fresh meat (93). L* and a* values are related to meat brightness and metmyoglobin content, respectively (93, 100). In the present meta-analysis, supplementation with FLAs did not affect L* and a* in meat, indicating that FLAs do not affect metmyoglobin formation or appearance in beef. Furthermore, the lower b* observed in response to FLAs supplementation is positive, as consumers expect to find low b* values in fresh meat (101).

FLAs supplementation did not affect the meat's protein, moisture, and ash content; however, the IMF increased. These results indicate that FLAs do not negatively affect the nutritional value of beef since the protein and ash content of the meat are related to its nutritional value (93, 102). In contrast, the higher IMF observed could be positive since IMF correlates positively with beef's tenderness and juiciness (103). In addition, some FLAs increase the adipogenesis of bovine preadipocytes (104), which participate in the deposition of IMF (105). In pigs, FLAs supplementation increases skeletal muscle PPARγ mRNA expression levels (106), which positively correlates with IMF (107). Similar effects of FLAs consumption in the present study partially explain the higher IMF observed.

4.6. Milk production and quality

It has been mentioned that increasing the utilization efficiency of ingested feed is necessary to improve milk production in ruminants (108). In the present study, FLAs supplementation increased DMD, OMD, CPD, NDFD, ADFD, and EED. These results indicate that FLAs increase the utilization efficiency of ingested feed and partially explain the higher milk production observed in response to FLAs supplementation. In addition, the higher milk production could be related to the increased ruminal propionate concentration observed since milk production in dairy cows increases curvilinearly in response to the supply of gluconeogenic precursors (109). In lactating buffaloes, it has been reported that supplementation with FLAs-rich plants increases serum somatotropin levels by up to 50% (110), which positively correlates with milk production in dairy cows (111). It has been documented that FLAs decrease the ruminal abundance of *Clostridium* microorganisms (112), which negatively correlates with milk production in bovines (113). In growing sheep, FLAs supplementation increases the relative abundance of the *Ruminococcaceae* microbial family (47), which positively correlates with milk production in dairy cows (113). Consequently, similar effects of FLAs consumption in the present meta-analysis partially explain the higher milk production observed.

Higher protein and fat content in milk was observed in response to FLAs supplementation. Under *in vitro* conditions, FLAs decrease the relative abundance of *Clostridium* (112) and *Methanobrevibacter* spp. (114), which negatively correlates with the percentage of milk protein in ruminants (115, 116). In beef cattle, FLAs supplementation increases the ruminal presence of the microbial family *Succinivibrionaceae* (57), which positively correlates with the protein content in milk from dairy cows (116). In the present study, the FLAs decreased the ruminal concentration of total protozoa, which negatively correlates with the fat content in the milk of small ruminants (117). In dairy cows, Kong et al. (59) detected that FLAs supplementation increases the relative rumen abundance of the microbial genus *Butyrivibrio*, which has a positive correlation with the fat percentage in dairy cows (116). In dairy goats, FLAs supplementation increases the expression of genes involved in milk fat synthesis, such as genes related to *de novo* fatty acid synthesis [acetyl-CoA carboxylase α (ACACA), fatty acid synthase (FASN), and stearoyl-CoA desaturase (SCD1)] and triglyceride synthesis [diacylglycerol Oacyltransferase 1 (DGAT1), diacylglycerol O-acyltransferase 2 (DGAT2), and glycerol-3-phosphate acyltransferase 1 (GPAM)] (118). Briefly, acetyl-CoA and malonyl-CoA are condensed under FASN catalysis, two carbon atoms are added to the carboxyl of the fatty acid, the ACACA gene limits the rate of the process, and the SCD1 gene catalyzes the synthesis of monounsaturated fatty acids (118). Likewise, the GPAM gene catalyzes the acyl group transfer from acyl-CoA to generate 1-acylglycerol-3-phosphate, while the GDAT1 and DGAT2 genes catalyze the formation of triglycerides with fatty acyl-CoA (118). Therefore, similar effects of FLAs consumption in the present study partially explain the increased milk fat and protein content observed. On the other hand, FLAs supplementation did not affect the lactose content in milk. This result was not expected since the FLAs increased the rumen concentration of propionate, which is the primary short-chain fatty acid required for lactose biosynthesis (108).

Tong et al. (119) mention that SCC is a widely used indicator to assess the health of the mammary gland and the quality of milk in bovines. For example, an increase in SCC is associated with intramammary infection and negatively affects raw milk quality (120). In the present meta-analysis, lower SCC was observed in response to FLAs supplementation, indicating that FLAs improve mammary gland health and milk quality in cattle. In dairy cows, FLAs supplementation decreases the presence of *Staphylococcus* bacteria in milk (121), which positively correlates with SCC in ruminant milk (122). In addition, IgA has been reported to be involved in the protection of mucous membranes, IgM is the first line of defense against infections, and IgG plays an important role in the immune response against infections (75, 121). In the present meta-analysis, IgG, IgA, and IgM serum levels increased in response to FLAs supplementation. Therefore, similar effects of FLAs consumption in the present study partially explain the lower SCC observed.

4.7. Limitations and strengths of the meta-analysis

The present meta-analysis was limited to research conducted only in beef cattle and dairy cows and may not apply to other ruminant species. In addition, high heterogeneity was detected in most of the response variables evaluated, which may represent a limitation in applying the global results obtained. However, this problem was diminished with the use of subgroup analysis, which allowed us to identify the specific conditions under which FLAs could be used successfully to improve different important parameters in beef cattle and dairy cows. Finally, this meta-analysis also establishes the steps for implementing future standardized experimental designs on the

use of FLAs as growth promoters and natural antioxidants in beef cattle and dairy cows.

5. Conclusions

The results obtained in the present meta-analysis indicate that FLAs can be used as natural growth promoters in beef cattle and, at the same time, improve feed conversion. The best result for daily weight gain is obtained with FLAs supplementation periods up to 75 days and diets high in concentrate (>700 g/kg DM). Likewise, including FLAs in bovine diets improves dry matter intake and nutrient digestibility. The best dry matter intake is obtained with periods up to 75 days and when the FLAs used are puerarin, anthocyanin, and daidzein. Furthermore, supplementation with FLAs improves total antioxidant status and immune response in cattle by reducing serum concentration of malondialdehyde and increasing serum levels of antioxidant enzymes and immunoglobulins. The best results for serum concentration of superoxide dismutase are obtained with FLAs extracts and when the FLAs used are puerarin or daidzein. At the same time, FLAs supplementation improves meat quality by reducing shear force and malondialdehyde content. In addition, FLAs improve milk production and composition. The highest milk production is obtained when FLAs extracts are used, with daidzein or mixtures of FLAs, and low doses of FLAs (≤600 mg/kg DM). The best results for milk protein content are obtained with supplementation periods longer than 75 days, diets with moderate levels of concentrate (400–700 g/kg DM), and daidzein or mixtures of FLAs. Likewise, the best fat content in milk is achieved with daidzein or mixtures of FLAs and using cows with more than 100 days in milk. Finally, FLAs supplementation improves ruminal fermentation in cattle through increased ruminal propionate concentration and reduced total rumen protozoa. The best rumen propionate concentration is obtained with supplementation periods of up to 75 days.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JO-O: conceptualization, methodology, data curation, formal analysis, investigation, visualization, writing—original draft preparation, and writing—review and editing. GD-I: methodology, data curation, formal analysis, validation, and writing—review and editing. AL-B: conceptualization, resources, writing—review and editing, supervision, project administration, resources, and funding acquisition. AC-C: methodology, investigation, data curation, and writing—review and editing. LM-R: data curation, supervision, and writing—review and editing. GM-M: software, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1134925/full#supplementary-material

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4. META-ANALYSIS OF HYDROXYCINNAMIC ACIDS INTO FINISHING LAMBS' DIET: GROWTH PERFORMANCE, ANTIOXIDANT STATUS, AND MEAT QUALITY

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Meta-analysis of hydroxycinnamic acids into finishing lambs' diet: Growth performance, antioxidant status, and meat quality

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ABSTRACT

The objective of this study was to evaluate the effects of dietary supplementation with hydroxycinnamic acids (HAs) on productive performance, antioxidant status in blood serum, and meat quality of finishing lambs using meta-analytic methods. Based on the selection criteria, 14 peer-reviewed publications were included in the final database. The weighted mean difference (WMD) between the experimental treatments (diets added with HAs) and the control treatments (diets without HAs) was evaluated using the random effects meta-analysis model. HAs supplementation had positive effects on daily weight gain (WMD = 13.46 g/d; $P < 0.001$), dry matter intake (WMD = 19.54 g/d; $P = 0.040$), and feed conversion rate (WMD = −0.249 kg/kg; $P = 0.001$). In addition, HAs supplementation decreased serum levels of malondialdehyde (WMD = −0.64 nmol/mL; $P < 0.001$) and increased ($P < 0.001$) serum levels of glutathione peroxidase (WMD = 1.25 ng/mL), superoxide dismutase (WMD = 41.34 pg/mL), catalase (WMD = 149.71 pg/mL) and total antioxidant capacity (WMD = 0.973 U/mL). In meat, HAs supplementation decreased the MDA content (WMD = 0.427 mg/kg; $P = 0.043$); however, it did not affect the pH, shear force, or meat color ($P > 0.05$). In conclusion, HAs supplementation improves the productive performance and antioxidant status of finishing lambs without affecting meat quality.

1. Introduction

In recent years, interest in the use of intensive lamb fattening systems has increased due to the growing demand for lamb meat at favorable prices in various parts of the world (Muñoz-Osorio et al., 2017). However, the high metabolic rate associated with the development and growth processes in fattening lambs increases the production of reactive oxygen species, which leads to oxidative stress and cell damage in the animal (Nussey et al., 2009). It has been reported that these oxidative stress conditions negatively affect the health status, animal performance, and meat quality of finishing lambs (Valadez-García et al., 2021a). Therefore, in recent years, interest in improving the antioxidant status of finishing lambs has increased, mainly through dietary supplementation with natural products (Liu et al., 2018a; Dorantes-Iturbide et al., 2022). Among these are hydroxycinnamic acids (HAs), which according to Peña Torres et al. (2019), are a group of phenolic compounds present in a wide variety of plant species that possess antioxidant, immunomodulatory, and anti-inflammatory properties.

The effects of dietary supplementation with HAs have been investigated mainly in broilers and finishing pigs (Liu et al., 2023; Wang et al., 2022); however, the information related to the effects of HAs in finishing lambs and other ruminants is still limited. In ruminants, the growing interest in the use of HAs is because, compared to other phenolic compounds, HAs can be absorbed and deposited in animal tissues more easily (Peña-Torres et al., 2019). Furthermore, previous studies (Peña-Torres et al., 2022; Wang et al., 2022) have reported that HAs exert anabolic effects at the muscle level, which has not yet been demonstrated with other phenolic compounds. The most common HAs are ferulic, chlorogenic, p-coumaric, and caffeic (Peña-Torres et al., 2019). However, in ruminants, ferulic acid has been used more

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frequently (Valadez-García et al., 2021b), which is present mainly in the cuticle of cereals such as corn (Peña-Torres et al., 2021).

Previous studies have evaluated the effects of some HAs on productive performance (Nicolás-López et al., 2023; Peña-Torres et al., 2022), antioxidant status in blood serum (Liu et al., 2018a; Wang et al., 2019a), and meat quality of finishing lambs (Tanóri-Lozano et al., 2022; Valadez-García et al., 2021a); however, some of these studies have reported inconsistent and contrasting results. For example, Wang et al. (2019a) observed better productive performance in lambs supplemented with HAs (80 mg/kg DM). Likewise, Peña-Torres et al. (2022) reported that supplementation with (300 mg/kg DM) HAs could effectively replace zilpaterol hydrochloride (one of the main β-adrenergic agonists used as growth promoters) without negatively affecting growth performance in lambs. In contrast, Tánori-Lozano et al. (2022) and Nicolás-López et al. (2023) did not detect significant effects of dietary supplementation with HAs (250 or 300 mg/kg DM) on animal performance and meat quality of finishing lambs. According to Valadez-García et al. (2021b), the dosage and bioavailability of the product containing HAs, the supplementation period, and the animal's physiological state are factors related to the variability observed among studies.

Some narrative review articles (Peña-Torres et al., 2019; Valadez-García et al., 2021b) have concluded that the inclusion of HAs in livestock diets can be a promising strategy to improve productive performance and meat quality. However, these reviews did not use meta-analytic methods and did not focus only on finishing lambs. It has been mentioned that narrative reviews lack a methodological focus and can lead to biased conclusions (Sauvant et al., 2008). In contrast, meta-analysis (MA) is a statistical tool that allows previously published data from various studies to be collected, combined, and statistically analyzed, thereby increasing statistical power (Ogbuewu et al., 2020). Additionally, the sources of heterogeneity between studies can be identified using MA (Higgins et al., 2003). According to Orzuna-Orzuna et al. (2023), there is a growing interest in the application of MA in research on the use of plant-derived products as dietary additives for ruminants. The hypothesis of this meta-analysis states that the inclusion of HAs in diets for finishing lambs will positively impact productive performance, and antioxidant status, without affecting meat quality. This meta-analysis aimed to evaluate the effects of dietary supplementation with hydroxycinnamic acids on productive performance, antioxidant status in blood serum, and meat quality of finishing lambs.

2. Materials and methods

2.1. Literature Search

The studies' identification, selection, choice, and inclusion were carried out following the PRISMA guidelines (Moher et al., 2009), as shown in Fig. 1. A systematic search was carried out in the Scopus, ScienceDirect, Google Scholar, and PubMed databases, where the results were restricted to studies published between January 2010 and January 2023. The keywords used in all the databases were the following: hydroxycinnamic acids, finishing lambs, growth performance,

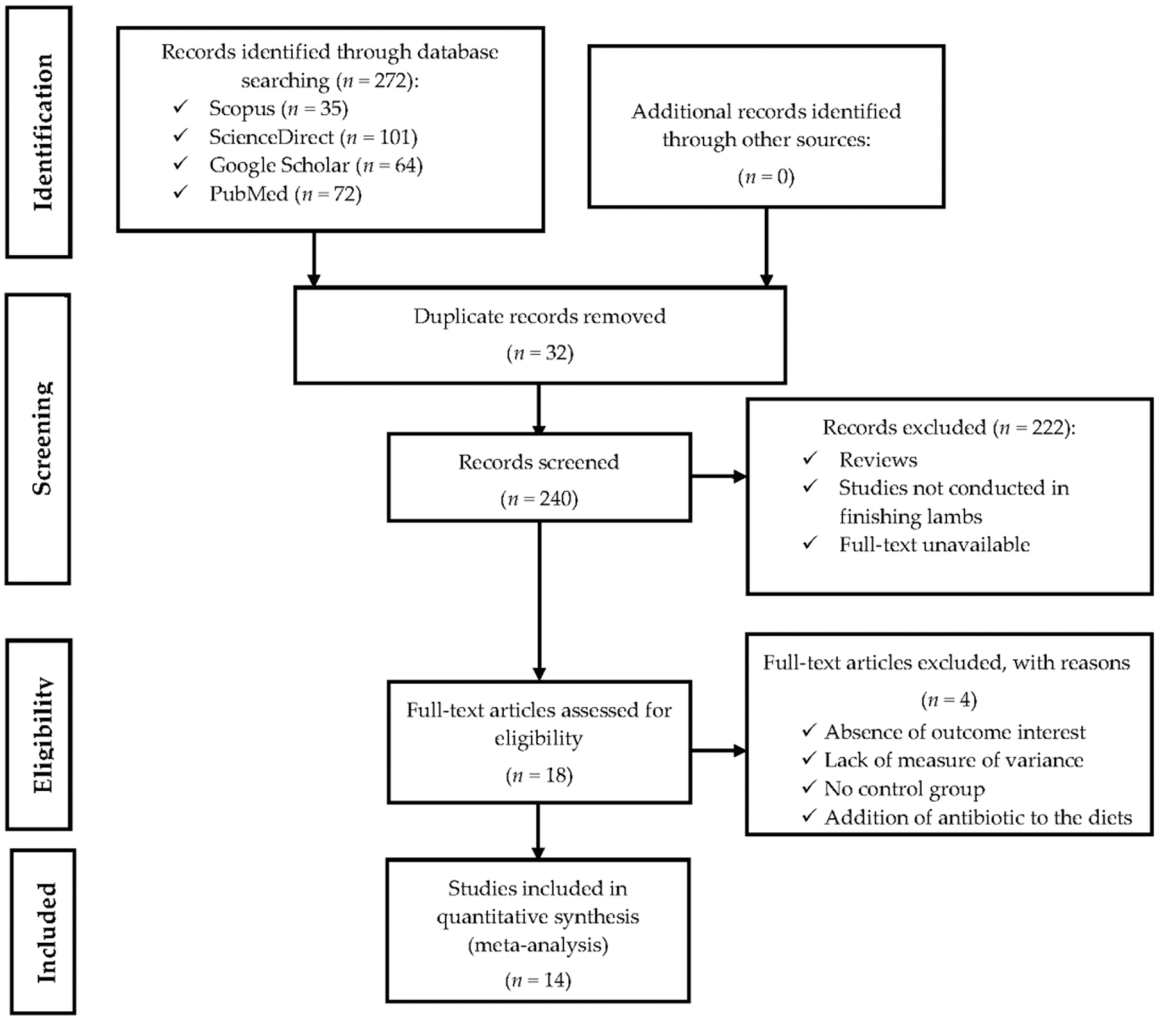

Fig. 1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

antioxidant status, meat quality, and the main representatives of the HAs (Peña-Torres et al., 2019), such as ferulic acid, chlorogenic acid, caffeic acid, and p-coumaric acid.

2.2. Inclusion and exclusion criteria

In total, 272 scientific publications were identified, from which all duplicate publications were excluded. A two-step selection process was then applied to the remaining publications, as reported in previous meta-analyses (Orzuna-Orzuna et al., 2022; Dorantes-Iturbide et al., 2022). During the first step, based on the titles and abstracts, review articles, in vitro experiments, and publications that did not use finishing lambs, did not measure any of the variables of interest, or used experimentally infected lambs, were excluded. During the second step, it was determined whether the remaining publications met the previously defined inclusion criteria to be considered in the final database. The inclusion criteria used were similar to those reported in previous meta-analyses (Dorantes-Iturbide et al., 2022; Orzuna-Orzuna et al., 2021): (1) studies that used finishing lambs; (2) data on productive performance, and antioxidant status in blood serum or meat quality; (3) studies using similar experimental and control diets, except for the presence of HAs in the diets; (4) studies that reported the doses (mg/kg DM) of HAs used or had the information necessary to estimate it; (5) studies published in peer-reviewed scientific journals and written in English; and (6) studies that reported the means of the control and HAs-supplemented treatments, the standard error or standard deviation, and the number of replicates.

2.3. Data extraction

Based on the inclusion criteria, only 14 peer-reviewed scientific articles were included in the final database (Table 1). Subsequently, only response variables reported in at least three independent articles were extracted (Orzuna-Orzuna et al., 2023; Sierra-Galicia et al., 2023). Data extracted from each study included average daily gain, dry matter intake, feed conversion ratio, malondialdehyde and antioxidant enzymes in blood serum, and meat physicochemical characteristics. From these response variables, the means of the experimental treatments (supplemented with HAs) and control (without HAs), the standard deviations (SD), and the number of replicates were obtained. When the SD was not reported, it was estimated with the standard errors of the treatment means (SEM) using the equation $SD = SEM \times \sqrt{n}$, where n = the number of replicates (Higgins & Thomas, 2019).

2.4. Calculations and statistical analysis

All statistical analyzes were performed in the R statistical software (version 4.1.2, R Core Team, Vienna, Austria), particularly with the "metafor" package (Viechtbauer, 2010). The effects of HAs as dietary additives for finishing lambs were evaluated using the weighted mean differences (WMD) between HAs-supplemented treatments (HAs diets) and control treatments (no HAs diets). Treatment means were weighted by the inverse of the variance of the random effects model, according to the methods proposed by DerSimonian & Laird (1986).

2.5. Heterogeneity and publication bias

Treatment effect heterogeneity (i.e., between-study variability) was assessed using the chi-square (Q) test, which due to its relatively low power, was declared significant when $P \leq 0.10$ (Egger et al., 2001). Additionally, the I^2 statistic was used to quantify the percentage of variation due to heterogeneity (Higgins et al., 2003). In this test, negative I^2 values were assigned as zero. An I^2 value of less than 25% indicates low heterogeneity, values between 25% and 50% indicate moderate heterogeneity, and I^2 values greater than 50% denote high heterogeneity (Higgins et al., 2003).

Table 1
Description of the studies included in the meta-analysis database.

Reference	HAs type	Duration, d	Dose (mg/kg DM)	Method of supplementation
Jordán et al. (2020)	Blend	80	100	Naturally present in diet
Lobo et al. (2021)	Blend ($n = 3$)	50	322, 644, 1288	Extract ($n = 3$)
Liu et al. (2018a)	Chlorogenic ($n = 2$)	56	821 and 4105	Extract ($n = 2$)
Liu et al. (2018b)	Chlorogenic ($n = 2$)	49	4105 and 8210	Extract ($n = 2$)
Macías-Cruz et al. (2014)	Ferulic	34	265	Extract
Nicolás-López et al. (2023)	Ferulic	40	250	Extract
Pena-Bermudez et al. (2020)	Blend ($n = 3$)	53	322, 644, 1288	Extract ($n = 3$)
Pena-Bermudez et al. (2022)	Blend ($n = 3$)	53	322, 644, 1288	Extract ($n = 3$)
Peña-Torres et al. (2022)	Ferulic ($n = 2$)	32	300 and 600	Extract ($n = 2$)
Salinas-Rios et al. (2015)	Blend ($n = 2$)	56	5600 and 11,200	Naturally present in diet ($n = 2$)
Tanóri-Lozano et al. (2022)	Ferulic	40	300	Extract
Valadez-García et al. (2021a)	Ferulic	40	250	Extract
Wang et al. (2019a)	Ferulic ($n = 4$)	56	50, 100, 200 and 400	Extract ($n = 4$)
Wang et al. (2019b)	Ferulic ($n = 3$)	30	80, 400 y 2000	Extract ($n = 3$)

HAs: Hydroxycinnamic acids; d: days; *n*: represents the number of comparisons between hydroxycinnamic acids treatment and control treatment.

Publication bias was assessed using two methods: (1) Egger's regression asymmetry test (Egger et al., 1997) and (2) Begg's adjusted rank correlation (Begg & Mazumdar, 1994). For both tests, a significance level of $P \leq 0.05$ was used.

2.6. Meta-regression and subgroup analysis

The possible sources of heterogeneity between studies were determined using meta-regression analysis. For this, the method of moments of Der-Simonian and Laird (1986) was used since it is well established to estimate the variance between studies. To apply meta-regression to a response variable, it had to have the following characteristics: (1) $P \leq 0.10$ for the Q test or $I^2 > 50\%$ (Egger et al., 2001); (2) $P > 0.05$ for the Egger and Begg tests (Begg & Mazumdar, 1994; Egger et al., 1997); and (3) to be reported in ten or more individual studies (Littell et al., 2008). HAs doses and experimental period were used as continuous covariates, while HAs type and supplementation method were used as categorical covariates. When the meta-regression was significant ($P \leq 0.05$) for the continuous covariates, the WMD was analyzed by subgroup analysis (Dorantes-Iturbide et al., 2022; Orzuna-Orzuna et al., 2023), as follows: the dose of HAs (≤500 and >500 mg/kg DM) and experimental period (≤50 and >50 days). Likewise, when the categorical covariates were significant ($P \leq 0.05$), the WMD was evaluated using the following subgroups: type of HAs (ferulic acid, chlorogenic acid, and mixtures) and method of supplementation (extract or naturally present in the diet).

3. Results

3.1. Growth performance

Table 2 shows that dietary supplementation with HAs increased ($P < 0.05$) dry matter intake (DMI) and average daily gain (ADG). In contrast, a lower feed conversion ratio (FCR) was observed in response to HAs supplementation ($P = 0.001$).

3.2. Antioxidant status

Table 3 shows that dietary supplementation with HAs decreased the serum concentration of malondialdehyde (MDA; $P < 0.001$). However, total antioxidant capacity (TAC) and serum levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) increased in response to dietary supplementation with HAs.

3.3. Meat quality

Dietary supplementation with HAs did not affect ($P > 0.05$) the pH, shear force (ShF), lightness (L*), redness (a*), and yellowness (b*) of the meat (Table 4). However, lower MDA content was observed in meat in response to dietary supplementation with HAs ($P = 0.043$).

3.4. Meta-regression and publication bias

Tables 2–4 show that Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant ($P > 0.05$) for any tested variables, indicating no publication bias.

On the other hand, Tables 2–4 show significant heterogeneity ($P \leq 0.10$) for DMI, ADG, FCR, MDA, TAC, SOD, GPx, CAT, meat pH, and ShF. However, meta-regression should only be applied to response variables reported in at least ten studies to obtain reliable results (Littell et al., 2008). Therefore, only meta-regression was performed on DMI, ADG, and FCR. Table 5 shows no significant relationship ($P > 0.05$) between the covariates used and DMI, ADG, or FCR.

4. Discussion

4.1. Growth performance

In general, it has been mentioned that HAs can improve the quality and palatability of livestock diets due to their antioxidant properties (Peña-Torres et al., 2019). In addition, some HAs (chlorogenic acid) act as bitter taste-blocking agents on the membrane surface of taste receptors (Andrews et al., 2021). This effect could mask a possible bad taste of the diet and result in a higher DMI. In goats, the dietary inclusion of by-products containing HAs increases the rumen presence of fungi (Maxiselly et al., 2022), which, according to Akin & Borneman (1990), improve feed fiber degradation. Higher fiber degradation in the rumen could increase the rate of passage of feed particles in the digestive tract and result in higher DMI.

In the present meta-analysis, HAs supplementation increased the DMI, which indicates a higher nutrient intake that would partially explain the higher ADG observed. In heifers, HAs supplementation increases serum levels of growth hormone (Gorewit, 1983), which is positively related to body weight gain in lambs (Rosales et al., 2019). Pena-Torres et al. (2022) reported that HAs supplementation improved the growth rate in lambs through increased gene expression of the β2 adrenergic receptor and insulin-like growth factor type 1 in skeletal muscle. In pigs, dietary supplementation with low doses (400 mg/kg DM) of HAs decreases muscle protein degradation and increases muscle protein biosynthesis in skeletal muscle through the Akt-mTOR-S6K1 4EBP1 signaling pathway (Wang et al., 2022). Similar effects of dietary supplementation with HAs in the present meta-analysis could be related to the positive effects observed in ADG.

According to Ponnampalam et al. (2022), high-grain diets used to feed finishing lambs increase animal oxidative stress. In small ruminants, oxidative stress can cause damage to the rumen and intestinal epithelium (Ma et al., 2021; Sejian et al., 2017), which decreases the absorption of volatile fatty acids and nutrients, and increases the penetration of endotoxins and pathogens. In non-ruminants, dietary supplementation with HAs (1000 mg/kg DM) improves the integrity of the digestive tract epithelium through a greater expression of the nuclear factor-erythroid 2 related factor 2 (Nrf2), which participates in the production of endogenous antioxidant defenses (Ma, 2013). Therefore, similar effects of HAs consumption in ruminants could increase the absorption of volatile fatty acids and nutrients, which would explain the positive effects observed in FCR in the present study.

4.2. Antioxidant status

In small ruminants, the overproduction of reactive oxygen species (ROS) leads to oxidative stress (Celi, 2010), which causes damage to various tissues and organs (Chen et al., 2022). The present meta-analysis showed higher serum concentrations of SOD, GPx, and CAT in response to HAs supplementation. These effects indicate that, in finishing lambs, the dietary inclusion of HAs decreases the oxidative stress caused by ROS since the simultaneous increase in SOD, GPx, and CAT protects cell membranes from oxidative damage caused by ROS (Celi, 2010). Furthermore, according to Mancuso and Santangelo (2014), HAs supplementation increases the expression of SOD, GPx, and CAT by activating the Nrf2 transcription factor/antioxidant-sensitive element pathway. Therefore, similar effects of HAs consumption in the present study partially explain the observed increase in SOD, GPx, and CAT.

In the present meta-analysis, HAs supplementation increased serum TAC levels, indicating that HAs improved the overall antioxidant status of finishing lambs. HAs are phenols with antioxidant properties, which are rapidly bioavailable in ruminants because, after being ingested, they can be easily absorbed and transported to the bloodstream (Valadez-García et al., 2021b). This effect could partially explain the higher TAC observed in the present study since the serum level of TAC serves as an indicator of the absorption and bioavailability of the antioxidant products consumed (Ghiselli et al., 2000).

The dietary inclusion of HAs decreased the serum levels of MDA. This effect indicates that HAs can be used effectively to reduce lipid

Table 2
Growth performance of finishing lambs supplemented with hydroxycinnamic acids.

Item	N (NC)				Heterogeneity		Egger test[1]	Begg test[2]
		Control means (SD)	WMD (95% CI)	*P*-value	*P*-value	I^2 (%)	*P*-value	*P*-value
DMI, g/d	10 (19)	1185.1 (127.0)	19.54 (0.86; 38.22)	0.040	< 0.001	96.01	0.808	0.339
ADG, g/d	10 (19)	195.1 (47.1)	13.46 (6.89; 20.03)	< 0.001	< 0.001	83.19	0.075	0.357
FCR, kg/kg	10 (19)	5.96 (1.34)	-0.249 (−0.400; −0.098)	0.001	< 0.001	79.88	0.127	0.832

N: represents the number of studies; NC: represents the number of comparisons between hydroxycinnamic acids treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with hydroxycinnamic acids; CI: confidence interval of WMD; P-value to χ2 (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; [2]: Begg's adjusted rank correlation; DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio.

Table 3
Antioxidant status in blood serum of finishing lambs supplemented with hydroxycinnamic acids.

Item	N (NC)				Heterogeneity		Egger test[1]	Begg test[2]
		Control means (SD)	WMD (95% CI)	*P*-value	*P*-value	I^2 (%)	*P*-value	*P*-value
MDA, nmol/mL	5 (10)	2.75 (1.0)	-0.64 (−0.94; −0.33)	< 0.001	< 0.001	96.88	0.982	0.408
TAC, U/mL	6 (14)	4.86 (1.93)	0.973 (0.529; 1.426)	< 0.001	< 0.001	91.44	0.065	0.537
SOD, pg/mL	5 (12)	310.1 (141.7)	41.34 (23.55; 59.31)	< 0.001	< 0.001	90.37	0.104	0.177
GPx, ng/mL	5 (12)	2.65 (1.45)	1.25 (0.85; 1.64)	< 0.001	< 0.001	96.53	0.071	0.136
CAT, pg/mL	3 (8)	391.9 (136.1)	149.71 (39.2; 260.4)	0.008	< 0.001	97.21	0.269	0.216

N: represents the number of studies; NC: represents the number of comparisons between hydroxycinnamic acids treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with hydroxycinnamic acids; CI: confidence interval of WMD; P-value to $\chi 2$ (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; [2]: Begg's adjusted rank correlation; MDA: malondialdehyde; TAC: total antioxidant capacity; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase.

Table 4
Meat quality of finishing lambs supplemented with hydroxycinnamic acids.

Item	N (NC)				Heterogeneity		Egger test[1]	Begg test[2]
		Control mean (SD)	WMD (95% CI)	*P*-value	*P*-value	I^2 (%)	*P*-value	*P*-value
Meat pH	6 (13)	5.60 (0.25)	-0.032 (−0.072; 0.008)	0.117	< 0.001	75.63	0.203	0.452
Shear force, N	4 (7)	32.18 (3.31)	-1.934 (−4.434; 0.548)	0.126	< 0.001	98.18	0.191	0.427
MDA, mg/kg	3 (5)	2.23 (1.19)	-0.427 (0.014; 0.841)	0.043	< 0.001	89.46	0.170	0.083
Lightness (L*)	6 (13)	40.56 (2.82)	0.173 (−0.374; 0.720)	0.536	0.495	0.0	0.208	0.134
Redness (a*)	6 (13)	15.99 (1.86)	0.161 (−0.102; 0.423)	0.231	0.997	0.0	0.850	0.708
Yellowness (b*)	6 (13)	9.54 (2.77)	0.197 (−0.028; 0.422)	0.086	0.310	13.71	0.078	0.079

N: represents the number of studies; NC: represents the number of comparisons between hydroxycinnamic acids treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with hydroxycinnamic acids; CI: confidence interval of WMD; P-value to $\chi 2$ (Q) test of heterogeneity; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; [2]: Begg's adjusted rank correlation; MDA: malondialdehyde.

Table 5
Meta-regression comparing the associations between covariates and measured outcomes.

Parameter	Covariates	QM	Df	*p*-Value	R^2 (%)
Dry matter intake (DMI)	HAs dose	1.751	1	0.186	0.40
	Supplementation period	2.201	1	0.138	8.26
	HAs type	2.708	2	0.258	0.0
	Method of inclusion	0.345	1	0.557	0.0
Average daily gain (ADG)	HAs dose	0.125	1	0.991	0.0
	Supplementation period	1.582	1	0.208	0.0
	HAs type	0.012	2	0.994	0.0
	Method of inclusion	0.004	1	0.949	0.0
Feed conversion ratio (FCR)	HAs dose	0.005	1	0.946	0.0
	Supplementation period	0.041	1	0.840	0.0
	HAs type	0.940	2	0.625	0.0
	Method of inclusion	0.165	1	0.685	0.0

QM: coefficient of moderators; QM is considered significant at $P \leq 0.05$; df: degree of freedom; R2: the amount of heterogeneity accounted for; HA: hydroxycinnamic acid.

peroxidation in the blood serum of finishing lambs since low serum levels of MDA indicate low lipid peroxidation (Nielsen et al., 1997).

4.3. Meat quality

Evaluating the meat quality of finishing lambs is important because it influences the economic benefits obtained in sheep production systems. In the present meta-analysis, HAs supplementation did not affect the pH of the meat, which also had values within the range considered adequate to obtain an acceptable flavor and color by consumers (Mariezcurrena-Berasain et al., 2022). Similarly, the ShF values of meat were not affected by HAs and were within the normal range reported for sheep meat (Corazzin et al., 2019). In goats, supplementation with products containing HAs does not affect the collagen content of meat (Cimmino et al., 2018), which is related to ShF (Orzuna-Orzuna et al., 2022). Therefore, a similar effect of HAs consumption in finishing lambs partially explains the absence of changes observed in ShF in the present study.

In meat, the MDA content serves as an indicator of lipid peroxidation, which, according to Falowo et al. (2014), is the main non-microbial cause that decreases meat's quality and shelf life. Therefore, the lower MDA content observed in the present study indicates that HAs can be used as a dietary additive to improve the quality and shelf life of finishing lamb meat. Furthermore, it has been reported that dietary supplementation with mixtures of HAs increases the activity of SOD, GPx, and CAT in sheep skeletal muscle (Pena-Bermudez et al., 2020), which would explain the reduction in MDA observed in the present study.

Meat color is the most important sensory attribute since it directly influences consumer purchasing decisions (Pena-Bermudez et al., 2022). In the present study, the meat color parameters (L*, a*, and b*) were not affected by HAs and were within the normal ranges reported for sheep meat (Corazzin et al., 2019). These results indicate that HAs can be used in finishing lambs without impairing the appearance of the meat. Corazzin et al. (2019) mention that the amount and chemical state of muscle myoglobin, pH, and intramuscular fat are the main factors that influence meat color. In the present study, HAs neither affected the pH of the meat. Likewise, Cimmino et al. (2018) reported that supplementation with products containing HAs did not affect intramuscular fat content nor the amount and chemical status of muscle myoglobin in goats. Similar effects of the consumption of HAs in the present study partially explain the absence of changes observed in L* , a* , and b* .

5. Conclusions

The results of the present meta-analysis indicate that the dietary inclusion of HAs can be used as a nutritional strategy to stimulate feed intake, improve growth rate, and reduce feed conversion ratio in finishing lambs. In addition, HAs supplementation improves the

antioxidant status in the blood serum and the oxidative stability of the meat of finishing lambs. However, more studies are needed to evaluate and confirm the effects of the different individual HAs since, in the present study, the HAs were evaluated together without giving a relative weight to each of them.

Ethics approval

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CRediT authorship contribution statement

José Felipe Orzuna-Orzuna: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Alejandro Lara-Bueno:** Resources, Writing – review & editing, Supervision, Project administration, Resources, Funding acquisition. **Germán David Mendoza-Martínez:** Data curation, Supervision, Writing – review & editing. **Luis Alberto Miranda-Romero:** Software, Supervision, Writing – review & editing.

Conflict of interest

The authors declare that they have no competing interests.

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5. META-ANALYSIS OF DIETARY SUPPLEMENTATION WITH SEAWEED IN DAIRY COWS: MILK YIELD AND COMPOSITION, NUTRIENT DIGESTIBILITY, RUMENFERMENTATION, AND ENTERIC METHANE EMISSIONS

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Meta-Analysis of Dietary Supplementation with Seaweed in Dairy Cows: Milk Yield and Composition, Nutrient Digestibility, Rumen Fermentation, and Enteric Methane Emissions

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Abstract: This study used a meta-analytic approach to evaluate the effects of dietary supplementation with seaweed on milk yield, milk composition, nutrient digestibility, ruminal fermentation, and enteric methane (CH_4) emissions of dairy cows. Data used in statistical analyses were obtained from 23 peer-reviewed scientific articles. Effect size was assessed using weighted mean differences (WMD) between seaweed-supplemented and control treatments. Dietary supplementation with seaweed decreased ($p < 0.05$) dry matter intake, milk protein content, milk urea nitrogen, and somatic cell count. In contrast, milk fat content, milk lactose content, and milk iodine increased ($p < 0.05$) in response to dietary supplementation with seaweed. Dietary supplementation with seaweed did not affect ($p > 0.05$) nutrient digestibility, total volatile fatty acids, acetate, and propionate. Dietary supplementation with seaweeds increased ($p < 0.05$) ruminal pH and ruminal concentration of butyrate and valerate. In contrast, lower ($p < 0.05$) ruminal ammonia nitrogen concentration, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity were observed in response to dietary supplementation with seaweeds. In conclusion, dietary supplementation with seaweed modifies milk composition, improves ruminal fermentation, and decreases enteric methane emissions without negatively affecting milk yield or feed efficiency.

Keywords: macroalgae; *Asparagopsis taxiformis*; *Ascophyllum nodosum*; milk quality; meta-regression

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1. Introduction

Dairy cow production systems are important, as cow's milk is the most widely produced and consumed milk globally due to its high content of high-quality protein, bioavailable amino acids, vitamins, and minerals essential for humans [1]. However, enteric methane (CH_4) is produced in the rumen of dairy cows due to microbial fermentation of ingested feed [2]. Bačėninaitė et al. [3] indicate that CH_4 emitted by dairy cows contributes approximately 35–55% of total greenhouse gas (GHG) emissions from dairy farms worldwide. Consequently, in recent years, the interest of governments and researchers in finding solutions to reduce CH_4 emissions has increased dramatically [4]. To date, a large number of dietary additives have been evaluated for mitigating enteric CH4 in

dairy cows, such as essential oils, condensed tannins, saponins, flavonoids, ionophores, 3-nitrooxypropanol, and seaweed [3–5]. Among these additives, seaweed is one of the most effective in mitigating CH_4 emissions in dairy cows without negatively affecting milk yield or feed efficiency [6,7].

Seaweeds (also known as macroalgae) are photosynthetic multicellular eukaryotic organisms that can have different sizes and morphologies [8]. According to some authors [6,9], seaweeds grow on the ocean bottom and are generally classified into three types: red seaweeds (Rhodophyta), brown seaweeds (Ochrophyta), and green seaweeds (Chlorophyta). Although their chemical profile may vary by seaweed type, seaweeds generally contain a wide variety of bioactive compounds, such as polysaccharides, phlorotannins, and halogenated alkanes (mainly bromoform) [6]. According to Glasson et al. [10], halogenated alkanes from seaweed can inhibit CH_4 emission in ruminants by blocking the activity of coenzyme M methyltransferase and the enzyme methyl-coenzyme M reductase (MCR), which are required in ruminal CH_4 formation [7]. On the other hand, seaweed polysaccharides have antimicrobial, antioxidant, antiviral, and anti-inflammatory properties in livestock [6]. Furthermore, seaweed phlorotannins improve ruminal protein metabolism and have antioxidant, anthelmintic, and anti-methanogenic activity in ruminants [7].

Specifically in dairy cows, a growing number of experiments have evaluated the effects of dietary seaweed supplementation on milk yield [11,12], milk composition [2,13], ruminal fermentation and nutrient digestibility [14,15], and enteric CH_4 emissions [16,17]. However, no fully reliable conclusions have been obtained regarding the effect of seaweed treatment on dairy cows because the results reported across studies have been contradictory. For example, some studies reported an increase in milk yield [13], improved milk composition [18], and a decrease in enteric CH_4 emissions [14] from dairy cows supplemented with seaweed. In contrast, other authors [2,17,19] show that seaweed negatively affects milk yield and milk composition of dairy cows. Likewise, other studies [11,15] reported that seaweed did not affect milk yield, milk composition, ruminal fermentation, nutrient digestibility, and CH_4 methane emissions of dairy cows.

Previously reported results from two meta-analyses [20,21] suggest that seaweeds can mitigate enteric CH_4 emissions in ruminants without negatively affecting productive performance. However, these two meta-analyses [20,21] only included a small number of studies (between six and 11) of dairy cows in their database. Furthermore, the meta-analysis by Lean et al. [21] only evaluated five response variables related to dairy cows. Likewise, in the meta-analysis by Sofyan et al. [20], the results for milk yield, milk composition, rumen parameters, and enteric CH_4 emissions were obtained by combining data from small ruminants, dairy cows, and beef cattle (in some response variables such as CH_4). According to Ioannidis [22], using databases with few studies or combining data from different animal species (e.g., small ruminants, dairy cows, and beef cattle) decreases the likelihood of obtaining robust and scientifically reliable results. On the other hand, Kebreab et al. [23] mention that periodically updating meta-analyses on a specific topic using larger databases can help obtain conclusive findings. In response to the increasing number of scientific articles published in recent years on the use of seaweed as a dietary additive for dairy cows, the current study aimed to evaluate the effects of dietary supplementation with seaweed on milk yield, milk composition, nutrient digestibility, ruminal fermentation, and enteric methane emissions of dairy cows through meta-analytical statistical procedures. The hypothesis of the present meta-analysis states that the inclusion of seaweed in diets for dairy cows will positively impact milk yield, milk composition, nutrient digestibility, and ruminal fermentation and will decrease methane emissions.

2. Materials and Methods

2.1. Literature Search

The research question was formulated using the PICO strategy proposed by Nishikawa-Pacher [24], in which P is the population, I is the intervention, C is the comparison, and O is the outcome. In the current study, the population was dairy cows, the intervention

was the inclusion of seaweed in the diet, the comparison was between diets supplemented with seaweed and diets without seaweed, and the outcomes were the means of treatments obtained in milk yield and composition, nutrient digestibility, ruminal fermentation, and enteric CH_4 emissions. Subsequently, the scientific documents that evaluated the effects of dietary supplementation with seaweed in dairy cows were identified, selected, chosen, and included in the database following the guidelines of the PRISMA protocol [25], as shown in Figure 1. The identification of literature was carried out through systematic searches using the search engines Scopus, ScienceDirect, PubMed, and Web of Science. The keywords used in the searches were the following: (1) seaweed, (2) macroalgae, (3) dairy cows, (4) dairy cattle, (5) milk yield, (6) milk production, (7) milk composition, (8) milk quality, (9) nutrient digestibility, (10) ruminal fermentation, and (11) methane emission. To obtain updated information, literature searches were restricted to studies published in the recent decade (January 2014 to May 2024).

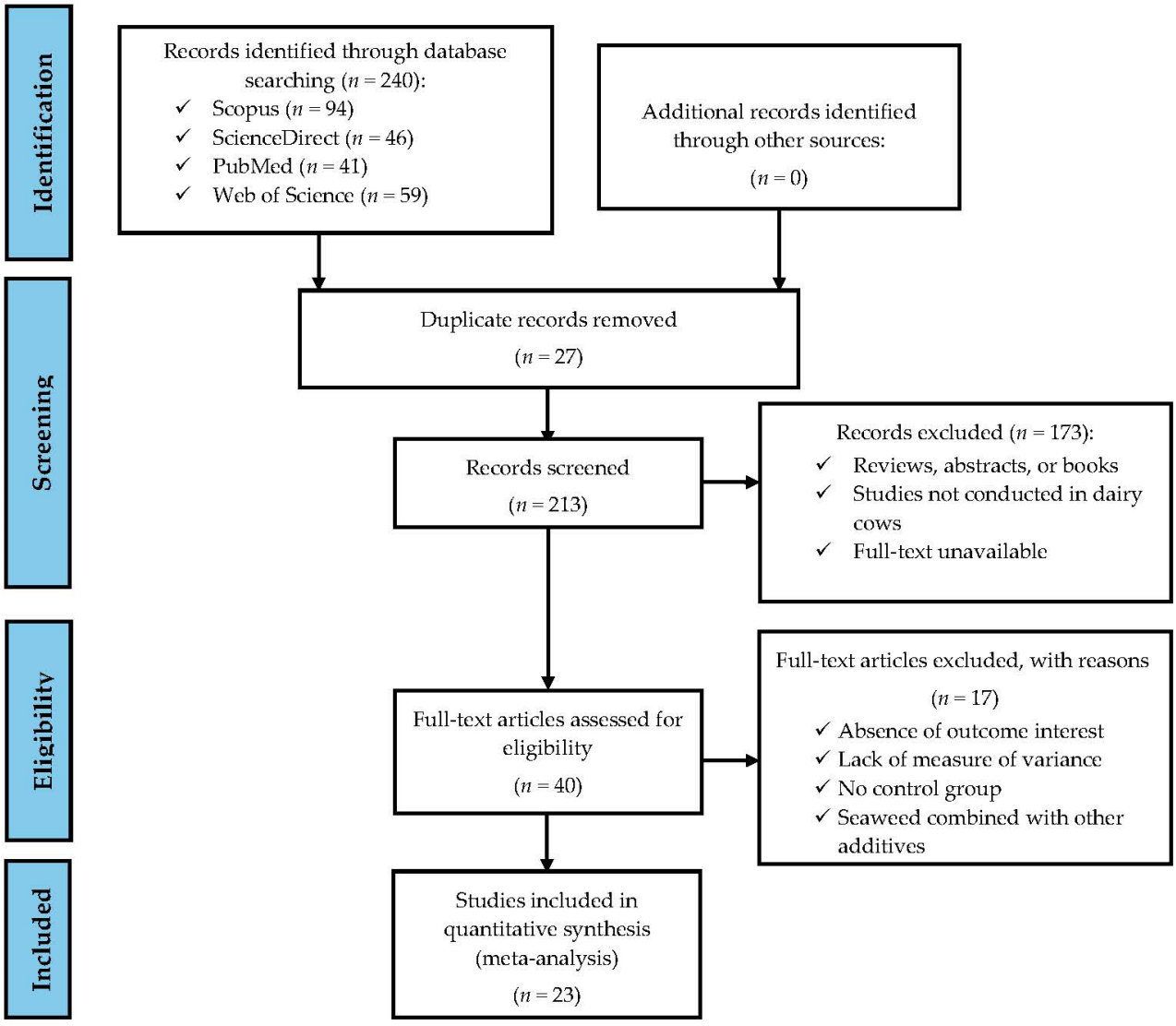

Figure 1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

2.2. Inclusion and Exclusion Criteria

Through the searches, 240 scientific documents were identified, which, after eliminating duplicate documents, were reduced to 213. After this, documents that had any of the following traits were eliminated: (1) conference proceedings, books, theses, review

articles, and simulations; (2) studies that did not use dairy cows as experimental units; and (3) studies that did not use seaweed or combined seaweed with antibiotics, organic acids, or other products. The remaining documents were evaluated and only those that met all of the following inclusion criteria were used to form the meta-analysis database [26,27]: (1) scientific articles written in the English language and peer-reviewed; (2) studies that used dairy cows as experimental units and randomized experiments; (3) studies that compared the effect of dietary supplementation with seaweed against a control treatment (diets without the addition of seaweed) in dairy cows fed with the same basal diet; (4) studies reporting data on parameters related to milk yield and composition, nutrient digestibility, ruminal fermentation, and enteric CH_4 emissions; (5) studies that indicated the duration of the seaweed supplementation period and the doses of seaweed added to diets; and (6) studies that included data on standard deviation (SD), standard error (SEM), number of replicates (*n*), and means of treatments supplemented with seaweed and control (diets without the addition of seaweed).

2.3. Data Extraction

Table 1 shows the 23 scientific articles used to build the meta-analysis database. The following characteristics of each selected scientific article were extracted into an Excel spreadsheet: (1) name of the author, (2) year of publication, (3) breed of dairy cows, (4) experimental design (continuous or rotative), (5) days in milk from dairy cows, (6) duration (days) of the period of dietary supplementation with seaweed, (7) dose (g/kg DM) of seaweed added to the diets, (8) seaweed species (i.e., *Asparagopsis taxiformis*, *Ascophyllum nodosum*, among others), (9) type of seaweed (red or brown), and (10) level of forage (g/kg DM) included in diets. The variables extracted from the scientific articles were grouped as follows: (1) dry matter intake (DMI), milk yield (MY); energy-corrected milk yield (ECMY); feed efficiency (FE), milk fat yield (MFY), milk protein yield (MPY), milk lactose yield (MLY), milk fat content (MFC), milk protein content (MPC), milk lactose content (MLC), total solids (TC), milk urea nitrogen (MUN), somatic cell count (SCC), iodine and bromoform in milk; (2) dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), acid detergent fiber digestibility (ADFD), ruminal pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA), acetate, propionate, butyrate, valerate, acetate/propionate ratio, daily CH_4 emission (g/d), CH_4 yield (g/kg DMI), CH_4 intensity (g/kg ECMY). The SEM, number of replicates, and treatment means were obtained for each response variable. The WebPlot-Digitizer software version 4.4 was used to extract the data reported in graphs, following the procedures described by Drevon et al. [28].

Table 1. Description of the studies included in the meta-analysis database.

Reference	Breed	ED	DIM, d	SP, d	SW Dose (g/kg DM)	SW Specie	SW Type	Forage, g/kg DM
Antaya et al. [11]	Jersey	Rotative	40	21	3.2, 6.3, 9.7	*Ascophyllum nodosum*	Brown	642
Antaya et al. [29]	Jersey	Continuous	142	28	5.8, 6.6, 6.8	*Ascophyllum nodosum*	Brown	700
Bendary et al. [30]	Holstein	Continuous	7	150	2.9	*Ascophyllum nodosum*	Brown	600
Bošnjaković et al. [18]	Holstein	Rotative	174	21	20, 40	SL, SM, AN	Brown	611
Eikanger et al. [2]	Norwegian	Continuous	95	39	1.25, 2.50	*Asparagopsis taxiformis*	Red	650
Hong et al. [12]	Holstein	Continuous	30	360	20, 40	Blend-1	Brown	650
Katwal et al. [31]	Crossbreed	Rotative	NR	45	80.00	*Sargassum johnsonii*	Brown	600
Kidane et al. [32]	Norwegian	Rotative	164	28	8.9	*Ascophyllum nodosum*	Brown	840
Krizsan et al. [14]	Nordic Red	Rotative	122	21	5.0	*Asparagopsis taxiformis*	Red	600
López et al. [33]	Holstein	Continuous	302	38	4.8	*Ascophyllum nodosum*	Brown	770
Muizelaar et al. [16]	Holstein	Rotative	91	63	6.6, 6.5, 6.7	CHC, SL, Blend-2	Red, Brown	750
Newton et al. [13]	Iceland	Continuous	168	49	0.9, 3.5	Blend-3	Brown	550
Newton et al. [34]	Holstein	Continuous	168	63	13.4	*Ascophyllum nodosum*	Brown	710
Nyloy et al. [35]	Norwegian	Continuous	95	52	1.25, 2.5	*Asparagopsis taxiformis*	Red	650
Qin et al. [36]	Holstein	Rotative	NR	28	2.1	SL	Brown	750
Reyes et al. [37]	Holstein and Jersey	Continuous	150	84	60	CHC	Red	670
Roque et al. [19]	Holstein	Rotative	201	21	5.0, 10.0	*Asparagopsis armata*	Red	NR
Rey-Crespo et al. [38]	Holstein	Continuous	154	70	5.3	Blend-4	Blend	765
Sharma y Datt [39]	Crossbreed	Continuous	NR	150	15, 30	*Laminaria digitata*	Brown	NR

Table 1. *Cont.*

Reference	Breed	ED	DIM, d	SP, d	SW Dose (g/kg DM)	SW Specie	SW Type	Forage, g/kg DM
Silva et al. [40]	Jersey	Rotative	102	28	2.7, 5.4, 8.1	*Ascophyllum nodosum*	Brown	663
Singh et al. [41]	Sahiwal	Continuous	56	56	20	*Sargassum wightii*	Brown	750
Stefenoni et al. [17]	Holstein	Rotative	95	28	2.5, 5.0	*Asparagopsis taxiformis*	Red	603
Thorsteinsson et al. [15]	Holstein	Continuous	174	21	0.23, 0.46	*Ascophyllum nodosum*	Brown	534

ED: experimental design; DIM: days in milk; d: days; SP: supplementation period; SW: seaweed; NR: not reported; CHC: *Chondrus crispus*; SL: *Saccharina latissima*; SM: *Sargassum muticum*; AN: *Ascophyllum nodosum*; Blend-1: unreported species; Blend-2: SL and *Fucus serratus*; Blend-3: *Ascophyllum nodosum* and *Laminaria digitata*; Blend-4: SM, *Ulva rígida*, and *Sacchorhiza polyschides*.

2.4. Calculations, Statistical Analysis, Heterogeneity, and Publication Bias

The "meta" [42] and "metafor" [43] packages available in the R statistical software (version 4.1.2) were used to analyze all data in the current study. The effect size (ES) of seaweed supplementation in dairy cow diets was assessed by examining the weighted mean differences (WMD) between the experimental (diets with seaweed) and control (diets without seaweed) treatments. According to Takeshima et al. [44], WMD have greater statistical power and are easier to interpret than other ES measures, which makes their use advisable. The methods and procedures previously proposed by Der-Simonian and Laird [45] for random effects models were used to weight the treatment means by inverse variance.

Heterogeneity between studies was rigorously assessed using I^2 and Cochran's Q statistics, and significant heterogeneity was declared when Q had $p \leq 0.05$ and $I^2 > 50\%$ [46,47]. Furthermore, publication bias was assessed with Rosenberg's fail-safe number (NFS) using a significance level of $p \leq 0.05$ [48]. Although publication bias was detected in one response variable, the result was considered valid and robust when NFS > [5 (n) + 10], where n is the number of comparisons included in the analysis [49].

2.5. Meta-Regression and Subgroup Analysis

Univariate meta-regression analyses were used to test the effects of experimental design, days in milk, level of forage in the diet, seaweed dose, supplementation period, seaweed type, and seaweed species on the observed heterogeneity in response variables. Meta-regression analyses were performed using Der-Simonian and Laird's [45] method of moments only on response variables that had the following traits: (1) being reported in at least ten different scientific articles [26,27,46]; (2) have Cochran's Q statistic with $p \leq 0.05$ and I^2 statistic > 50% [46,47]; and (3) have $p > 0.05$ in the Rosenberg's fail-safe number [48]. The covariates evaluated were divided as follows: (1) experimental design (continuous and rotative); (2) days in milk (≤100 and >100 days); (3) level of forage in the diet (≤600 and >600 g/kg DM); (4) doses of seaweed added to diets 0.23–5.0, 5.1–10.0, and >10.0 g/kg DM; (5) dietary supplementation period with seaweed ≤ 30 and >30 days; (6) type of seaweed (red seaweed and brown seaweed); and (7) species of seaweed (i.e., *Asparagopsis taxiformis*, *Ascophyllum nodosum*, among others). The significant covariates ($p \leq 0.05$) were evaluated through subgroup analysis, including only subgroups with at least three comparisons, as recommended by other authors [27,50].

3. Results

3.1. Milk Yield and Composition

Table 2 shows that dietary supplementation with seaweeds decreased ($p < 0.05$) DMI, MPC, MUN, and SCC. In contrast, MFC, MLC, and milk iodine increased ($p < 0.05$) in response to dietary seaweeds supplementation. On the other hand, MY, ECMY, FE, MFY, MPY, MLY, TS, and bromoform content in milk were not affected ($p > 0.05$) by dietary supplementation with seaweeds. Table 2 shows that there was significant heterogeneity (Q ≤ 0.05 and $I^2 > 50\%$) in DMI, MY, ECMY, and iodine.

Table 2. Milk yield and milk composition in dairy cows supplemented with seaweed.

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
DMI, kg/d	21 (41)	19.85 (4.93)	−0.218 (−0.488; 0.052)	0.113	<0.001	67.99
MY, kg/d	18 (35)	26.17 (7.43)	−0.100 (−0.718; 0.519)	0.752	<0.001	78.85
ECMY, kg/d	11 (21)	25.59 (8.55)	−0.525 (−1.823; 0.774)	0.428	<0.001	91.92
FE, DMI/MY	9 (17)	1.164 (0.351)	−0.010 (−0.028; 0.009)	0.296	0.309	12.39
MFY, kg/d	8 (18)	1.06 (0.33)	−0.004 (−0.037; 0.030)	0.829	0.769	0.00
MPY, kg/d	8 (18)	0.87 (0.31)	0.008 (−0.013; 0.029)	0.446	0.978	0.00
MLY, kg/d	7 (17)	1.17 (0.46)	0.016 (−0.018; 0.049)	0.356	0.998	0.00
MFC, g/kg	17 (33)	4.13 (0.55)	0.070 (0.046; 0.095)	<0.001	0.506	0.00
MPC, g/kg	17 (33)	3.34 (0.30)	−0.039 (−0.068; −0.009)	0.010	0.071	40.93
MLC, g/kg	15 (32)	4.67 (0.15)	0.015 (0.001; 0.029)	0.040	0.506	0.00
TS, g/100 g	6 (13)	12.61 (1.38)	0.122 (−0.038; 0.282)	0.135	0.989	0.00
MUN, mg/dL	10 (19)	12.27 (2.58)	−0.478 (−0.755; −0.201)	<0.001	0.060	37.63
SCC, $\times 10^3$ cell/mL	9 (17)	4.98 (2.55)	−0.275 (−0.424; −0.125)	<0.001	0.073	48.34
Iodine, mg/dL	9 (15)	0.32 (0.19)	0.805 (0.574; 1.036)	<0.001	<0.001	96.87
Bromoform, µL	3 (4)	0.20 (0.11)	0.047 (−0.008; 0.102)	0.096	0.917	0.00

N: number of studies; NC: number of comparisons between seaweed treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with seaweed; CI: confidence interval of WMD; [1] *p*-Value to Cochran's Q statistic; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; DMI: dry matter intake; MY: milk yield; ECMY: energy-corrected milk yield; FE: feed efficiency; MFY: milk fat yield; MPY: milk protein yield; MLY: milk lactose yield; MFC: milk fat content; MPC: milk protein content; MLC: milk lactose content; TS: total solids; MUN: milk urea nitrogen; SCC: somatic cell count.

3.2. Nutrient Digestibility, Ruminal Fermentation, and Enteric Methane Emissions

Dietary supplementation with seaweeds did not affect ($p > 0.05$) DMD, OMD, CPD, NDFD, ADFD, TVFA, acetate, and propionate (Table 3). Dietary supplementation with seaweeds increased ($p < 0.05$) ruminal pH and ruminal concentration of butyrate and valerate. In contrast, lower ($p < 0.05$) ruminal NH_3-N concentration, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity were observed in response to dietary supplementation with seaweeds. Likewise, Table 3 shows that significant heterogeneity ($Q \leq 0.05$ and $I^2 > 50\%$) was observed in NH_3-N, TVFA, acetate, propionate, butyrate, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity. In the current study, meta-regression analyses were only performed on DMI, MY, ECMY, and daily CH_4 emissions. This is justified because the other response variables that had significant heterogeneity were reported in fewer than ten studies, and under these conditions, the power of the test is low [46].

Table 3. Nutrient digestibility, ruminal fermentation, and enteric methane emission in dairy cows supplemented with seaweed.

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
DMD, g/100 g	5 (11)	69.95 (3.56)	−0.241 (−0.639; 0.156)	0.234	0.724	0.00
OMD, g/100 g	7 (15)	72.63 (2.91)	−0.113 (−0.498; 0.272)	0.565	0.660	0.00
CPD, g/100 g	7 (15)	66.67 (5.37)	−0.230 (−1.199; 0.740)	0.642	0.065	43.97
NDFD, g/100 g	6 (15)	62.42 (5.75)	0.308 (−0.366; 0.981)	0.371	0.327	11.27
ADFD, g/100 g	3 (9)	62.02 (5.93)	0.248 (−1.113; 1.609)	0.721	0.236	23.29
Ruminal pH	4 (9)	6.21 (0.37)	0.074 (0.014; 0.134)	0.016	0.242	22.61
NH_3-N, mg/dL	4 (6)	11.88 (4.98)	−1.643 (−3.270; −0.016)	0.048	<0.001	83.53
TVFA, Mm	6 (12)	122.73 (16.94)	−4.427 (−11.877; 3.023)	0.244	<0.001	90.85
Acetate, mol/100 mol	5 (10)	61.69 (5.02)	−1.204 (−2.412; 0.003)	0.061	<0.001	68.73
Propionate, mol/100 mol	5 (10)	21.00 (2.57)	0.978 (−0.219; 2.175)	0.109	<0.001	71.80
Butyrate, mol/100 mol	5 (10)	13.51 (2.22)	1.073 (0.385; 1.761)	0.002	<0.001	85.87
Valerate, mol/100 mol	5 (10)	1.59 (0.41)	0.141 (0.025; 0.257)	0.017	0.063	47.05

Table 3. *Cont.*

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
Acetate/propionate	4 (8)	3.25 (0.70)	−0.321 (−0.550; −0.093)	0.006	<0.001	82.42
CH_4 production, g/d	10 (22)	411.00 (87.40)	−29.422 (−41.565; −17.280)	<0.001	<0.001	89.18
CH_4 yield, g/kg DMI	9 (20)	18.06 (3.61)	−1.578 (−2.204; −0.951)	<0.001	<0.001	81.01
CH_4 intensity, g/kg ECMY	9 (19)	15.82 (5.26)	−1.710 (−2.344; −1.076)	<0.001	<0.001	66.10

N: number of studies; NC: number of comparisons between seaweed treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with seaweed; CI: confidence interval of WMD; [1] *p*-Value to Cochran's Q statistic; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids; CH_4: methane; DMI: dry matter intake; ECMY: energy-corrected milk yield.

3.3. Publication Bias and Meta-Regression

Table 4 shows that the Rosenberg fail-safe number was only significant ($p < 0.05$) for MFC, MPC, MUN, SCC, iodine, daily CH_4 emission, and CH_4 yield, which suggests that there was publication bias in these response variables. However, the results obtained can be considered solid because NFS > [5 (n) + 10] was observed in all these variables [49].

Table 4. Analysis of publication bias.

Outcomes	Observed Significance	Target Significance	NFS Number	Number of Comparisons (n)	NFS > [5 (n) + 10]
DMI	0.2878	0.05	0	41	NA
MY	0.1915	0.05	0	35	NA
ECMY	0.2708	0.05	0	21	NA
FE	0.0692	0.05	0	17	NA
MFY	0.8589	0.05	0	18	NA
MPY	0.4246	0.05	0	18	NA
MLY	0.3332	0.05	0	17	NA
MFC	<0.001	0.05	283	33	175
MPC	<0.001	0.05	247	33	175
MLC	0.0775	0.05	0	32	NA
TS	0.6746	0.05	0	13	NA
MUN	0.0008	0.05	253	19	105
SCC	<0.001	0.05	324	17	95
Iodine	<0.001	0.05	1010	15	85
Bromoform	0.0962	0.05	0	4	NA
DMD	0.2671	0.05	0	11	NA
OMD	0.6511	0.05	0	15	NA
CPD	0.1890	0.05	0	15	NA
NDFD	0.4562	0.05	0	15	NA
ADFD	0.6531	0.05	0	9	NA
Ruminal pH	0.0648	0.05	0	9	NA
NH_3-N	0.0729	0.05	0	6	NA
TVFA	0.3391	0.05	0	12	NA
Acetate	0.1211	0.05	0	10	NA
Propionate	0.0634	0.05	0	10	NA
Butyrate	0.1941	0.05	0	10	NA
Valerate	0.0740	0.05	0	10	NA
Acetate/propionate	0.0647	0.05	0	8	NA
CH_4 production	<0.0001	0.05	201	22	120
CH_4 yield	<0.0001	0.05	187	20	110
CH_4 intensity	0.3276	0.05	0	19	NA

DMI: dry matter intake; MY: milk yield; ECMY: energy-corrected milk yield; FE: feed efficiency; MFY: milk fat yield; MPY: milk protein yield; MLY: milk lactose yield; MFC: milk fat content; MPC: milk protein content; MLC: milk lactose content; TS: total solids; MUN: milk urea nitrogen; SCC: somatic cell count; DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids; CH_4: methane; n: number of comparisons; NFS: fail-safe number; NA: Rosenberg's test does not apply since the observed significance level was greater than 0.05.

Table 5 shows that the covariate of experimental design, days in milk, level of forage in the diet, seaweed dose, and supplementation period did not have a significant relationship

($p > 0.05$) with any of the response variables evaluated. The seaweed type covariate explained ($p < 0.001$) 61.18, 66.68, 72.40, and 58.26% of the heterogeneity observed in DMI, MY, ECMY, and daily CH_4 emission, respectively. The seaweed species covariate explained ($p < 0.001$) 75.97, 66.10, 83.08, and 39.68% of the heterogeneity observed in DMI, MY, ECMY, and daily CH_4 emission, respectively.

Table 5. Meta-regression of the effects of dietary seaweed supplementation on growth performance of dairy cows.

Outcomes		QM	Df	*p*-Value	R^2 (%)
Dry matter intake (DMI)	Experimental design	2.879	1	0.090	3.53
	Days in milk	1.470	1	0.480	0.62
	Forage level	0.738	1	0.607	0.00
	Seaweed dose	2.133	2	0.344	4.27
	Suplementation period	0.037	1	0.848	0.00
	Seaweed type	19.683	1	<0.001	61.18
	Seaweed specie	38.266	8	<0.001	75.97
Milk yield (MY)	Experimental design	0.847	1	0.357	0.00
	Days in milk	0.028	1	0.986	0.00
	Forage level	4.184	1	0.123	0.00
	Seaweed dose	1.551	2	0.460	2.41
	Suplementation period	0.080	1	0.778	0.00
	Seaweed type	28.541	1	<0.001	66.68
	Seaweed specie	44.459	10	<0.001	66.10
Energy corrected milk yield (ECMY)	Experimental design	0.041	1	0.840	0.00
	Days in milk	1.544	1	0.642	1.74
	Forage level	0.003	1	0.955	0.00
	Seaweed dose	3.212	2	0.201	1.07
	Suplementation period	1.631	1	0.202	3.18
	Seaweed type	25.074	1	<0.001	72.40
	Seaweed specie	44.150	5	<0.001	83.08
Methane (CH_4) production, g/d	Experimental design	2.327	1	0.127	2.79
	Days in milk	0.536	1	0.464	0.00
	Forage level	0.032	1	0.858	0.00
	Seaweed dose	2.836	2	0.242	3.17
	Suplementation period	0.269	1	0.604	0.00
	Seaweed type	12.184	1	<0.001	39.68
	Seaweed specie	27.526	7	<0.001	58.26

QM: coefficient of moderators; QM is considered significant at $p \leq 0.05$; df: degree of freedom; R^2: the amount of heterogeneity accounted for.

3.4. Subgroup Analysis

Figure 2a shows that DMI decreased (WMD = −1.507 kg/d; $p = 0.010$) when red seaweeds were used. However, when brown seaweeds were used, DMI was not affected (WMD = 0.059 kg/d; $p = 0.501$). Figure 2b shows that MY decreased when red seaweed was used (WMD = −1.542 kg/d; $p = 0.006$). In contrast, when brown seaweeds were used, MY increased (WMD = 0.595 kg/d; $p = 0.003$). Figure 2c shows that ECMY decreased (WMD = −2.780 kg/d; $p < 0.001$) when red seaweeds were used. However, when brown seaweeds were used, ECMY was not affected (WMD = 0.489 kg/d; $p = 0.125$). Daily CH_4 emissions decreased ($p < 0.01$) regardless of the type of seaweed used (Figure 2d). However, the effect was greater when studies used red seaweed (WMD = −104.020 g/d) than when they used brown seaweed (WMD = −13.755 g/d).

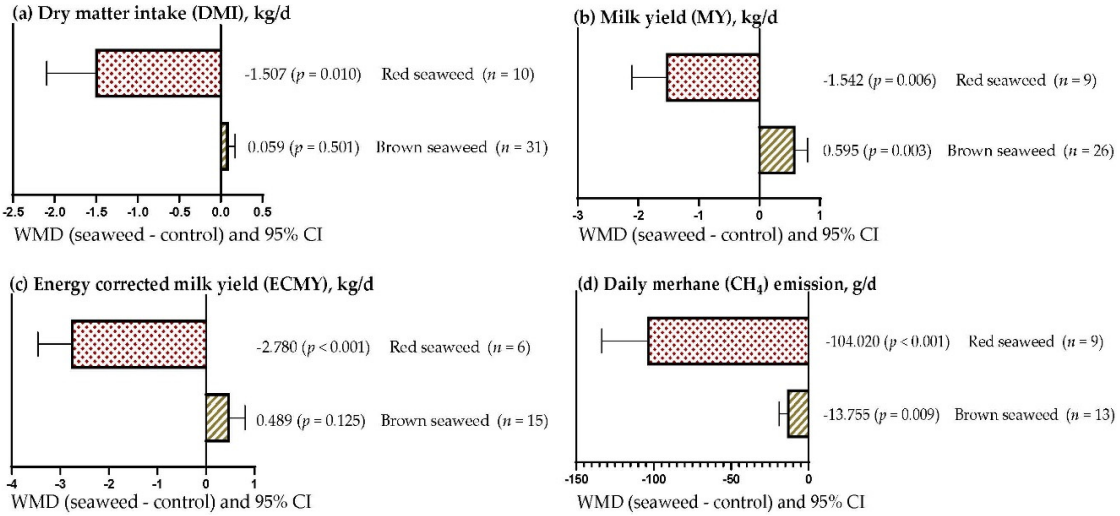

Figure 2. Subgroup analysis (subgroup = seaweed type) of the effect of including seaweed in dairy cows' diets, WMD = weighted mean differences between seaweed treatments and control.

Figure 3a shows that DMI was not affected (WMD = 0.198 kg/d; $p = 0.561$) when the seaweed used was *Saccharina latissima*. However, DMI decreased (WMD = −1.812 kg/d; $p = 0.003$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, DMI increased (WMD = 0.277 kg/d; $p = 0.049$) when seaweed *Ascophyllum nodosum* was added to the diets. Figure 3b shows that MY increased (WMD = 1.004 kg/d; $p = 0.038$) when the seaweed used was *Saccharina latissima*. However, MY decreased (WMD = −2.215 kg/d; $p < 0.001$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, MY increased (WMD = 0.823 kg/d; $p < 0.001$) when seaweed *Ascophyllum nodosum* was added to the diets. Figure 3c shows that ECMY was not affected (WMD = 0.054 kg/d; $p = 0.958$) when the seaweed used was *Saccharina latissima*. However, ECMY decreased (WMD = −3.402 kg/d; $p < 0.001$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, ECMY increased (WMD = 0.734 kg/d; $p = 0.050$) when seaweed *Ascophyllum nodosum* was added to the diets. Daily CH_4 emissions decreased ($p < 0.01$) regardless of the seaweed type used (Figure 3d). However, the effect was greater when studies used seaweed *Asparagopsis taxiformis* (WMD = −105.673 g/d) than when they used seaweed *Ascophyllum nodosum* (WMD = −24.451 g/d).

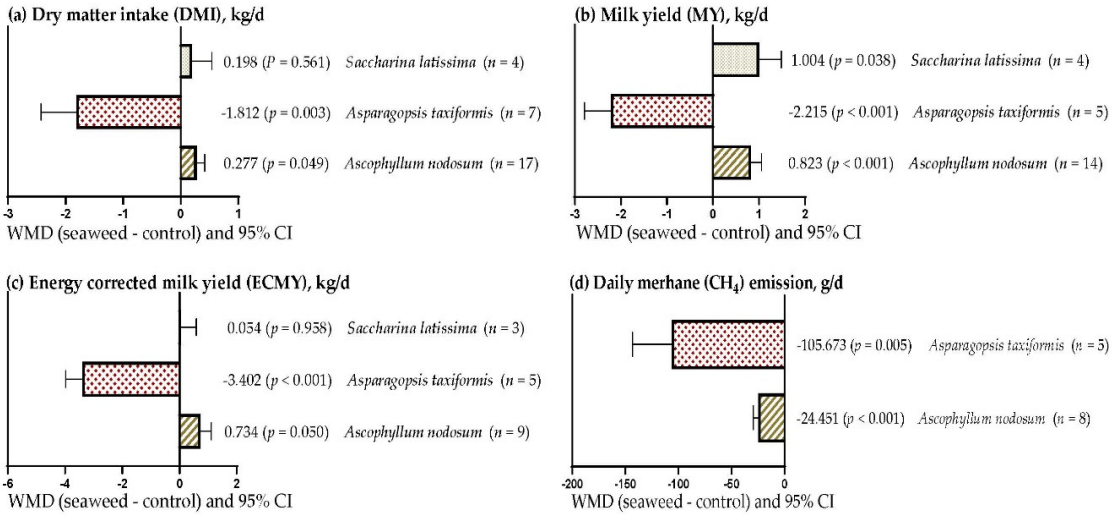

Figure 3. Subgroup analysis (subgroup = seaweed species) of the effect of seaweed supplementation in dairy cows' diets; WMD = weighted mean differences between seaweed treatments and control.

4. Discussion

4.1. Milk Yield and Composition

Dietary supplementation with seaweed did not affect DMI, suggesting that seaweed does not negatively affect dietary palatability or nutrient intake in dairy cows. However, subgroup analyses revealed that DMI decreased when red seaweed *Asparagopsis taxiformis* was used and increased when brown seaweed *Ascophyllum nodosum* was used. An in vitro study [51] reported that several brown seaweeds (*Undaria pinnatifida*, *Sargassum fusiforme*, and *A. nodosum*) can increase the relative abundance of bacteria *Fibrobacter succinogenes* (+19.4 to +58.4%), *Ruminococcus albus* (+7.4 to +11.1%), *Ruminococcus flavefaciens* (+4.8 to +14.9%), and rumen fungi (+86.8 to +100%) in ruminal fluid of beef cattle fed high forage diets (600 g/kg DM). Similar effects of brown seaweed consumption on dairy cows could explain the higher DMI observed since, according to Comtet-Marre et al. [52], a high abundance of *F. succinogenes*, *R. albus*, *R. flavefaciens*, and rumen fungi can decrease the residence time of feed particles in the rumen and lead to higher DMI. On the other hand, under in vitro conditions [53], *A. taxiformis* decreases (−41.1 to −64.2%) the relative abundance of the microbial genera *Fibrobacter* and *Ruminococcus* in the ruminal fluid of steers fed high forage diets (500 g/kg DM). Similar effects of *A. taxiformis* on dairy cows could reduce fiber digestibility and passage rate and result in low DMI.

MY, ECMY, FE, MFY, MPY, MLY, and TS were not affected by seaweed supplementation. However, subgroup analyses showed that MY and ECMY decreased when red seaweed *A. taxiformis* was used and increased when brown seaweed *A. nodosum* was added to the diets. These differences could be related to the effects of *A. taxiformis* and *A. nodosum* on DMI. Likewise, under in vitro conditions, *A. taxiformis* and other red seaweed decrease the relative abundance of bacteria related to the degradation of fiber, carbohydrates, and proteins [53]. A similar effect of *A. taxiformis* in dairy cows would decrease nutrient digestibility and rumen production of volatile fatty acids, which could decrease the metabolic availability of nutrients and energy and negatively affect MY. In contrast, several brown seaweeds, including *A. nodosum*, improve (+12.7 to +22.5%) serum levels of antioxidant enzymes (glutathione peroxidase and catalase) and total antioxidant capacity (TAC) in the blood serum of dairy ruminants [40,54]. These effects of the brown seaweed *A. taxiformis* partially explain the higher MY detected in the present study since, according to Dong et al. [55], the serum concentration of catalase, superoxide dismutase, and TAC have a positive correlation (r of 0.38 to 0.64) with MY in dairy cows.

Dietary supplementation with seaweed increased MFC and MLC but decreased MPC. Although an increase in MFC is not considered a nutritional benefit in humans, it plays an essential role in milk quality because it directly influences its visual attributes and flavor [56]. For example, high MFC is positively associated with milk creaminess and flavor [57]. On the other hand, an increase in MLC could negatively affect the quality of some dairy products. For example, Portnoy and Barbano [58] indicate that high MLC can induce the excessive formation of lactose and lactate crystals, negatively affecting ice cream's texture and consumers' acceptability of cheddar cheese. Likewise, Osorio et al. [56] mention that a low MPC decreases the quality and value of cow's milk and derived dairy products.

The higher MFC observed in the current study could be related to the effects of seaweed on lipid metabolism. For example, a recent study [18] shows that dietary supplementation with seaweed increases serum triglyceride (TG) concentration by up to 61.5% in dairy cows. Likewise, another study [11] reported that the dietary inclusion of seaweed decreases serum levels of non-esterified fatty acids (NEFA) between 12.0 and 19.5% in dairy cows. According to Andjelić et al. [59], in dairy cows, MFC has a significant positive (r = 0.304) and negative correlation (r = −0.324) with serum levels of TG and NEFA, respectively. On the other hand, recent studies [18,40] reported that seaweed increases (4.2 to 13.4%) the serum glucose concentration in dairy cows. This seaweed effect could explain the increased MLC because lactose synthesis in the mammary gland is carried out mainly from plasma glucose [60]. Furthermore, the lower MPC observed in the current study could be explained

by the reduction in DMI of cows supplemented with seaweed. This hypothesis is justified because a low DMI decreases crude protein intake (CPI) and lactation net energy intake (NE_LI) and, according to Hristov et al. [61], DMI, CPI, and NE_LI are positively correlated (r of 0.57 to 0.62) with MPC in dairy cows. Likewise, a recent study [40] reported that seaweed supplementation decreases dairy cows' serum insulin levels by up to 22.5%. A low serum insulin concentration can decrease the absorption of amino acids in the mammary gland [56], which could result in low MPC.

Hossein-Zadeh [62] mentions that MUN is a normal component in ruminant milk, which varies from 20 to 75% of the non-protein nitrogen of milk, and its values can be used as indicators of the efficiency of utilization of protein ingested in dairy cows. In the present study, lower MUN was observed in response to dietary supplementation with seaweed, suggesting that seaweed improves the utilization efficiency of dietary protein. Recent studies [29,40] reported that dietary supplementation with seaweed decreases blood urea nitrogen (BUN) levels in dairy cows by up to 16.4%. This effect of seaweed could explain the lower MUN observed in the current meta-analysis since, according to a recent study [62], BUN has a high positive correlation with MUN in dairy cows. On the other hand, SCC values are a simple and helpful tool to periodically evaluate ruminants' udder health and milk quality [63]. Although somatic cells are a naturally present component in milk, an increase in SCC values is generally associated with a health problem in the udder and negatively affects MY and milk quality [64]. In the current study, dietary supplementation with seaweed decreased SCC, suggesting that seaweed could reduce SCC in milk from dairy cows. Lopez et al. [33] reported that seaweed supplementation decreases the abundance of *Staphylococcus* spp. bacteria (including *Staphylococcus aureus*) in the milk of dairy cows. This effect could explain the lower SCC observed in the current study since, according to Kaskous et al. [64], *S. aureus* has a positive correlation with SCC in ruminants.

Dietary supplementation with seaweed increased milk iodine content but did not affect milk bromoform content. These effects were expected since, in dairy cows supplemented with seaweed, the transfer efficiency of dietary iodine to milk is high (37.5 to 41.8%) [13,34], while the transfer efficiency of dietary bromoform to milk is low (4.44%) [65]. A high iodine content in milk could be positive for human health since, according to Han et al. [66], more than 2 billion people are at risk of consuming amounts of iodine below their requirements worldwide. According to Newton et al. [13], the high iodine content in milk could affect consumers' health in human populations without iodine deficiencies. However, the maximum dose of iodine allowed in milk for human consumption is 500 mg/L [6], which is higher than the range iodine content (1.0 to 29.6 mg/L) observed in the milk of dairy cows supplemented with seaweed in the current study. On the other hand, the lack of significant changes in bromoform content in milk suggests that the inclusion of seaweed in diets for dairy cows does not represent any apparent risk of bromoform contamination [10].

4.2. Nutrient Digestibility, Ruminal Fermentation and Enteric Methane Emissions

In the current study, dietary supplementation with seaweed did not affect DMD, OMD, CPD, NDFD, and ADFD. Similarly, Maheswari et al. [54] also did not detect significant changes in DMD, OMD, CPD, NDFD, and ADFD of lactating buffaloes supplemented with high doses (25 g/kg DM for 90 days) of combinations of red and brown seaweeds (*Kappaphycus alvarezii*, *Gracilaria salicornia*, *Turbinaria conoids*). Likewise, dietary supplementation with up to 300 g/kg DM of various seaweeds (*Ruppia* sp., *Ulva* sp., or *Chaetomorpha* sp.) does not affect DMD, OMD, CPD, NDFD, and ADFD in sheep fed high forage diets [67].

Ruminal pH increased, and ruminal NH_3-N concentration decreased in response to dietary supplementation with seaweed. These effects could be positive since a low rumen pH is associated with rumen acidosis [68], while a high NH_3-N concentration generally indicates a low absorption of ammonia in the rumen, excessive deamination of amino acids or slow utilization of ammonia by ruminal microorganisms [69]. Some authors [70,71] reported that seaweed increases (40.0 to 142.1%) the rumen relative abundance of *Selenomonas ruminantium* and *Megasphera elsdenii* bacteria. A higher concentration of these bacteria could

increase the ruminal pH because they are lactate-consuming, and the ruminal pH decreases when lactic acid accumulates [68]. On the other hand, Rémond et al. [72] found that a high ruminal concentration of butyrate stimulates ruminal absorption of NH_3-N. Therefore, the lower ruminal NH_3-N concentration could be partially explained by the increased ruminal butyrate concentration of dairy cows supplemented with seaweed. Likewise, several studies [40,51,70] show that seaweed decreases (−23.1 to −54.0%) the population of rumen protozoa, which have a positive correlation (r = 0.609) with the rumen concentration of NH_3-N in ruminants [73].

In the current study, dietary supplementation with seaweed increased the rumen concentration of butyrate and valerate but decreased the acetate/propionate ratio. The higher ruminal concentration of butyrate and valerate could be closely related to the reduction in CH_4 emission of dairy cows supplemented with seaweed. This hypothesis is justified because when ruminal methanogenesis decreases, the ruminal concentration of butyrate and valerate increases since it acts as an alternative sink for H_2 [74]. According to Min et al. [9], the acetate/propionate ratio changes when the individual rumen concentration of acetate and propionate is modified. These two volatile fatty acids did not change significantly in the present study. However, dietary supplementation with seaweed numerically modified the rumen concentration of acetate (−1.9%) and propionate (+4.7%), which could be related to the observed lower acetate/propionate ratio.

A recent study [3] shows that enteric CH_4 emitted by dairy cows accounts for a high proportion (between 35 and 55%) of total GHG emissions from dairy farms worldwide. According to recent reviews [10,75], mitigation of enteric CH_4 emissions in dairy cows is essential to improve environmental sustainability in dairy farms. In the present meta-analysis, dietary supplementation with seaweed decreased daily CH_4 emission (−7.16%), CH_4 yield (−8.74%), and CH_4 intensity (−10.81%). The simultaneous reduction of these three types of enteric CH_4 shows that seaweed has a consistent anti-methanogenic effect and could improve environmental sustainability in dairy farms. Similar to our results, Roque et al. [76] reported that dietary supplementation with low doses (2.5 and 5.0 g/kg DM for 147 days) of seaweed in beef cattle fed high forage diets (600 g/kg DM) decreased the daily emission of CH_4 (−36.28 to −58.64), CH_4 yield (−32.58 to 52.03%), and CH_4 intensity (−36.93 to −54.46%). Likewise, Li et al. [77] observed lower daily CH_4 emission (−14.92 to −81.34%) and CH_4 yield (−15.33 to −80.66%) in sheep fed with 40% forage in the diet and supplemented with increasing doses (5.0 to 30.0 g/kg DM for 72 days) of seaweed. Wanapat et al. [75] mention that the anti-methanogenic effects of seaweeds are related to their bromoform and phlorotannin content. Bromoform is a halogenated alkane that competitively binds to coenzyme M methyltransferase, inhibiting methyl transfer in methanogenesis [10]. Likewise, bromoform blocks the enzyme methyl-coenzyme M reductase (MCR) [6], which plays an essential role because it catalyzes the last step in the formation of ruminal CH_4 [10]. On the other hand, phlorotannins are compounds analogous to condensed tannins [5], which can inhibit the growth of methanogenic archaea in the rumen [78].

Although daily CH_4 emission decreased regardless of seaweed type, subgroup analyses showed that daily CH_4 emission decreased almost four times more when red seaweed *A. taxiformis* was used than when brown seaweed *A. nodosum* was added to the diets. Hutchings et al. [79] reported that *A. taxiformis* has a high bromoform content (10–50 g/kg DM), while *A. nodosum* has a low concentration of phlorotannins (34.9 mg/kg DM) but does not have bromoform [5]. This variation in the concentration of anti-methanogenic compounds in seaweed could explain the differences in their effects on daily CH_4 emissions.

5. Conclusions

The overall results indicate that dietary supplementation with seaweed does not affect dry matter intake, milk yield, energy-corrected milk yield, or feed efficiency in dairy cows. However, the use of red seaweed, specifically *Asparagopsis taxiformis*, decreases dry matter intake, milk yield, and energy-corrected milk yield in dairy cows. Furthermore, brown

seaweed *Ascophyllum nodosum* increases dry matter intake, milk yield, and energy-corrected milk yield in dairy cows. Likewise, dietary supplementation with seaweed increases milk's fat, lactose, and iodine content while decreasing milk protein content, milk urea nitrogen, and somatic cell count. On the other hand, the inclusion of seaweed in dairy cow diets does not affect nutrient digestibility but improves ruminal fermentation through an increase in pH, butyrate, and valerate and a reduction in ruminal ammonia nitrogen concentration and acetate/propionate ratio. Furthermore, dietary supplementation with seaweed can be used as a nutritional strategy to reduce enteric methane emissions in dairy cows. The best enteric methane mitigation effect is achieved using red seaweed *Asparagopsis taxiformis*.

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6. GROWTH PERFORMANCE, DIETARY ENERGETICS, BLOOD METABOLITES, CARCASS TRAITS, MEAT QUALITY, AND GENE EXPRESSION OF LAMBS SUPPLEMENTED WITH A POLYHERBAL PHYTOGENIC ADDITIVE

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Growth Performance, Dietary Energetics, Blood Metabolites, Carcass Traits, Meat Quality, and Gene Expression of Lambs Supplemented with a Polyherbal Phytogenic Additive

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Simple Summary: The inclusion of phytogenic additives in diets for ruminants has shown a positive impact on the growth and health of animals. However, information on the effects of phytogenic additives on dietary energetics, meat quality, and gene expression in ruminants is limited. This study aimed to evaluate the effects of a polyherbal phytogenic additive on the growth performance, dietary energetics, carcass and meat traits, blood metabolites, and gene expression of finishing lambs. The polyherbal phytogenic additive improves feed intake, weight gain, feed efficiency, and dietary energy utilization efficiency. Likewise, the effects observed at the genetic level indicate that the polyherbal phytogenic additive improves energy production and antioxidant status in finishing lambs.

Abstract: This study aimed to evaluate the effects of supplementation with a polyherbal phytogenic additive (PPA) on the productive performance, dietary energetics, blood metabolites, carcass traits, meat quality, and gene expression of finishing lambs. Thirty-six male Pelibuey lambs (23.61 ± 0.57 kg body weight (BW)) were housed in individual pens and assigned to four treatments (n = 9) with different doses of PPA: 0 (CON), 2.5 (PPAL), 5 (PPAM), and 7.5 (PPAH) g of PPA/kg of DM for 56 days. Average daily gain, dry matter intake, and observed dietary net energy for maintenance and weight gain increased linearly ($p < 0.05$) in lambs supplemented with PPAH. A linear reduction ($p = 0.02$) in FCR was detected in lambs fed PPAM and PPAH. The PPAH supplementation linearly increased ($p < 0.001$) *Longissimus dorsi* muscle area, but other carcass traits were not affected ($p > 0.05$) by PPA doses. The physicochemical characteristics of the meat and the hematological parameters of the lambs were not affected ($p > 0.05$) by the PPA doses. The glucose, uric acid, creatinine, and bilirubin serum concentrations decreased linearly ($p < 0.05$) in lambs supplemented with PPAM and PPAH. Gene ontology analyses showed that nine biological processes were modified ($p < 0.05$), including DNA replication, drug metabolism–cytochrome P450, oxidative phosphorylation, and chemical carcinogenesis–reactive oxygen species. In conclusion, high doses (7.5 g/kg DM) of PPA can improve growth performance and dietary energy utilization efficiency in finishing lambs. Likewise, gene expression analysis indicates that supplementation with high doses of PPA could improve energy production and antioxidant status in finishing lambs.

Keywords: finishing lambs; bioactive compounds; phosphatidylcholine; blood biochemistry; hematological profile; nutrigenomics

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1. Introduction

Since the 1950s, antibiotics have been used successfully in preventing, controlling, and treating infectious diseases in domestic animals [1]. Furthermore, the inclusion of low doses of antibiotics in livestock feeds has been documented to improve the health, feed efficiency, growth rate, and carcass quality of animals [2,3]. However, the irrational and irresponsible use of antibiotics has contributed to the emergence of bacterial strains resistant to their effects [1]. This effect has led to the prohibition of antibiotics as growth promoters in several countries and has increased interest in the search for natural products that improve animal health and performance [4]. Among the natural products currently available are phytogenic additives, which, according to Windisch et al. [5], are products derived from plants that can be used in livestock feeding to improve their productivity.

Among the phytogenic additives are polyherbal phytogenic additives (PPAs), which consist of plant mixtures and differ from plant extracts in that they contain several bioactive metabolites with pharmaceutical properties [6]. Previous studies have shown the positive impact of dietary supplementation with PPAs on animal performance [7], dietary energy utilization efficiency [8], and meat quality [9] of finishing lambs. Other PPAs have been successfully used to improve the production of volatile fatty acids in the rumen [10], intestinal morphology and immune response [11], and the antioxidant status of blood serum in growing lambs [12]. BioCholine® is a PPA made from medicinal plant parts of *Andrographis paniculata*, *Azadirachta indica*, *Achyrantes aspera*, and *Trachyspermum ammi*, which is standardized to contain 16 g of phosphatidylcholine (PtdCho) per kilogram of product.

In non-ruminants, individual supplementation with *A. aspera* or *T. ammi* has been reported to improve animal performance, feed digestibility, and intestinal microbiota [13,14]. However, based on the literature reviewed, no previously published information exists on the effects of *A. aspera or T. ammi* in ruminants. Dietary inclusion of *A. paniculata* has been successfully used to improve rumen parameters, rumen microbiota, and meat quality in goats [15,16]. Previous studies [17,18] show the positive impact of supplementation with A. indica on nutrient digestibility, weight gain, and carcass traits of small ruminants. According to Hassan et al. [19], plant combinations provide complex mixtures of bioactive compounds with synergistic effects. Therefore, using a PPA containing a combination of *A. aspera*, *T. ammi*, *A. paniculata*, and A. indica could be a better strategy to improve livestock health and productivity than the individual administration of those plants.

On the other hand, it has been documented that dietary supplementation with products containing PtdCho improves animal health and performance in non-ruminants through changes in gene expression [20,21]. Likewise, Diaz et al. [22] reported that a PPA containing PtdCho improved calves' growth rate and health status through changes in the expression of lipid, carbohydrate, and protein metabolism genes. Other products containing PtdCho have shown a positive impact on rumen microbial populations [23], the antioxidant status of blood serum [24], and the nutrient digestibility [25] of ruminants. However, based on the literature reviewed, no information is available on the effects of dietary supplementation with PPAs or PtdCho on the gene expression of finishing lambs.

Based on the above background, we hypothesize that supplementation with a PPA made from medicinal plants and containing PtdCho will benefit the animal performance, blood metabolites, meat and carcass quality, and gene expression of finishing lambs. The objective of this study was to evaluate the effects of increasing levels of a polyherbal phytogenic additive made from medicinal plants (*Andrographis paniculata*, *Azadirachta indica*, *Achyrantes aspera*, and *Trachyspermum ammi*) and containing phosphatidylcholine on the productive performance, dietary energetics, blood metabolites, carcass traits, meat quality, and gene expression of finishing lambs.

2. Materials and Methods

2.1. Experimental Location

The present experiment was carried out from June to August 2022 at the Experimental Farm of the Universidad Autónoma Chapingo, located in Texcoco, State of Mexico, Mexico (Latitude: 19°30′45″ N; Longitude: 98°52′47″ W; altitude: 2250 masl). The average annual temperature of Texcoco is 18.2 °C, and the average annual precipitation is 665 mm [8]. All lamb care procedures were carried out following the federal guidelines for the use and care of animals [26] and were approved by the Research Ethics and Bioethics Committee of the Autonomous University of the State of Mexico (Protocol #25-05 2022).

2.2. Characterization of the Polyherbal Phytogenic Additive

The polyherbal phytogenic additive (PPA) used was BioCholine® (Nuproxa S. de RL. de CV., Querétaro, México). BioCholine® is a commercial product composed of plant parts of *Andrographis paniculata*, *Azadirachta indica*, *Achyrantes aspera*, and *Trachyspermum* ammi, and is standardized to contain 16 g of phosphatidylcholine (PtdCho) per kilogram of product. The methodology previously described by other authors [6,27] was followed to extract and characterize the bioactive compounds of the PPA used. Briefly, bioactive compounds were extracted from PPA using an ultrasonic processor (GEX130, 115 V 50/60 Hz) equipped with a 3 mm titanium tip and mechanical stirrers (Cole-Parmer, Vernon Hills, IL, USA). Subsequently, one gram of PPA was mixed with 10 mL of hexane, and the organic phase was separated, concentrated to 1 mL, and then evaporated with a rotary evaporator (model R-200, Büchi, Flawil, Switzerland) to obtain the necessary material for the final analysis.

The characterization of the PPA was carried out by gas chromatography (Agilent 7890B, Agilent, Santa Clara, CA, USA) coupled to mass spectrophotometry (Agilent 5977A), equipped with a capillary column of a 30 m length, 250 µm diameter, and 0.25 µm film thickness (HP-5MS Ultra Inert, Agilent, Santa Clara, CA, USA). The temperature program was 40 °C for 3 min, and then increased to 250 °C at a rate of 10 °C/min and held there for 36 min. The temperature used in the injector was 240 °C in split mode, the helium flow rate was 1 mL/min, and the mass spectrophotometry was programmed in SCAN mode (35–550 m/z) to identify the PPA compounds. Table 1 shows the main compounds detected in PPA (BioCholine®).

Table 1. Gas chromatographic–mass spectral analysis of the bioactive components of BioCholine®.

Component	Retention Time (min)	Molecular Formula	Molecular Weight (g/mol)	Proportion of Total Area (%)
3-Hexanol	3.86	$C_6H_{14}O$	102.1	0.15
Pentanoic acid	5.89	$C_5H_{10}O_2$	102.1	0.04
Hexanoic acid	7.82	$C_6H_{12}O_2$	116	0.67
Undecane	9.62	$C_{11}H_{24}$	156.1	0.06
Octanoic acid	10.86	$C_8H_{16}O_2$	144.1	0.09
5-Hydroxymethylfurfural	11.65	$C_6H_6O_3$	126	0.07
Thymoquinone	11.96	$C_{10}H_{12}O_2$	164.1	0.16
Nonanoic acid	12.23	$C^9H^{18}O_2$	158.1	0.09
Carvacrol	12.57	$C_{10}H_{14}O_2$	150.1	1.75
n-Decanoic-acid	13.53	$C_{10}H_{20}O_2$	172.1	0.06
p-Cymene	15.98	$C_{10}H_{14}O_2$	166.1	0.13
Dodecanoic acid	16.05	$C_{12}H_{24}O_2$	200.2	0.46
Tetradecanoic acid	18.31	$C_{14}H_{28}O_2$	228.2	1.31
Pentadecanoic acid	19.31	$C_{15}H_{30}O_2$	242.2	0.33
Hexadecanoic acid, methyl ester	19.98	$C_{17}H_{34}O_2$	270.3	0.65
n-Hexadecanoic acid, palmitico	20.48	$C_{16}H_{32}O_2$	256.2	16.24
12-Octadecadienoic acid, methyl ester	21.62	$C_{19}H_{34}O_2$	1.77	1.77
9-Octadecenoic acid, methyl ester	21.68	$C_{19}H_{36}O_2$	1.44	1.44
Oleic acid	21.90	$C_{18}H_{34}O_2$	282.3	0.98
12-Octadecanoic acid, linoleico	22.33	$C_{18}H_{32}O_2$	280.2	58.94

2.3. Animals, Experimental Design, and Diet Composition

Thirty-six male Pelibuey lambs (23.61 ± 0.57 kg BW, 4–5 months old) were distributed using a completely randomized experimental design into one of four treatments (n = 9): (1) basal diet without PPA (CON); (2) PPAL, CON + 2.5 g of PPA/kg of dry matter (DM); (3) PPAM, CON + 5 g of PPA/kg DM; and (4) PPAH, CON + 7.5 g of PPA/kg DM. The 5 g dose of PPA (BioCholine®) was chosen based on a previous experiment in which this dose improved rumen propionate concentration and antioxidant status in the blood serum of growing lambs [24]. However, the animal performance of dairy cows and calves supplemented with the same PPA used in the present study has shown dose-dependent responses [22,25]. Therefore, the PPA doses in the present study included a low dose (2.5 g/kg DM) and a high dose (7.5 g/kg DM). All lambs were adapted to individual pens and the basal diet for 12 days and were subsequently subjected to an experimental phase of 56 days. At the beginning of the adaptation period, all lambs received a subcutaneous administration of 0.5 mL/lamb of ivermectin (Closantil® 5%, Chinoin Labs, Mexico City, Mexico), an intramuscular injection with 500,000 IU of vitamin A, 75,000 IU of vitamin D, and 50 mg of vitamin E (Vigantol®, Bayer Labs, Mexico City, Mexico), and were vaccinated intramuscularly against Pasteurella and Clostridium (2.5 mL/lamb, Multibacterina® 7, MSD Labs, Mexico City, Mexico). The individual pens had an area of 2 m^2 with walls (1.2 m high) and a concrete floor, galvanized sheet shade (2 m high), automatic waterer, and individual feeder (40 cm bunk space/lamb).

PPA was supplied to the lambs through the basal diet, formulated to obtain weight gains of 300 g/d [28]. The ingredients used and the nutritional composition of the experimental diets are shown in Table A1. First, PPA doses (2.5, 5, or 7.5 g/kg DM) were mixed with the minor ingredients of the diet (mineral premix, calcium carbonate, and common salt). Subsequently, these amounts were mixed with the rest of the ingredients until the complete experimental diets were obtained. Food was offered every day at 7:00 a.m. and 3:00 p.m. To guarantee ad libitum consumption, an amount of food 5% greater than the previous day's consumption was always assigned. Water was available ad libitum throughout the experiment. Figure 1 shows the experimental design used and the sampling carried out.

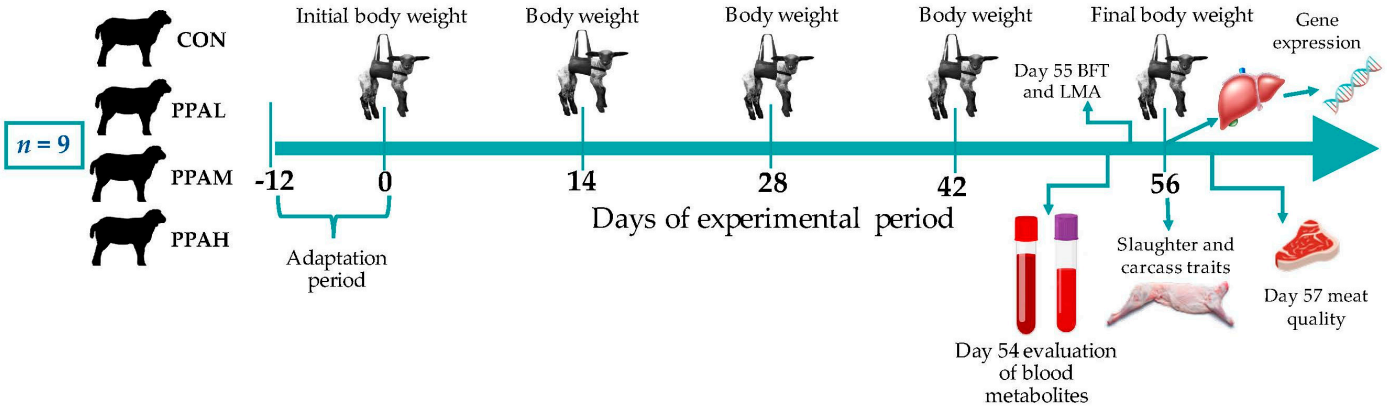

Figure 1. Diagram of the experimental design and the samplings carried out during the experiment. CON: basal diet without polyherbal phytogenic additive (PPA); PPAL, CON + 2.5 g of PPA/kg dry matter (DM); PPAM, CON + 5 g of PPA/kg DM; and PPAH, CON + 7.5 g of PPA/kg DM. BFT: back fat thickness; LMA: *Longissimus dorsi* muscle area.

Samples of the experimental diets were collected weekly and stored in polyethylene bags at −20 °C. Before chemical analyses, subsamples were thawed for 12 h at room temperature. Subsequently, equal amounts of the subsamples collected weekly were mixed to obtain a composite sample. Samples were dried at 55 °C for 72 h in a forced-air oven and then ground with a Wiley mill (model 4, Arthur Thomas Co., Philadelphia, PA, USA) using a 1 mm sieve. Finally, the contents of dry matter, crude protein, ether extract, and ash were determined following the procedures described by AOAC [29]. The contents of

neutral detergent fiber and acid detergent fiber were determined using the methods of Van Soest et al. [30].

2.4. Calculations

In the experimental phase, the body weight (BW) of each of the lambs was recorded one hour before morning feeding on days 1, 14, 28, 42, and 56 using a WeiHeng® brand digital scale (model WH-C100, Guangzhou Weiheng Electronics Co., Ltd., Guangzhou, China). The BWs recorded on days 1, 14, 28, and 42 were converted to reduced body weight (SBW) with the equation [31] SBW = BW × 0.96 to adjust for gastrointestinal filling. Before recording the final body weight (FBW) on day 56, all lambs were fasted for 18 h. Consequently, the dietary net energy and weight gain estimates were made based on SBW. Daily weight gain (ADG, kg/d) was calculated for periods of 14 days using the SBW data recorded on days 1, 14, 28, 42, and 56 with the following equation: ADG = (final SBW − initial SBW)/14. Each lamb's feed offered and rejected was recorded daily to estimate dry matter intake (DMI, kg/d). The lambs' feed conversion ratio (FCR) was calculated with the following equation: FCR = DMI/ADG.

Previous studies [8,32] mention that, in animal performance trials with lambs, the relationship between observed and expected DMI and the relationship between observed and expected dietary net energy (NE) serve as indicators to evaluate the efficiency of dietary energy utilization. The expected DMI estimate was based on the average ADG and SBW observed and the NE values calculated for the experimental diets (Table A1). For this, the following equation was used: expected DMI, kg/d = (EM/NEm) + (EG/NEg), where EM (energy required for maintenance, expressed in Mcal/d) = 0.056 × $SBW^{0.75}$ [NRC, 1985] and EG (energy gain, Mcal/d) = ADG × 0.276 × $SBW^{0.75}$ [33]. The values of NEm (NE maintenance, Mcal/kg of DM) and NEg (NE gain, Mcal/kg of DM) were 1.81 and 1.26, respectively, and were calculated with tabular values based on the ingredient composition of the experimental diets [28]. The coefficient of 0.276 was taken from the NRC [33], assuming a medium mature weight for male Pelibuey lambs [34]. Finally, with the DMI values observed in the present study and the ME and EG values, the observed dietary NE was calculated using the quadratic formula x = (−b − √(b^2 − 4ac))/2c, where x = NEm (Mcal/kg), a = −0.41 ME, b = 0.877 ME + 0.41 DMI + EG, and c = −0.877 DMI [35].

2.5. Carcass Traits, Carcass Morphometry, and Non-Carcass Components

Back fat thickness (BFT) and Longissimus dorsi muscle area (LDMA) were measured on day 55 of the experimental phase with the procedures described by Silva et al. [36]. For this, all the lambs were shaved between the 12th and 13th ribs, and subsequently a Sonovet 600 brand ultrasound (Medison, Inc., Cypress, CA, USA) with a 7.5 Mhz transducer was placed. The yield grade (YG) of the carcass was estimated with the equation YG = 0.4 + (10 × BFT, in cm) [37].

After obtaining FBW on day 56 of the experiment, all lambs were slaughtered on the same day according to the procedures of the Mexican Official Standard [38]. Immediately after completing the slaughter and bleeding of the lambs, the head, legs, skin, and all internal organs were separated from the carcass, and the hot carcass weight (HCY) was recorded. The equation HCY = (HCW/FBW) × 100 was used to estimate the hot carcass yield (HCY). Subsequently, all carcasses were cooled for 24 h at 4 °C, weighed individually, and then the cold carcass weight (CCW) was recorded. The cold carcass yield was estimated with the CCY = (CCW / FBW) × 100 equation.

For each of the lambs, the individual weight of the legs, skin, head, empty stomach complex (rumen, reticulum, omasum, and abomasum), empty small intestine (duodenum, jejunum, and ileum), empty large intestine (cecum, colon, and rectum), liver, kidneys, lungs, heart, and spleen were recorded. With the procedures described by Yañez et al. [39], in the cold carcass of each lamb, the following morphometric measurements were taken: internal carcass length (ICL), external carcass length (ECL), leg perimeter (LP), leg length (LL), chest girth (CG), and carcass compactness index (CCI), where CCI (kg/cm) = CCW/ILC.

2.6. Meat Quality

After keeping the carcasses at 4 °C for 24 h, the right *Longissimus dorsi* muscle (approximately 600 g) between the 4th and 12th rib was removed from each lamb with a scalpel. Subsequently, all samples were stored in identified polyethylene bags and frozen at −20 °C for one week. Before meat quality analyses, all samples were thawed at 4 °C for 24 h, and a scalpel was used to remove fat and subcutaneous tissue from each sample. Cooking loss (CL) was determined in duplicate following the procedures previously described by other authors [9,40]. Briefly, two 2.5-cm thick fillets were cut from each sample and grilled with an electric grill (Toastmaster cool-edge grill, Macon, MO, USA). The internal temperature of the fillets was monitored using a Taylor® brand thermometer (model 99878, Seattle, WA, USA), which was placed at the geometric center of each sample. When the internal temperature reached 70 °C, the samples were removed from the rack and cooled to room temperature (19–24 °C). Finally, CL was calculated with the equation [40] CL (%) = ((Wr − Wc)/Wr) × 100, in which Wr is the raw weight and Wc is the cooked weight of the meat samples used.

Meat color was evaluated with the procedures described by Miltenburg [41]. Approximately 3 cm slices were first cut from each sample, and the freshly cut surfaces were exposed to atmospheric air in a laboratory room for 30 min at room temperature. Subsequently, lightness (L*), redness (a*), and yellowness (b*) were measured in triplicate in different positions throughout the sample with a HunterLab brand colorimeter (Model MiniScan XE Plus, Reston, VA, USA).

To determine the chemical composition of the meat, a 300 g sample was taken from each lamb and ground individually with a Ship to Shore brand meat grinder (Model 99598, Camarillo, CA, USA) until a homogeneous mixture was obtained [40]. After this, the content (g/100 g) of moisture, fat, protein, and collagen was determined in triplicate with a FOSS brand near-infrared spectrophotometer (FoodScan™ Lab, Hillerod, Denmark), according to the procedures described by Anderson [42].

2.7. Blood Metabolites

In the last week of the experimental phase (day 54), two blood samples were taken from the jugular vein of each lamb before morning feeding (06:30 h). The first blood sample (4 mL/lamb) was collected using BD Vacutainer® K2 EDTA anticoagulant tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). These samples were transported at 4 °C for approximately 1 h to the laboratory, where they were analyzed with an EasyVet® hematology analyzer (QS Kontrolab, Hamburg, Germany), as reported by Dorantes-Iturbide et al. [8]. The hematological variables obtained included hematocrit, complete blood count, and differential leukocyte count.

The second blood sample (6 mL/lamb) was collected with BD Vacutainer® anticoagulant-free tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). These samples were centrifuged with a refrigerated centrifuge (Sigma 2–16 k, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 3500 rpm for 20 min to obtain blood serum, which was stored at −20 °C in Eppendorf tubes. Finally, an EasyVet® autoanalyzer (QS Kontrolab, Hamburg, Germany) was used to determine the contents of glucose, cholesterol, triglycerides, total protein, albumin, globulin, urea, uric acid, creatinine, bilirubin, liver enzymes (alkaline phosphatase, lactate dehydrogenase, and aspartate aminotransferase), calcium, and phosphorus in the blood serum, as reported in previous studies [6,7,40].

2.8. Liver Samples, RNA Extraction and Microarrays

Immediately after being slaughtered, liver samples were taken from the CON- and PPAH-supplemented lambs to extract RNA. For this, samples of 100 mg of liver tissue were taken in triplicate and placed in 2 mL microtubes (cap with O-ring) with 1 mL of DNA/RNA Shield™ reagent (Zymo Research, Irvine, CA, USA). The samples were placed in a cooler for transport to the laboratory. Subsequently, RNA extraction was carried out with the phenol–chloroform method, following the step-by-step protocol described by Toni

et al. [43]. The integrity and purity of the RNA were evaluated following the procedures described in a previous study by our research team [22].

From each experimental treatment (CON and PPAH), a group of 30,000 ng of RNA was obtained. The replicates (lambs) contributed an equal concentration to form the total RNA in each group. Transcriptome analysis was performed using a heterologous mouse chip (M22K) with 24,341 applications at the Microarray Unit facilities of the Institute of Cellular Physiology of the National Autonomous University of Mexico (UNAM). GenArise software was used to identify differentially expressed genes (DEGs; z score 1.5) on the microarray. Furthermore, the bioinformatics tool DAVID 6.8 (Database for Annotation, Visualization, and Integrated Discovery) was used to evaluate gene sets' enrichment, as Sheman and Lempicki suggested [44]. Enrichment analysis of gene sets through gene ontology was used to summarize the biological meaning of the identified differentially expressed transcripts, as reported by recent studies [21,22].

2.9. Statistical Analysis

2.9.1. Performance, Dietary Energetics, Blood Metabolites, Carcass Traits, Non-Carcass Components and Meat Quality

All statistical analyses were performed using SAS statistical software [45]. First, the Shapiro–Wilk normality test was applied to all variables evaluated with the PROC UNIVARIATE procedure. Data on productive performance and dietary energetics were analyzed with the PROC MIXED procedure, using a completely randomized experimental design with measures repeated over time, in which each lamb was considered an experimental unit. The initial BW covariate was not included in the statistical model because it was not significant ($p > 0.05$). The statistical model was adjusted by testing different variance–covariance structures, and the best fit was obtained with the compound symmetry structure because the lowest values of AIC and BIC were detected [46]. The structure of the statistical model used was

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

where Y_{ijk} = value observed in treatment i and period j for lamb k; μ = overall mean; T_i = fixed effect of the ith treatment; P_j = fixed effect of the jth period (period 1: 1–14, . . . , period 4: 43–56 d); $(T \times P)_{ij}$ = fixed effect of the interaction between the jth treatment and the ith period; L_k = random effect of the lamb (repetition) within the treatment (k = 1, 2, 3,..., 36); and e_{ijk} = random error.

Data on carcass traits, organs, meat quality, and blood metabolites were analyzed with the PROC GLM procedure, using a completely randomized experimental design where each lamb was considered an experimental unit. The FBW covariate was not included in the statistical model because it was not significant ($p > 0.05$). The statistical model used was the following:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} represents the observations, μ = general mean, T_i = fixed effect of the ith treatment, and eij = random error.

In all the variables evaluated, polynomial orthogonal contrasts were used to determine the linear and quadratic effects of the inclusion levels (0, 2.5, 5, and 7.5 g/kg DM) of PPA [47]. Significant differences were declared when $p \leq 0.05$.

2.9.2. Gene Enrichment Analysis

The gene enrichment analysis was performed considering at least two genes for each biological group, as reported by Mendoza-Martínez et al. [21]. Subsequently, to identify the enriched annotation terms that belonged to the tested gene sets and the biological closeness between the enriched processes, an EASE (modified Fisher Exact) score with $p \leq 0.05$ and the fold enrichment value were used [44], respectively.

3. Results

3.1. Growth Performance and Dietary Energetics

Compared to the CON treatment, ADG and DMI increased linearly ($p < 0.05$) in lambs fed PPAH (Table 2). In contrast, a linear reduction ($p = 0.02$) was detected in the FCR of lambs fed PPAM and PPAH compared to CON lambs. However, the variation in DMI was not affected by the doses of PPA added to the diets ($p > 0.05$).

Table 2. Growth performance of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value			
	CON	PPAL	PPAM	PPAH		Per	Trat × Per	Linear	Quadratic
Initial body weight, kg	23.22	23.91	23.52	23.78	0.597	-	-	-	-
Final body weight, kg	40.49	41.35	41.96	44.32	1.573	<0.0001	0.99	0.08	0.73
Daily weight gain (DWG), kg/d	0.308 ^{b}	0.311 ^{b}	0.329 ^{b}	0.367 ^{a}	0.015	<0.0001	0.88	0.01	0.33
Dry matter intake (DMI), kg/d	1.333 ^{b}	1.328 ^{b}	1.344 ^{ab}	1.407 ^{a}	0.032	<0.0001	0.95	0.02	0.27
DMI variation (%) [1]	12.24	14.01	14.10	12.09	1.268	<0.0001	0.29	0.93	0.11
Feed conversion ratio (FCR), DMI/DWG	5.06 ^{a}	4.85 ^{ab}	4.37 ^{bc}	4.22 ^{c}	0.218	<0.0001	0.33	0.02	0.92
Observed dietary net energy, Mcal/kg									
Maintenance (ObsNEm)	1.793 ^{b}	1.840 ^{b}	1.892 ^{ab}	2.003 ^{a}	0.080	<0.0001	0.85	0.02	0.63
Gain (ObsNEg)	1.162 ^{b}	1.204 ^{b}	1.250 ^{ab}	1.346 ^{a}	0.070	<0.0001	0.85	0.02	0.63
Observed to expected diet net energy									
Maintenance (OExNEm)	0.991 ^{b}	1.017 ^{b}	1.046 ^{ab}	1.106 ^{a}	0.044	<0.0001	0.85	0.02	0.63
Gain (OExNEg)	0.922 ^{b}	0.955 ^{b}	0.992 ^{ab}	1.068 ^{a}	0.039	<0.0001	0.85	0.02	0.63
Observed to expected DMI	1.128 ^{a}	1.110 ^{a}	1.037 ^{ab}	0.976 ^{b}	0.059	<0.0001	0.75	0.01	0.65

BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. [1] Variation in daily feed intake among individuals between days; CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means; ^{a,b,c}—means within a row with different subscripts differ when $p \leq 0.05$.

Compared to the CON treatment, observed dietary NE for maintenance (ObsNEm) and weight gain (ObsNEg) increased linearly ($p < 0.05$) in lambs fed PPAH (Table 2). Similarly, the relationship between observed and expected dietary NE for maintenance (OExNEm) and weight gain (OExNEg) increased linearly ($p = 0.02$) in lambs fed PPAH compared to CON lambs. In contrast, a linear reduction ($p = 0.01$) in the ratio between observed and expected DMI was detected for lambs fed PPAH compared to CON lambs.

3.2. Carcass Traits and Carcass Morphometry

Table 3 shows that LDMA increased linearly ($p < 0.001$) compared to the CON treatment in lambs fed PPAM and PPAH. However, PPA supplementation did not affect BFT, HCW, HCY, CCW, CCY, YG, ELC, ILC, CG, LL, PL, and CI.

Table 3. Carcass traits of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Back fat thickness (BFT), mm	4.20	4.24	4.33	4.37	0.08	0.10	0.98
Muscle area *longissimus dorsi* (LDMA), cm^2	10.11 ^{c}	10.50 ^{bc}	10.73 ^{b}	11.23 ^{a}	0.16	<0.001	0.70
Hot carcass weight (HCW), kg	20.25	20.90	20.62	20.86	0.72	0.17	0.68
Hot carcass yield (HCY), %	50.26	50.74	49.48	29.29	0.99	0.35	0.74
Cold carcass weight (CCW), kg	18.62	19.31	19.26	20.37	0.77	0.14	0.79
Cold carcass yield (CCY), %	46.20	46.63	46.24	45.91	0.98	0.78	0.70
Yield grade (YG)	0.41	0.41	0.42	0.42	0.03	0.10	0.98
External length of the carcass (ELC), cm	57.33	57.44	57.33	58.33	0.78	0.41	0.57
Internal length of the carcass (ILC), cm	53.89	55.22	55.00	54.44	0.72	0.65	0.20
Chest girth (CG), cm	71.78	74.44	73.44	75.11	1.21	0.67	0.34

Table 3. *Cont.*

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Length of the leg (LL), cm	32.22	33.77	33.00	33.33	0.59	0.83	0.78
Perimeter of the leg (PL), cm	34.66	35.00	36.88	36.89	0.63	0.10	0.79
Compactness index (CI), kg/cm	0.34	0.35	0.35	0.37	0.01	0.10	0.41

[1] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means; [a,b,c]—means within a row with different subscripts differ when $p \leq 0.05$.

3.3. Non-Carcass Components and Meat Quality

The weights of the stomach complex, small intestine, large intestine, lungs and trachea, heart, liver, kidneys, spleen, testicles, skin, head, and feet were not affected ($p > 0.05$) by the doses of PPA added to the diets (Table 4).

Table 4. Organ weights of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Stomach complex (empty), kg	1.451	1.406	1.441	1.512	0.068	0.48	0.40
Small intestine (empty), kg	0.887	0.896	0.926	0.965	0.054	0.08	0.28
Large intestine (empty), kg	1.040	1.117	1.291	1.222	0.136	0.24	0.11
Lungs and trachea, kg	0.639	0.666	0.635	0.646	0.042	0.96	0.85
Heart, kg	0.191	0.190	0.179	0.200	0.013	0.81	0.39
Liver, kg	0.898	0.897	0.899	0.882	0.049	0.84	0.87
Kidneys, kg	0.634	0.669	0.683	0.714	0.048	0.46	0.33
Spleen, kg	0.079	0.084	0.078	0.076	0.006	0.59	0.57
Testicles, kg	0.565	0.592	0.594	0.586	0.046	0.12	0.15
Skin, kg	3.305	3.197	3.077	3.436	0.157	0.70	0.14
Head, kg	2.253	2.158	2.124	2.269	0.087	0.97	0.18
Feet, kg	0.965	0.889	0.913	0.987	0.045	0.96	0.11

[1] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means.

Table 5 shows that being supplemented with increasing doses of PPA did not affect CL, L*, a*, b*, protein, fat, moisture, and collagen content of meat ($p > 0.05$).

Table 5. Meat traits of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Cooking loss (CL), %	17.47	16.50	16.65	18.73	1.45	0.29	0.17
Lightness (L*)	34.95	34.02	34.57	35.69	1.23	0.96	0.26
Redness (a*)	10.47	10.45	9.40	10.60	0.50	0.76	0.22
Yellowness (b*)	10.08	9.93	10.22	10.94	0.68	0.73	0.12
Protein, g/100 g	19.73	19.80	20.19	19.98	0.17	0.22	0.17
Fat, g/100 g	3.29	3.44	3.19	3.03	0.27	0.77	0.10
Moisture, g/100 g	75.05	74.68	74.62	74.85	0.33	0.41	0.16
Collagen, g/100 g	1.71	1.64	1.62	1.71	0.06	0.88	0.11

[1] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means.

3.4. Blood Metabolites

Dietary supplementation with increasing doses of PPA did not affect ($p > 0.05$) blood levels of hematocrit, hemoglobin, red blood cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, leukocytes, lymphocytes, monocytes, segmented neutrophils, band neutrophils, basophils, and plasma protein (Table 6).

Table 6. Hematological profile of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Hematocrit, %	35.44	38.00	36.11	36.44	1.30	0.85	0.39
Hemoglobin, g/dL	11.79	12.05	11.76	12.20	0.26	0.42	0.73
Red blood cells, 10^6/mL	8.27	8.38	8.63	9.15	0.34	0.08	0.36
Mean corpuscular volume, Fl	29.27	29.49	20.40	29.46	0.51	0.84	0.87
Mean corpuscular hemoglobin, pg	14.07	15.10	13.78	13.32	0.80	0.33	0.36
Mean corpuscular hemoglobin concentration, g/dL	48.31	51.66	47.10	45.57	2.86	0.32	0.39
Platelets, 10^3/mL	936.00	986.11	826.33	1004.67	52.22	0.84	0.22
Leukocytes, 10^3/mL	9.01	9.19	8.71	8.25	0.43	0.15	0.45
Lymphocytes, 10^3/mL	45.66	46.00	47.22	48.55	3.48	0.80	0.88
Monocytes, 10^3/mL	8.19	10.67	9.33	8.89	1.36	0.29	0.07
Segmented neutrophils, 10^3/mL	43.67	42.33	43.00	45.78	3.76	0.67	0.58
Band neutrophils, 10^3/mL	0	0	0	0	0	0	0
Eosinophils, 10^3/mL	2.67	1.00	0.89	1.33	0.62	0.14	0.09
Basophils, 10^3/mL	0.11	0.00	0.00	0.00	0.05	0.18	0.32
Plasma protein, g/dL	6.79	6.70	6.85	6.67	0.17	0.82	0.79

[1] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means.

Compared with CON treatment, serum concentrations of glucose, uric acid, creatinine, and bilirubin decreased linearly ($p < 0.05$) in lambs fed PPAM and PPAH (Table 7). However, serum concentrations of cholesterol, triglycerides, urea, total protein, albumin, globulin, albumin–globulin ratio, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, calcium, and phosphorus were not affected by the doses of PPA added to diets.

Table 7. Biochemistry of blood serum of finishing lambs supplemented with a polyherbal phytogenic additive [1].

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Glucose, mg/dL	65.44 [a]	64.12 [ab]	60.00 [b]	58.35 [b]	1.93	0.05	0.38
Cholesterol, mg/dL	40.33	37.11	45.77	37.11	4.06	0.95	0.50
Triglycerides, mg/dL	18.77	17.88	19.89	17.33	1.81	0.77	0.55
Urea, mg/dL	42.11	44.44	47.89	40.33	3.17	0.89	0.12
Uric acid, mg/dL	0.47 [a]	0.46 [a]	0.25 [b]	0.32 [b]	0.04	0.001	0.35
Creatinine, mg/dL	0.87 [a]	0.78 [a]	0.62 [b]	0.46 [c]	0.05	<0.0001	0.55
Total protein, g/dL	5.45	5.23	5.48	5.19	0.35	0.74	0.91
Albumin, g/dL	2.82	2.74	2.88	2.84	0.16	0.78	0.90
Globulin, g/dL	2.62	2.48	2.60	2.46	0.18	0.63	0.99
Albumin/globulin	1.08	1.10	1.11	1.13	0.03	0.36	0.92
Bilirubin, mg/dL	0.51 [a]	0.50 [a]	0.39 [b]	0.28 [b]	0.05	0.006	0.43
Alkaline phosphatase, UI/dL	463.55	451.00	481.00	486.11	48.91	0.65	0.85
Lactate dehydrogenase, UI/dL	395.78	434.67	445.89	394.78	29.59	0.89	0.11
Aspartate aminotransferase, UI/dL	87.78	95.55	93.55	90.67	6.89	0.83	0.44

Table 7. *Cont.*

Parameter	Treatments				SEM	*p*-Value	
	CON	PPAL	PPAM	PPAH		Linear	Quadratic
Calcium, mg/dL	9.24	8.63	9.44	9.35	0.32	0.43	0.42
Phosphorus, mg/dL	3.84	3.69	3.87	3.99	0.15	0.36	0.38

[1] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*. CON—basal diet without polyherbal phytogenic additive (PPA); PPAL—basal diet + 2.5 g of PP/kg DM; PPAM—basal diet + 5 g of PPA/kg DM; PPAH—basal diet + 7.5 g of PPA/kg DM. SEM—standard error of the treatment means; [a,b,c]—means within a row with different subscripts differ when $p \leq 0.05$.

3.5. Gene Expression

Compared to CON treatment, PPAH supplementation modified the expression of 2312 genes at least 1.5 times, of which 1135 and 1177 were downregulated and upregulated, respectively. Table 8 shows that the biological processes enriched with the downregulated DEGs were DNA replication (p = 0.003), tyrosine metabolism (p = 0.006), metabolism of drugs (p = 0.003), the intestinal immune network for immunoglobulin A production (p = 0.009), TGF-β signaling pathway (p = 0.007), and amoebiasis (p = 0.0056). On the other hand, Table 9 shows that DEGs upregulated the biological processes of non-alcoholic fatty liver disease (p = 0.00003), oxidative phosphorylation (p = 0.0033), and chemical carcinogenesis–ROS (p = 0.0016).

Table 8. Biological processes enriched with differentially down-expressed genes in liver tissue from lambs supplemented with PPAH.

DNA Replication (FC = 4.7; *p*-Value = 0.003)			Tyrosine Metabolism (FC = 4.1; *p*-Value = 0.006)		
Symbol	**Gene Name**	**FC**	**Symbol**	**Gene Name**	**FC**
Prim2	DNA primase, p58 subunit	−2.81	Aldh3a1	Aldehyde dehydrogenase family 3, subfamily A1	−2.62
Rfc1	Replication factor C (activator 1) 1	−2.31	Adh4	Alcohol dehydrogenase 4 (class II), pi polypeptide	−2.15
Mcm4	Minichromosome maintenance complex component 4	−2.01	Aox1	Aldehyde oxidase 1	−2.14
Rnaseh2b	Ribonuclease H2, subunit B	−1.94	Fahd1	Fumarylacetoacetate hydrolase domain containing 1	−2.09
Pold1	Polymerase (DNA directed), delta 1, catalytic subunit	−1.83	Got2	Glutamatic-oxaloacetic transaminase 2, mitochondrial	−1.86
Rfc4	Replication factor C (activator 1) 4	−1.55	Maoa	Monoamine oxidase A	−1.77
Rfc3	Replication factor C (activator 1) 3	−1.54	4930438A08Rik	RIKEN cDNA 4930438A08 gene	−1.60
Drug metabolism—Cytochromo P450 (FC = 3.3; *p*-Value = 0.003)			**TGF-β Signaling Pathway (FC = 2.7; *p*-Value = 0.007)**		
Symbol	**Gene Name**	**FC**	**Symbol**	**Gene Name**	**FC**
Gsta3	Glutathione S-transferase, alpha 3	−2.86	Acvr2a	Activin receptor IIA	−2.78
Aldh3a1	Aldehyde dehydrogenase family 3, subfamily A1	−2.62	Tgfbr2	Transforming growth factor, beta receptor II	
Gsta4	Glutathione S-transferase, alpha 4	−2.56	Ltbp1	Latent transforming growth factor beta binding protein 1	−2.50
Gstm3	Glutathione S-transferase, mu 3	−2.50	Tgfb1	Transforming growth factor, beta 1	−3.31
Adh4	Alcohol dehydrogenase 4 (class II), pi polypeptide	−2.15	E2f5	E2F transcription factor 5	−1.93
Aox1	Aldehyde oxidase 1	−2.14	Inhba	Inhibin beta-A	−1.80
Fmo1	Flavin containing monooxygenase 1	−1.91	Acvr1b	Activin A receptor, type 1B	−1.79
Maoa	Monoamine oxidase A	−1.77	Rbl1	RB transcriptional corepressor like 1	−1.76
Fmo2	Flavin containing monooxygenase 2	−1.60	Bmpr1a	Bone morphogenetic protein receptor, type 1A	−1.69
			Tgfb2	Transforming growth factor, beta 2	−1.64

Table 8. *Cont.*

Intestinal Immune Network for Immunoglobulin A Production (FC = 3.8; *p*-Value = 0.009)			Amoebiasis (FC = 2.6; *p*-Value = 0.006)		
Symbol	**Gene Name**	**FC**	**Symbol**	**Gene Name**	**FC**
Tgfb1	Transforming growth factor, beta 1	−3.31	Il6	Interleukin 6	−2.30
Cd40lg	CD40 ligand	−2.84	Serpinb9c	Serine (or cysteine) peptidase inhibitor, clade B, member 9c	−2.28
Il6	Interleukin 6	−2.30	Rab5a	RAB5A, member RAS oncogene family	−2.21
Tnfsf13	Tumor necrosis factor (ligand) superfamily, member 13	−1.93	Tgfb1	Transforming growth factor, beta 1	−3.31
H2-Oa	Histocompatibility 2, O region alpha locus	−1.77	Gnaq	Guanine nucleotide-binding protein, alpha q polypeptide	−1.75
Cxcl12	Chemokine (C-X-C motif) ligand 12	−1.62	Muc2	Mucin 2	−1.99
Tnfrsf13b	Tumor necrosis factor receptor superfamily, member 13b	−1.55	Rab7	RAB7, member RAS oncogene family	−1.84
			Lamb1	Laminin B1	−1.69
			Gna11	Guanine nucleotide-binding protein, alpha 11	−1.67
			Tgfb2	Transforming growth factor, beta 2	−1.64
			Lama1	Laminin, alpha 1	

PPAH—basal diet + 7.5 g of polyherbal phytogenic additive (PPA)/kg DM; PPA—based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*; FC—fold change.

Table 9. Biological processes enriched with differentially upregulated genes in liver tissue from lambs supplemented with PPAH.

Non-Alcoholic Fatty Liver Disease (FC = 3.5; *p*-Value = 0.00003)			Oxidative Phosphorylation (FC = 2.8; *p*-Value = 0.003)		
Symbol	**Gene Name**	**FC**	**Symbol**	**Gene Name**	**FC**
Ndufc1	NADH:ubiquinone oxidoreductase subunit C1	2.66	Ndufc1	NADH:ubiquinone oxidoreductase subunit C1	2.66
Ndufa1	NADH:ubiquinone oxidoreductase subunit A1	2.47	Ndufa1	NADH:ubiquinone oxidoreductase subunit A1	2.47
Jun	Jun proto-oncogene	2.25	Cyct	Cytochrome c, testis	2.22
Cyct	Cytochrome c, testis	2.22	Uqcr10	Ubiquinol-cytochrome c reductase, complex III subunit X	1.99
Rxra	Retinoid X receptor alpha	2.06	Ndufb4	NADH:ubiquinone oxidoreductase subunit B4	1.90
Uqcr10	Ubiquinol-cytochrome c reductase, complex III subunit X	1.99	Uqcrfs1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	1.87
Ndufb4	NADH:ubiquinone oxidoreductase subunit B4	1.90	Ndufv2	NADH:ubiquinone oxidoreductase core subunit V2	1.85
Uqcrfs1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	1.87	Uqcrq	Ubiquinol-cytochrome c reductase, complex III subunit VII	1.78
Ndufv2	NADH:ubiquinone oxidoreductase core subunit V2	1.85	Ndufa3	NADH:ubiquinone oxidoreductase subunit A3	1.77
Pik3cd	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	1.83	Atp5l	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	1.70
Xbp1	X-box binding protein 1	1.79	Cox7a2l	Cytochrome c oxidase subunit 7A2-like	1.55
Uqcrq	Ubiquinol-cytochrome c reductase, complex III subunit VII	1.78	Cox6b1	Cytochrome c oxidase, subunit 6B1	1.51
Ndufa3	NADH:ubiquinone oxidoreductase subunit A3	1.77			
Lep	Leptin	1.59			
Cox7a2l	Cytochrome c oxidase subunit 7A2-like	1.55			
Cox6b1	Cytochrome c oxidase, subunit 6B1	1.51			

Table 9. *Cont.*

Chemical Carcinogenesis–Reactive Oxygen Species (FC = 2.4; *p*-Value = 0.002)		
Symbol	**Gene Name**	**FC**
Ndufc1	NADH:ubiquinone oxidoreductase subunit C1	2.66
Sos2	SOS Ras/Rho guanine nucleotide exchange factor 2	2.50
Ndufa1	NADH:ubiquinone oxidoreductase subunit A1	2.47
Raf1	V-raf-leukemia viral oncogene 1	2.46
Mapk9	Mitogen-activated protein kinase 9	2.42
Jun	Jun proto-oncogene	2.25
Uqcr10	Ubiquinol-cytochrome c reductase, complex III subunit X	1.99
Gstm2	Glutathione S-transferase, mu 2	1.94
Ndufb4	NADH:ubiquinone oxidoreductase subunit B4	1.90
Uqcrfs1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	1.87
Ndufv2	NADH:ubiquinone oxidoreductase core subunit V2	1.85
Pik3cd	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	1.83
Uqcrq	Ubiquinol-cytochrome c reductase, complex III subunit VII	1.78
Ndufa3	NADH:ubiquinone oxidoreductase subunit A3	1.77
Ptpn11	Protein tyrosine phosphatase, non-receptor type 11	1.58
Cox7a2l	Cytochrome c oxidase subunit 7A2-like	1.55
Cox6b1	Cytochrome c oxidase, subunit 6B1	1.51

PPAH—basal diet + 7.5 g of polyherbal phytogenic additive (PPA)/kg DM; PPA—based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera*, and *Azadirachta indica*; FC—fold change.

4. Discussion

4.1. Growth Performance and Dietary Energetics

In the present study, the higher DMI observed could be associated with the presence of nonanoic acid in the PPA used since, according to recent studies [48,49], this acid can be used as a flavoring in foods for domestic animals with positive effects on food intake. Likewise, PPAH supplementation decreased (fold change = −2.11) the expression of the *Tas2r119* gene (taste receptor, type 2, member 119). Lower expression of *Tas2r119* in mammals decreases cholecystokinin production in neuroendocrine cells and leads to higher food intake [50]. In contrast, PPA doses did not affect the variation in DMI between individuals, which indicates that the evaluated PPA does not alter the well-being of the lambs [8,51]. On the other hand, in the current study, higher DMI and ObsNEg were observed in response to PPAH supplementation. Furthermore, previous studies have reported that some bioactive metabolites (carvacrol and PtdCho) and plants (*A. paniculata*) present in the PPA evaluated in the current study increase nutrient digestibility in ruminants [8,15,25]. These effects could result in greater metabolic availability of nutrients and energy to form new body tissues, which would explain the better ADG and FCR detected in PPAH lambs.

In the current study, higher ObsNEm, ObsNEg, OExNEm, and OExNEg were observed in lambs fed the PPAH diet. Similarly, Dorantes-Iturbide et al. [8] observed between 17.7 and 23.2% higher ObsNEm, ObsNEg, OExNEm, and OExNEg in finishing lambs supplemented with low doses (1 and 2 g/kg DM for 56 days) of PPA based on *A. paniculata*, *A. indica*, *Tinospora cordifolia*, *Ocimum sanctum*, and *Whitania somnifera*. The increases in ObsNEm, ObsNEg, OExNEm, and OExNEg detected in lambs fed PPAH could be related to the positively regulated DEGs within the oxidative phosphorylation process since, according to Sieck et al. [52], increased enzymatic activity in oxidative phosphorylation increases energy (ATP) production in ruminant cells. Furthermore, the presence of carvacrol in the PPA used could explain the positive effects detected in ObsNEm, ObsNEg, OExNEm, and OExNEg in the present study. This is because, in finishing lambs, carvacrol increases the rumen production of total volatile fatty acids, which are the primary source of energy in small ruminants [53]. Furthermore, the values of OExNEm and OExNEg detected in lambs fed with PPAH were greater than 1.0, which indicates greater efficiency in the use of dietary energy for maintenance and weight gain [8,54].

4.2. Carcass Traits and Carcass Morphometry

In vivo measurements of LDMA and BFT, and some carcass traits, such as HCW, HCY, CCW, and CCY, are important in the sheep meat industry because they influence the yield of prime cuts (e.g., loin and anterior ribs) [55,56]. In the current study, LDMA increased in response to PPAM and PPAH supplementation; however, BFT, HCW, HCY, CCW, CCY, and YG were not affected by increasing levels (2.5, 5.0, and 7.5 g/kg DM) of PPA added to the diets. The increase in LDMA observed in lambs supplemented with PPAM and PPAH is positive since, according to a recent study [57], LDMA is positively correlated (r = 0.43) with total muscle content in the lamb carcass. The increase in LDMA could be associated with the greater expression of the *Lep* gene observed in the current study since, according to Rosales et al. [58], the *Lep* gene encodes leptin, and serum levels of this hormone have a positive correlation (r = 0.55) with muscle accumulation in lambs.

Morphometric measurements in the sheep carcass can be used in equations to predict the amount of adipose tissue, muscle, and bone [57,59]. In the current study, PPA doses did not affect ELC, ILC, CG, LL, PL, and CI. In recent studies, Dorantes-Iturbide et al. [8] and Orzuna-Orzuna et al. [7] also did not detect changes in ELC, ILC, CG, LL, and PL of finishing lambs supplemented with various doses (between 1 and 3 g/kg DM) of different PPAs in the diet.

Our study's growth performance and carcass trait results imply that high doses (7.5 g/kg DM) of BioCholine® PPA can successfully improve growth rate, feed efficiency, and rib-eye cut quantity in finishing lambs. These effects could lead to increased economic profitability of the fattening process and positively impact the economic income of sheep producers and the meat industry.

4.3. Non-Carcass Components and Meat Quality

In the present study, the weights of lambs' internal and external organs were not affected by supplementation with increasing doses of PPA. These effects indicate that the PPA used can be consumed by finishing lambs without affecting the development of their organs and body tissues [60]. Several previous studies [7–9] also did not detect changes in the weights of internal and external organs of finishing lambs supplemented with increasing doses (between 1 and 3 g/kg DM) of different PPAs containing *A. paniculata*, *A. indica*, *Acacia concinna*, among others.

From the point of view of meat science, the pH, CL, color, and chemical composition of meat influence the quality of sheep meat [61]. In the present study, the doses of PPA evaluated did not alter the pH, CL, color (L*, a*, and b), or chemical composition of the meat, which suggests that the PPA used can be included in lamb diets without affecting the quality of the meat. Previous studies also did not detect changes in pH, CL, color, and chemical composition of meat from finishing lambs supplemented with a PPA (1, 2, and 3 g/kg DM for 56 days) based on *A. concinna*, *Saccharum officinarum*, and *Balanites roxburghi* [9] or with a PPA (5, 10, and 15 g/kg DM for 60 days) based on *Emblica officinalis* and *Ocimum sanctum* [51].

4.4. Blood Metabolites

According to Roland et al. [62], hematological parameters are useful to evaluate health in ruminants because they allow the detection of systemic diseases and disorders of the hematological system. In the current study, the lambs' hematological parameters were unaffected by the PPA doses used. Likewise, the average values of all hematological parameters were within the range reported as normal for clinically healthy growing male Pelibuey lambs [63]. These results indicate that the evaluated PPA can be included in diets for finishing lambs without altering the hematological system of the animals. Similarly, previous studies reported that dietary inclusion of other PPAs (between 1 and 15 g/kg DM) with different combinations of plants that included *A. paniculata*, *A. indica*, *A. concinna*, *S. officinarum*, *B. roxburghi*, *E. officinalis*, and *O. sanctum* also did not affect the normal ranges of hematological parameters in lambs [8,64] and calves [6,27].

Braun et al. [65] indicate that, in sheep, blood chemistry parameters are mainly used as a diagnostic tool for nutritional, liver, and muscle disorders. In the current study, supplementation with PPAM and PPAH reduced glucose, uric acid, creatinine, and bilirubin serum concentrations. However, the average values of these and the other serum metabolites were within the range reported as normal for clinically healthy lambs [66]. This effect suggests that the evaluated PPA can be used in lamb diets without the risk of causing nutritional, liver, and muscle disorders. The lower serum glucose detected in the PPAM and PPAH lambs could be related to the presence of oleic acid in the PPA used since, according to a recent study [67], the intake of oleic acid decreases serum glucose in ruminants through an increase in serum insulin levels. The PPA evaluated also contains p-cymene, an aromatic monoterpene with hypoglycemic effects in mammals [68]. Furthermore, the lower serum concentration of uric acid observed in the current study could be explained by the presence of thymoquinone in the PPA used. This is because thymoquinone inhibits the activity of the enzyme xanthine oxidase, which is necessary to produce uric acid through the breakdown of purines [69].

On the other hand, the lower serum creatinine detected in the current study could be associated with the high linoleic acid content in the PPA evaluated. This is because linoleic acid consumption increases the glomerular filtration rate [70], which is inversely related to serum creatinine [71]. Furthermore, the PPA used in this study contains terpenes (carvacrol and p-cymene), which can inhibit the expression of the *UGT1A9* gene (member A9 of family 1 of UDP glucuronosyltransferase) [72]. This mechanism could explain the lower serum bilirubin observed in lambs fed with PPAM and PPAH since, according to Zhou et al. [73], the *UGT1A9* gene is necessary for the formation of direct and conjugated bilirubin through the glucuronidation process.

4.5. Gene Expression

In the current study, dietary PPAH supplementation decreased the expression of DNA damage-related genes within the biological process of DNA replication. Among these genes, *Prim2* encodes to produce a DNA replication-promoting protein, mismatch repair, and cell activation [74]. Likewise, the genes *Rfc1*, *Rfc3*, and *Rfc4* encode replication factors C that participate in the excision repair of damaged DNA [75]. The gene *Mcm4* encodes one of the six proteins that make up the mini-chromosome maintenance complex essential for DNA strand separation during chromosome replication, an essential process for cell viability [76]. On the other hand, the *Rnaseh2b* gene encodes an RNase H2 responsible for initiating the ribonucleotide excision repair pathway, which is incorporated into genomic DNA in large quantities during replication, causing DNA destabilization [77]. Finally, the *Pold1* gene encodes the catalytic and error-correcting subunit of DNA polymerase delta, which is responsible for the synthesis of lagging-strand DNA during DNA replication [78]. These effects of PPAH intake could improve DNA integrity in lambs, and good DNA integrity is positively associated with better health status in ruminants [22].

The second most down-enriched biological process was tyrosine metabolism, within which several genes (*Aldh3a1*, *Adh4*, *Aox1*, *Fahd1*, *Got2*, *Maoa*) with amino acid oxidative activity are grouped [79]. Among these genes, the *Maoa* gene encodes for the production of monoamine oxidases, which produce hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) when they metabolize dopamine in the cytosol of cells [80]. According to Ahmed and Younus [79], the *Aldh3a1* and *Adh4* genes encode to produce alcohol dehydrogenase and aldehyde dehydrogenase enzymes, which can reduce and inactivate H_2O_2 and ROS in cells. This effect suggests that dietary supplementation with PPAH could help to decrease oxidative stress (OS) in finishing lambs.

Within the biological process of drug metabolism cytochrome P450, dietary supplementation with PPAH decreased the expression of the genes *Gsta3*, *Gsta4*, *Gstm3*, *Fmo1*, and *Fmo2*. The genes *Gsta3*, *Gsta4*, and *Gstm3* belong to the detoxification enzyme family Glutathione S-Transferase, and a reduction in their expression could decrease the conjugation of glutathione into toxins for their excretion [81]. Likewise, the genes *Fmo1* and *Fmo2*

encode for NADPH-dependent flavin monooxygenases, which are enzymes that catalyze the oxidation of drugs, pesticides, and xenobiotics [82].

Within the intestinal immune network for IgA production, dietary supplementation with PPAH decreased the expression of the genes *Tgfβ1*, *Cd40lg*, *Il6*, *Tnfsf13*, *H2-Oa*, *Cxcl12*, and *Tnfrsf13b*. IgA is the main isotype in the intestine and is responsible for maintaining intestinal homeostasis [83]. According to Inamine and Schnabl [84], any disruption in the intestine directly affects the liver because the liver receives the products absorbed in the intestine via the portal circulation. In contrast, liver tissue can alter intestinal homeostasis through the secretion of hepatic IgA and bile acids [85]. In the current study, the gene with the greatest reduction in response to PPAH supplementation was *Tgfβ1*. In mammals, *Tgfβ1* plays an important role in systemic IgA expression and production, but overproduction of *Tgfβ1* can induce fibrosis in the liver, kidney, and lung [86]. Leite et al. [87] mention that low expression of the *Cd40lg* gene decreases serum IgA levels and causes defects in T-cell proliferation. Likewise, lower expression of the *Il6* gene negatively affects IgA secretion [88]. In addition, the genes *Tnfsf13*, *H2-Oa*, *Cxcl12*, and *Tnfrsf13b* are related to B cell activating factors as IgA precursors [89].

On the other hand, dietary supplementation with PPAH decreased the expression of several genes (*Tgfβr2*, *Ltbp1*, *Tgfβ1*, *E2f5*, *Bmpr1a*, *Smurf2*, *Tgfβ2*, *Acvr2a*, *Acvr1b*, and *Inhba*) within the biological process of TGF-β signaling. Among these genes, the most biologically important are *Tgfβr1*, *Tgfβr2*, *Acvr2a*, and *Acvr1b* since, according to Utoh et al. [90], the genes of the activin family, such as *Acvr2a* and *Acvr1b*, are potent inhibitors of hepatocyte proliferation. Likewise, Morikawa et al. [86] mention that the genes *Tgfβr1* and *Tgfβr2* act as potent immunosuppressive cytokines.

Within the biological process of amoebiasis, dietary supplementation with PPAH decreased the expression of several genes, including *Il6*, *Serpinb9c*, *Rab5a*, *Muc2*, *Rab7*, *Lamb1*, and *Lama1*. Portunato et al. [91] mention that amoebiasis is an infection caused by some protozoa that reach the liver through the hepatic portal circulation and form abscesses. A low concentration of *Il6* facilitates the development of abscesses in the liver [92], while a low expression of *Serpinb9c* increases the sensitivity of hepatocytes to death by natural killer cells [93]. Verma et al. [94] state that the *Rab5a* and *Rab7* genes are required for the biogenesis of giant endocytic vacuoles in the liver. Likewise, the *Muc2* gene encodes for the mucin 2 protein, which is necessary for the synthesis of a biofilm essential for the colonization and invasion of bacteria [95]. In addition, the *Lamb1* and *Lama1* genes are related to the progression of liver fibrosis [94].

Within the biological process of non-alcoholic fatty liver disease, dietary supplementation with PPAH increased the expression of the *Lep* and *Xbp1* genes. The *Lep* gene encodes for the production of leptin, which is an anti-steatotic hormone that stimulates lipid mobilization and prevents its accumulation in the liver [96]. Likewise, the *Xbp1* gene encodes for the X-box binding protein 1, which has a hepatoprotective function against non-alcoholic steatohepatitis [97]. These effects suggest that dietary supplementation with PPAH could improve liver health in finishing lambs. The PPA used in the current study contains PtdCho, which is essential for the export of triglycerides from the liver [25]. However, non-alcoholic fatty liver disease develops when the amount of fatty acids, lipogenesis, or the synthesis of triglycerides from diglycerides exceeds the export or oxidation of fatty acids [98].

In small ruminants, when the oxidative activity of ROS exceeds the neutralizing capacity of the body's antioxidant defense system, OS is produced [99]. According to Carvajal [100], most ROS are generated at the mitochondrial level in complexes I and III of the electron transport chain through the transfer of electrons to molecular oxygen. In the current study, within the biological processes of oxidative phosphorylation and chemical carcinogenesis, dietary supplementation with PPAH increased the expression of genes involved in the electron transport chain at the level of complex I–NADH:ubiquinone oxidoreductase (*Ndufc1*, *Ndufa1*, *Ndufb4*, *Ndufv2*, *Ndufa3*), complex III–cytochrome c reductase (*Uqcr10*, *Uqcrfs1*, *Uqcrq*), and complex IV–cytochrome c oxidase (*Cox7a2l*, *Cox6b1*). An increased expression of genes in complex I (NADH:ubiquinone oxidoreductase) of the

electron chain suggests that dietary supplementation with PPAH could increase oxidative efficiency in finishing lambs since, according to Weiss et al. [101], NADH:ubiquinone oxidoreductase is one of the two rate-limiting enzymes for substrate de-electronation. Likewise, increased expression of genes in the NADH:ubiquinone oxidoreductase complex could decrease the production of some ROS (e.g., superoxides) in lamb cells since, according to Bazil et al. [102], the NADH:ubiquinone oxidoreductase complex is one of the sites with the highest electron leak before oxygen is reduced to water by cytochrome c oxidase.

On the other hand, a higher expression of the genes of the enzymatic complexes I, III, and IV of the electron transport chain is positively correlated with a higher mitochondrial respiratory capacity [103]. Ruminants' high mitochondrial respiration rate is associated with better feed efficiency [104]. Furthermore, dietary supplementation with PPAH increased the expression of the *Atp5l* gene within oxidative phosphorylation. This effect suggests that dietary supplementation with PPAH improves metabolic energy production in lambs because the *Atp5l* gene encodes for the G subunit of mitochondrial ATP synthase, which helps to synthesize ATP from ADP and inorganic phosphate [105].

5. Conclusions

Dietary supplementation with 7.5 g/kg DM of PPA (BioCholine®) improves dry matter intake, weight gain, feed efficiency, dietary energy utilization efficiency, and *Longissimus dorsi* muscle area without altering other carcass traits, meat quality, or blood metabolites. Likewise, gene expression analysis indicates that supplementation with high doses (7.5 g/kg DM) of BioCholine® could improve metabolic energy production and antioxidant status in finishing lambs.

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Informed Consent Statement: Not applicable.

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

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Appendix A

Table A1. Ingredients and nutrient composition of the basal diets.

Ingredients, g/kg of DM	Treatments			
	CON	**PPAL**	**PPAM**	**PPAH**
Oat straw	194	194	194	194
Ground corn	303	300.5	298	295.5
Ground sorghum	241	241	241	241
Soybean meal	81	81	81	81
Wheat bran	71	71	71	71
Corn gluten	74	74	74	74
Bypass fat	23	23	23	23
Common salt	5	5	5	5
Vitamin and mineral premix [a]	5	5	5	5
Calcium carbonate	3	3	3	3
Polyherbal phytogenic additive (PPA) [b]	0	2.5	5	7.5
Total	1000	1000	1000	1000
Nutrient composition, g/kg of DM				
Dry matter	892.3	893.2	888.0	889.5
Crude protein	158.1	157.9	157.7	157.4
Ether extract	25.5	25.4	25.3	25.3
Neutral detergen fiber	271.2	273.8	272.3	276.2
Acid detergent fiber	142.1	143.4	142.8	144.6
Ashes	55.2	54.2	49.6	50.1
Calculated net energy, Mcal/kg				
Maintenance [c]	1.79	1.79	1.79	1.79
Gain [c]	1.25	1.25	1.25	1.25

CON: basal diet without polyherbal phytogenic additive (PPA); PPAL: basal diet + 2.5 g of PPA/kg of DM; PPAM: basal diet + 5 g of PPA/kg of DM; PPAH: basal diet + 7.5 g of PPA/kg of DM; [a] vitamin and mineral premix (Ovino plus, Vitasal®, Texcoco, Mexico): calcium, 24%; chlorine, 12%; sodium, 8%; phosphorus, 3%; magnesium, 2%; potassium, 0.5%; sulfur, 0.5%; antioxidant, 0.5%; Lasalocid, 2000 ppm; zinc, 5000 ppm; manganese, 4000 ppm; iron, 2000 ppm; iodine, 100 ppm; selenium, 30 ppm; cobalt, 60 ppm; chromium, 5 ppm; vitamin A, 500 000 UI; vitamin D, 150 000 UI; vitamin E, 1000 UI. [b] BioCholine® based on *Trachyspermum ammi*, *Andrographis paniculata*, *Achyrantes aspera, and Azadirachta indica*. [c] The calculation is based on NRC [28].

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7. CONCLUSIONES GENERALES

Los aceites esenciales, flavonoides, ácidos hidroxicinámicos y el fitogénico poliherbal BioCholine® (7.5 g kg^{-1} MS) mejoran el comportamiento productivo y las características de la canal en bovinos, así como en corderos en finalización. Asimismo, los aceites esenciales y flavonoides mejoran los parámetros ruminales al disminuir la concentración ruminal de protozoarios totales y nitrógeno amoniacal y, al mismo tiempo, incrementar la concentración ruminal de propionato. Además, la suplementación dietética con aceites esenciales y flavonoides mejora la calidad de la carne y de la leche en bovinos, mientras que las algas marinas disminuyen las emisiones de metano entérico en vacas lecheras.