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

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Abstract: The environmental sustainability of beef production is a significant concern within the food production system. Tannins (TANs) can be used to minimize the environmental impact of ruminant production because they can improve ruminal fermentation and ruminants’ lifetime performances and mitigate methane (CH₄) emissions. The objective of this study was to evaluate the effects of dietary supplementation with TANs as sustainable natural alternative to reduce the environmental impact on growth performance, rumen fermentation, enteric CH₄ emissions, and nitrogen (N) use efficiency of beef cattle through a meta-analysis. A comprehensive search of studies published in scientific journals that investigated the effects of TANs’ supplementation on the variables of interest was performed using the Scopus, Web of Science, and PubMed databases. The data analyzed were extracted from 32 peer-reviewed publications. The effects of TANs were assessed using random-effects statistical models to examine the standardized mean difference (SMD) between TANs’ treatments and control (non-TANs). The heterogeneity was explored by meta-regression and subgroup analysis was performed for the covariates that were significant. TANs’ supplementation did not affect weight gain, feed consumption, feed efficiency, or N use efficiency (p > 0.05). However, it reduced the concentration of ammonia nitrogen in rumen (SMD = −0.508, p < 0.001), CH₄ emissions per day (SMD = −0.474, p < 0.01) and per unit dry matter intake (SMD = −0.408, p < 0.01), urinary N excretion (SMD = −0.338, p < 0.05), and dry matter digestibility (SMD = −0.589, p < 0.001). Ruminal propionate (SMD = 0.250) and butyrate (SMD = 0.198) concentrations and fecal N excretion (SMD = 0.860) improved in response to TANs’ supplementation (p < 0.05). In conclusion, it is possible to use TANs as a CH₄ mitigation strategy without affecting cattle growth rate. In addition, the shift from urinary to fecal N may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N. Therefore, the addition of tannins in the diet of beef cattle could be used as a sustainable natural alternative to reduce the environmental impact of beef production.

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

1. Introduction

Minimizing enteric methane (CH₄) emissions from ruminant production while improving feed conversion efficiency and growth rate is a goal for sustainable livestock production [1]. In addition, the nitrogen (N) excreted by ruminants is the main source of nitrous oxide (N₂O) emissions in livestock systems [2] and can contribute to air and water pollution [3]. Therefore, strategies based on changing the composition and concentration of
urinary compounds by diet manipulation could be considered potential options to mitigate urine N₂O emissions and consequently improve sustainability in ruminant production [4]. Among these strategies, dietary tannins’ (TANs’) supplementation has received special attention, particularly in ruminants [5]. TANs are a group of polyphenolic compounds that are present in a wide variety of plants and can have positive effects in animals, such as antimicrobial, antiparasitic, antioxidant, anti-inflammatory, and immunomodulatory [5]. According to Naumann et al. [6], TANs are generally classified based on their chemical structure into two groups: condensed tannins (CTs) and hydrolysable tannins (HTs). CTs consist of flavan-3-ol subunits linked together to form oligomers and polymers, whereas HTs are esters of gallic or ellagic acid linked to a polyol core [6].

In ruminants, previous studies [7–9] have shown that dietary supplementation with TANs improves the utilization efficiency of ingested feed. In addition, TANs have been successfully used to reduce enteric CH₄ production, urinary N excretion, and N₂O emissions [7,10] and to increase the duodenal flux of microbial protein and amino acids [11]. TANs-rich plants and TANs’ extracts have also shown positive impact on rumen microbial activity [12], ruminal fermentation rate [10], antioxidant status, and health of ruminants [13,14]. However, TANs can also reduce the digestion of protein in the rumen and the entire gastrointestinal tract [15]. Therefore, the intake of TANs in combination with a medium-poor quality diet (e.g., insufficient crude protein in the diet) may not generate nutritional benefits and is detrimental to performance ([6,8,15]. For example, some studies have reported negative effects of dietary supplementation with TANs on digestibility, productive performance, and ruminal fermentation [2,16], while other studies have not observed significant effects on digestibility, productive performance, CH₄ emissions, and urinary and fecal nitrogen excretion in response to TAN supplementation [7,11,16].

Particularly, in beef cattle, several studies have been conducted to evaluate the effect of dietary supplementation with TANs on the growth performance [17,18], nutrient intake and digestibility [19,20], ruminal parameters [21,22], enteric CH₄ emissions [23,24], and urinary and fecal N excretion [17,21]. However, the results observed to date have been non-conclusive because their effects vary widely, even within the same plant species [14]. The variations in the chemical and botanical origin of TANs, processing methods, feeding conditions, physiological state of animals, and supplementation levels used are factors that could contribute to the variability of the effects observed in animals supplemented with TANs [5,14,25]. Therefore, identifying and controlling this variability is a key aspect in the development of TANs-containing products that can be used as feed additives to improve the sustainability of beef production.

Although some classical reviews [5,6,14,25] previously suggested that dietary supplementation with TANs can improve productivity and decrease enteric CH₄ production in ruminants, these studies did not use a meta-analytical approach and none focused only on beef cattle. Meta-analysis (MA) is a statistical tool that allows combining and synthesizing data published in different studies in a quantitative way [26–28]. In addition, MA can be used to explore sources of heterogeneity, which provides additional information on factors contributing to the variability of the observed results [29], and it also helps to identify potential areas for further research [26]. MA has been frequently used in clinical and biomedical research, but its implementation in animal science-related research is still limited [30]. The objective of this meta-analysis was to evaluate the effect of dietary supplementation with tannins as sustainable natural alternative to reduce the environmental impact on the growth performance, nutrient intake and digestibility, ruminal parameters, enteric CH₄ emissions, and nitrogen use efficiency of beef cattle. In addition, we examined the heterogeneity of the responses by meta-regression analysis to identify factors contributing to the variability observed in the response variables.
2. Materials and Methods

2.1. Literature Search and Study Selection

A comprehensive literature search in the scientific databases of Web of Science, Scopus, and PubMed was carried out to identify studies that investigated the effect of TANs’ supplementation on growth performance, nutrient intake and digestibility, ruminal fermentation, and enteric CH$_4$ emissions in beef cattle. In all databases, the keywords “tannin, chestnut, quebracho, leucaena, birdsfoot, lotus, sainfoin, onobrychis, sulla, hedysarum, proanthocyanidin, growth, digestibility, fermentation, methane, bull, steer and cattle” were used, among which were TANs and the most common TANs-containing plants [31]. A total of 613 scientific publications published between 2010 and 2020 were identified. These publications went through a two-step selection process, as previously described by Herremans et al. [31]. First, a selection was performed using titles and abstracts excluding in vitro and simulation studies, reviews, and articles that did not measure the variables of interest. Subsequently, to be considered, studies had to meet several inclusion criteria previously reported by other authors [31,32]: (1) studies on adult (male, weaned or older) and confined beef cattle; (2) data on growth performance, nutrient intake and digestibility, ruminal fermentation, urinary and fecal excretion, or in vivo CH$_4$ emissions (measured with respirometry chambers, the sulfur hexafluoride “SF6” tracer technique, or the Green-Feed system (C-Lock Inc., Rapid City, SD, USA)); (3) similarity between control and experimental groups, except for the presence of TANs; (4) quantification or possible determination of dietary TANs’ doses; (5) peer-reviewed journal articles written in English; (6) experimental design employed (rotating or continuous); (7) least squares means of the control and experimental groups with variability measures (standard error or standard deviation); and (8) sample size used.

2.2. Data Extraction

Based on the selection criteria, only 32 articles were included in the database for the final analysis. The response variables extracted for the meta-analysis included daily weight gain, feed efficiency (determined as weight gain/feed intake (G:F), kg/kg), final body weight, intake and digestibility of dry matter (DM) and nutrients (organic matter, crude protein, ether extract, neutral detergent fiber digestibility, and acid detergent fiber digestibility), ruminal parameters (ruminal concentration of propionate, butyrate, acetate, total volatile fatty acids, ammonia nitrogen, and protozoa), in vivo CH$_4$ emissions (per day and per unit of dry matter intake), and urinary and fecal N excretion. Moreover, when available, additional data were collected, such as characteristics of the published study (author, year of publication), amount of forage in the diet (g/kg DM), source of chemical or botanical origin of TANs, experimental design used (rotational or continuous), period of TANs’ supplementation (days), chemical composition of diet, number of replicates, type of TANs (CTs, HTs, or mixture of both), method of TANs’ inclusion (extract or naturally present in the diet), and amount of TANs in the diet (g/kg DM). The references of the articles included in the data set are listed in Table A1 in Appendix A. Averages, standard deviation (SD), and number of repetitions for each treatment were extracted from these articles. When the articles presented the SD of each experimental group, these values were used directly in the meta-analysis. In cases where the SD was not reported, it was calculated by multiplying the standard error means (SEM) by the square root of the sample size, using the equation $SD = SEM \times \sqrt{n}$, as previously reported by Higgins and Thomas [33], where $n = \text{number of replicates}$.

2.3. Calculations and Statistical Analysis

Regarding the data involved in the meta-analysis and meta-regression, these were analyzed using the Open Meta-analyst for Ecology and Evolution software [34] and the statistical software R (version 3.6.3) using the “metafor” package [35]. The response variables were analyzed through the standardized mean difference (SMD), also called effect size (ES), in which the difference between the means of the experimental and control groups
was standardized using the SD of the groups with and without TANs [36]. The SMDs were calculated using the methods previously described by DerSimonian and Laird [37] for random effects models. The SMD is a more robust estimation of the ES when there is heterogeneity in the data set [38]. On the other hand, using the SAS statistical program [39], the chemical composition variables of the diets and the response parameters extracted were analyzed with the MEANS procedure to obtain descriptive statistics values. Differences in the composition of the diets of the control and TANs-supplemented treatments were evaluated by the MIXED procedure, using the studies as random effect and Tukey’s test to detect differences between treatments, as previously reported by Torres et al. [40].

2.4. Heterogeneity

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

2.5. Publication Bias

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

2.6. Meta-Regression

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

3.1. Study Attributes and Excluded Studies

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

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

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

| Parameter Dietary Features | NC | Mean | Minimum | Maximum | SD |
|----------------------------|----|------|--------|---------|----|
| Forage g/kg DM             | 105| 506.9| 509.1  | 498.0   | 425.0|
| DM, g/kg                  | 80 | 647.8| 700.0  | 600.0   | 425.0|
| OM, g/kg DM               | 50 | 927.5| 928.3  | 936.0   | 935.0|
| CP, g/kg DM               | 105| 124.4| 132.5  | 134.5   | 130.5|
| EE, g/kg DM               | 61 | 38.3| 32.10  | 35.50   | 30.10|
| NDF, g/kg DM              | 97 | 430.5| 423.7  | 404.4   | 358.9|
| ADF, g/kg DM              | 73 | 259.1| 259.7  | 226.5   | 211.7|
| Starch, g/kg DM           | 31 | 184.6| 184.6  | 184.6   | 184.6|
| Ca, g/kg DM               | 41 | 6.18 | 6.18   | 6.18    | 0.836|
| P, g/kg DM                | 41 | 4.11 | 4.11   | 4.11    | 0.313|
| Duration, days            | 99 | 93   | 90     | 28      | 12.29|
| FBW, kg                   | 31 | 457.5| 458.2  | 443.5   | 437   |
| DMI, kg/d                 | 73 | 8.357| 8.136  | 8.20    | 7.48  |
| OML, kg/d                 | 46 | 6.837| 6.820  | 6.540   | 6.18  |
| CPI, kg/d                 | 26 | 0.828| 0.957  | 0.900   | 0.900 |
| EEL, kg/d                 | 8  | 0.823| 0.823  | 0.823   | 0.823 |
| NDFI, kg/d                | 38 | 3.679| 3.524  | 3.760   | 3.679 |
| ADFI, kg/d                | 17 | 2.521| 2.453  | 2.850   | 2.521 |
| ADG, kg/d                 | 37 | 1.258| 1.273  | 1.370   | 1.258 |
| FE, kg/kg                 | 22 | 0.153| 0.150  | 0.160   | 0.150 |
| DMD, g/kg DM              | 49 | 622.0| 594.3  | 628.0   | 628.0 |
| OMD, g/kg DM              | 59 | 660.1| 632.0  | 660.0   | 646.3 |
| CPD, g/kg DM              | 43 | 571.6| 541.2  | 679.0   | 635.0 |
| EED, g/kg DM              | 23 | 689.4| 679.4  | 713.0   | 699.0 |
| NDFD, g/kg DM             | 47 | 561.4| 534.9  | 576.0   | 518.1 |
| ADFD, g/kg DM             | 24 | 494.1| 415.4  | 532.0   | 413.6 |
| Ruminal pH                | 57 | 6.637| 6.621  | 6.700   | 6.680 |
| NH3-N, mg/dL              | 57 | 11.25| 10.39  | 10.63   | 8.16  |
| Total VFA, mM             | 54 | 84.72| 86.49  | 74.01   | 78.42 |
| Acetate, % molar          | 54 | 60.39| 60.67  | 67.80   | 66.09 |
| Propionate, % molar       | 54 | 19.39| 19.85  | 18.74   | 18.47 |
| Butyrate, % molar         | 54 | 11.94| 12.38  | 10.33   | 11.79 |
| Protozoa, log10/mL        | 26 | 5.508| 5.306  | 5.480   | 5.595 |
| CH4, L/d                  | 26 | 150.6| 135.7  | 128.8   | 107.0 |
| CH4, L/DMI                | 28 | 19.93| 18.76  | 20.10   | 14.78 |
Table 1. Cont.

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

NC: number of comparisons; SD: standard deviation; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; Ca: calcium; P: phosphorus; ADG: average daily gain; FE: feed efficiency; FBW: final body weight; DMI: DM intake; OMI: OM intake; CPI: CP intake; NDFI: NDF intake; ADFI: ADF intake; EDF: EE intake; EED: EE digestibility; NEE: NE digestibility; NH3-N: nitrogen ammonia; VFA: volatile fatty acids; CH4: methane; FE: determined as weight gain/feed intake (G:F), kg/kg; UNE: urinary nitrogen excretion; FNE: fecal nitrogen excretion; NUE: nitrogen use efficiency; a, b: in the same row (only applies to dietary features), means followed by different letters differ significantly by the Tukey’s test (p < 0.05).

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

### 3.2. Growth Performance and Nutrient Intake

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

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

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

### 3.3. Digestibility, Ruminal Parameters, and Methane Emissions

There were no significant effects of TANs’ inclusion in beef cattle diets (p > 0.05) for ether extract digestibility (EED), ruminal pH, ruminal concentration of total volatile fatty acids (VFA), acetate and protozoa, or for nitrogen use efficiency (NUE; Table 3). However, we observed a negative impact (p < 0.05) of TANs’ inclusion in the diets on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), and acid detergent fiber digestibility (ADFD). On the other hand, rumen propionate, butyrate concentration, and fecal nitrogen excretion...
(FNE) increased \((p < 0.05)\) in response to TANs’ supplementation. We observed a positive impact (reduction) of TANs’ inclusion \((p < 0.05)\) in the diets for ruminal ammonia nitrogen concentration \((\text{NH}_3\text{-N})\), urinary nitrogen excretion (UNE), and for enteric CH\(_4\) emissions per day (MED) and per unit of dry matter intake (MEDMI; Table 3).

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

| Parameter                                      | N   | NC  | SMD    | SE  | 95% CI Lower | 95% CI Upper | p-Value | Q     | p-Value | I\(^2\) (%) |
|------------------------------------------------|-----|-----|--------|-----|--------------|--------------|---------|-------|---------|-------------|
| Dry matter digestibility                      | 17  | 49  | −0.589 | 0.124 | −0.833       | −0.346       | <0.001  | 97.833| <0.001  | 50.94       |
| Organic matter digestibility                  | 21  | 59  | −0.612 | 0.108 | −0.825       | −0.400       | <0.001  | 108.599| <0.001  | 46.59       |
| Crude protein digestibility                   | 15  | 43  | −0.903 | 0.210 | −1.315       | −0.492       | <0.001  | 173.687| <0.001  | 75.82       |
| Ether extract digestibility                   | 8   | 23  | −0.328 | 0.215 | −0.750       | 0.094        | 0.12    | 61.615| <0.001  | 64.29       |
| NDFD                                          | 20  | 57  | −0.508 | 0.128 | −0.759       | −0.258       | <0.001  | 148.223| <0.001  | 62.22       |
| ADFD                                          | 19  | 54  | 0.041  | 0.115 | −0.184       | 0.267        | 0.72    | 120.090| <0.001  | 55.87       |
| Ruminal pH                                     | 19  | 54  | 0.250  | 0.107 | 0.040        | 0.460        | 0.02    | 103.404| <0.001  | 48.74       |
| Ruminal NH\(_3\)-N                            | 19  | 54  | 0.198  | 0.079 | 0.042        | 0.354        | 0.01    | 61.204| 0.20    | 13.40       |
| Total VFA                                      | 26  | 82  | 0.745  | 0.397 | −1.523       | 0.033        | 0.06    | 235.732| <0.001  | 89.39       |
| Methane emissions/day                          | 9   | 26  | −0.474 | 0.155 | −0.178       | −0.171       | 0.002   | 50.007| <0.05   | 48.01       |
| Methane emissions/unit of DMI                 | 10  | 28  | −0.408 | 0.155 | −0.712       | −0.105       | 0.008   | 56.848| <0.001  | 52.50       |
| Urinary nitrogen excretion                    | 12  | 35  | −0.338 | 0.149 | −0.630       | −0.046       | 0.023   | 83.931| <0.001  | 59.49       |
| Fecal nitrogen excretion                      | 11  | 31  | 0.860  | 0.138 | 0.589        | 1.131        | <0.001  | 48.304| 0.018   | 37.89       |
| Nitrogen use efficiency                       | 8   | 22  | −0.273 | 0.262 | −0.786       | 0.239        | 0.296   | 75.726| <0.001  | 72.27       |

\(N\): number of studies; \(NC\): number of comparisons; \(SMD\): standardized mean difference; \(CI\): confidence interval of \(SMD\); \(SE\): standard error; \(Q\): chi-squared statistic and associated significance level \((p\)-value\); \(I^2\): percentage of variation; \(NDFD\): neutral detergent fiber digestibility; \(ADFD\): acid detergent fiber digestibility; \(\text{NH}_3\)-N: ammonia nitrogen; \(\text{VFA}\): volatile fatty acids; \(\text{DMI}\): dry matter intake.

### 3.4. Analysis of Publication Bias

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

### 3.5. Meta-Regression

Significant heterogeneity \((Q)\) was observed for DMI, FE \((p < 0.05); Table 2\), DMD, OMD, CPD, EED, NDFD, ADFD, ruminal pH, ruminal \(\text{NH}_3\)-N concentration, total VFA, acetate, propionate and protozoa, MED, and MEDMI, as well as for UNE and FNE \((p < 0.001); Table 3\). Although significant heterogeneity existed, it is not advisable to use meta-regression when there are fewer than 10 studies that reported the response variable of interest [43]. Consequently, this analysis was only performed for the variables DMI, DMD,
OMD, CPD, NDFD, ruminal pH, ruminal concentration of NH$_3$-N, total VFA, acetate and propionate, MEDMI, and UNE as well as for the FNE.

Except for age, there was no significant relationship ($p > 0.05$) between DMI and the moderators used (level of supplementation, period of supplementation, type of TANs, method of TANs’ supply, source of botanical or chemical origin of TANs, NDF content in the diet, and experimental design). The dose of TANs supplied in the diets explained 63.4, 69.1, 25.8, 33.4, 17.2, and 31.7% of the observed heterogeneity for DMD, OMD, NDFD, ruminal acetate and propionate concentration, and FNE, respectively ($p < 0.05$). The period of TANs’ supplementation only had a significant relationship ($p < 0.05$) with the MEDMI, explaining only 21.95% of the observed heterogeneity. The type of TANs explained ($p < 0.05$) 7.25, 7.16, 19.5, 6.7, and 17.4% of the observed heterogeneity in CPD, NDFD, NH$_3$-N, total VFA, and UNE, respectively. A significant relationship ($p < 0.05$) was observed between CPD and MEDMI with the method of inclusion of TANs in the diet (extract or naturally present in plant parts), where the inclusion method explained 14.5, 23.2, 70.3, and 84.85% of the observed heterogeneity in CPD, MEDMI, UNE, and FNE, respectively. The source of botanical or chemical origin of TANs explained ($p < 0.05$) 48.7, 13.3, 83.7, 17.3, 18, 61, 82.3, and 100% of the heterogeneity observed in CPD, NDFD, NH$_3$-N, total VFA, propionate, MEDMI, UNE, and FNE, respectively. A significant relationship ($p < 0.05$) was observed between CPD and MEDMI with the NDF content of the diets, where variation in NDF content explained 16 and 48.8% of the heterogeneity observed in CPD and MEDMI, respectively. The experimental design used (rotating or continuous) explained ($p < 0.05$) 19.8, 42, 29.7, 48.2, and 33.1% of the observed heterogeneity for DMD, OMD, NDFD, ruminal pH, and MEDMI, respectively. The age ($\leq$ 12 and > 12 months old) explained ($p < 0.05$) 31.4, 100, 49.7, 55.1, and 79.7% of the heterogeneity observed in DMI, CPD, ruminal pH, NH$_3$-N, and total VFA, respectively.

3.6. Subgroup Analysis

Regarding the type of TANs, supplementation with HTs and mixture of CTs with HTs decreased CPD ($p < 0.001$), while there was no change in CPD in animals supplemented with CTs ($p > 0.05$; Figure 1). NDFD decreased (SMD = $-0.633$; $p < 0.001$) in beef cattle supplemented with CTs, but there was no change in NDFD with supplementation of HTs and mixture of CTs with HTs ($p > 0.05$; Figure S7). Ruminal NH$_3$-N concentration decreased ($p < 0.001$) with supplementation of HTs (SMD = $-0.980$) and mixture of CTs with HTs (SMD = $-0.582$). However, NH$_3$-N was not affected in animals supplemented with CTs ($p > 0.05$; Figure S8). The ruminal concentration of total VFA increased in study animals using CTs (SMD = $0.253$; $p = 0.04$) but decreased when using HTs (SMD = $-0.491$; $p = 0.03$). No significant changes in ruminal concentration of total VFA were observed in study animals using mixtures of CTs and HTs ($p > 0.05$; Figure 2). UNE decreased with supplementation of HTs and mixture of CTs (SMD = $-0.445$; $p = 0.03$) with HTs (SMD = $-0.900$; $p < 0.001$). However, UNE was not affected in animals supplemented with CTs (SMD = $-0.338$; $p > 0.05$).

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

Figure 4 shows that NDFD decreased ($p < 0.05$) only when TANs came from Acacia mearnsii and quebracho. Ruminal NH$_3$-N concentration was not affected by TANs when they came from a mixture of plants ($p > 0.05$). However, it increased when the TANs came from Leucaena leucocephala (SMD = 76.47; $p < 0.001$) and decreased in studies using Acacia mearnsii, quebracho, chestnut, pistachio, and tannic acid as a source of TANs ($p < 0.05$; Figure S9).
Figure 1. Forest plot of the effect size or standardized mean difference and 95% confidence interval of tannin type on crude protein digestibility (CPD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of total CPD, while points to the right of the line indicate increase in total CPD concentration.
Figure 2. Forest plot of effect size or standardized mean difference and 95% confidence interval of tannin type on ruminal concentration of total volatile fatty acids (VFA) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of total VFA, while points to the right of the line indicate increase in total VFA concentration.
Figure 3. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on crude protein digestibility (CPD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduced CPD, while points to the right of the line indicate increased CPD.
Figure 4. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on neutral detergent fiber digestibility (NDFD) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduced NDFD, while points to the right of the line indicate increased NDFD.

Supplementation with TANs decreased the concentration of total VFA in ruminal liquid when tannic acid was the source of chemical origin of the TANs (SMD = −0.886; p = 0.004). On the other hand, the ruminal concentration of total VFA increased (SMD = 0.431; p = 0.018) when quebracho was used as the source of TANs (Figure 5).

Dietary supplementation with TANs increased ruminal propionate concentration only when TANs were obtained from quebracho tree and pistachio (p < 0.001), while ruminal
propionate concentration was reduced (SMD = \(-1.104\); \(p = 0.046\)) when TANs were from plant mixtures (Figure 6).
Figure 6. Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of chemical or botanical origin of tannin on ruminal propionate concentration in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction of propionate, while points to the right of the line indicate increase of propionate.
Dietary supplementation with TANs significantly reduced MEDMI only in animals from studies using tannic acid and *Leucaena leucocephala* as a source of TANs ($p < 0.001$; Figure 7).

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

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

![Figure 7](image-url)

**Figure 7.** Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical or chemical origin of tannin on enteric methane emissions per unit dry matter intake (MEDMI) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in MEDMI, while points to the right of the line indicate increase in MEDMI.
**Figure 8.** Forest plot of the effect size or standardized mean difference and 95% confidence interval of the source of botanical or chemical origin of tannin on urine nitrogen excretion (UNE) in beef cattle. The solid vertical black line represents the mean difference of zero or no effect. Points to the left of the solid vertical line represent reduction in UNE, while points to the right of the line indicate increase in UNE.

| Studies                              | Estimate (95% CI) |
|--------------------------------------|------------------|
| Abrego et al. 2010-1                 | -0.888 (-1.730, 0.013) |
| Abrego et al. 2010-2                 | -0.794 (-1.636, 0.049) |
| Abrego et al. 2010-3                 | -0.913 (-1.759, 0.032) |
| Subgroup OECD (P=0.46% , Ph=0.464)   | -0.931 (-1.867, -0.035) |
| Avesta et al. 2015-1                 | -0.216 (-1.026, 0.015) |
| Avesta et al. 2015-2                 | -0.030 (-0.880, 0.820) |
| Koeing and Bøhnchen 2016             | -1.287 (-2.644, 0.1) |
| Ortegal et al. 2015-1                | -0.428 (-1.827, 0.970) |
| Ortegal et al. 2015-2                | -0.937 (-2.397, 0.520) |
| Ortegal et al. 2015-3                | -0.871 (-2.347, 0.605) |
| Tasci et al. 2015-1                  | -0.398 (-1.558, 0.762) |
| Tasci et al. 2015-2                  | -0.787 (-2.004, 0.430) |
| Tasci et al. 2015-3                  | -1.267 (-2.341, -0.207) |
| Subgroup Acacia marina (P=0.4% , P=0.409) | -0.779 (-1.678, -0.079) |
| Elbert et al. 2017-1                 | 0.212 (0.725, 1.198) |
| Elbert et al. 2017-2                 | -0.462 (-1.887, 0.963) |
| Elbert et al. 2017-3                 | 0.346 (-0.505, 1.207) |
| Elbert et al. 2017-4                 | -0.894 (-2.058, 0.280) |
| Horner et al. 2005-1                 | -0.125 (-1.569, 0.325) |
| Horner et al. 2005-2                 | -0.358 (-1.557, 0.841) |
| Horner et al. 2005-3                 | -0.489 (-1.474, 0.504) |
| Horner et al. 2005-4                 | -0.524 (-1.628, 0.580) |
| Horner et al. 2005-5                 | -0.937 (-1.999, 0.134) |
| Horner et al. 2005-6                 | -1.276 (-2.655, -0.083) |
| Subgroup Guayacans (P=0.4% , P=0.490) | -0.381 (-1.486, 0.724) |
| Pifre-Palquis et al. 2015a-1         | 0.635 (0.219, 1.093) |
| Pifre-Palquis et al. 2015a-2         | 0.848 (0.607, 1.125) |
| Pifre-Palquis et al. 2015a-3         | 0.398 (0.120, 0.684) |
| Pifre-Palquis et al. 2015a-4         | 0.758 (0.505, 1.014) |
| Subgroup Lecanea racemosa (P=0.4% , P=0.57B) | -0.378 (0.900, 1.658) |
| Overall (P=0.4% , P=0.001)           | -0.361 (-0.839, 0.117) |

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

| Studies                              | Estimate (95% CI) |
|--------------------------------------|------------------|
| Avila et al. 2015-1                  | 1.569 (0.823, 2.316) |
| Avila et al. 2015-2                  | 1.796 (0.849, 2.962) |
| Koenig and Baehrens 2018             | 1.946 (0.987, 2.904) |
| Ortegal et al. 2015-1                | 0.646 (-0.776, 2.067) |
| Ortegal et al. 2015-2                | 0.985 (-0.571, 2.526) |
| Ortegal et al. 2015-3                | 0.673 (-0.571, 1.913) |
| Tasci et al. 2015-1                  | 0.235 (0.385, 0.745) |
| Tasci et al. 2015-2                  | 0.495 (0.647, 2.323) |
| Tasci et al. 2015-3                  | 0.934 (0.983, 2.003) |
| Subgroup Acacia marina (P=0.6% , P=0.644) | 1.846 (0.983, 2.099) |
| Elbert et al. 2017-1                 | 0.704 (-0.165, 1.572) |
| Elbert et al. 2017-2                 | 1.010 (0.269, 1.751) |
| Elbert et al. 2017-3                 | 0.965 (-0.968, 2.899) |
| Elbert et al. 2017-4                 | 0.904 (-0.387, 1.746) |
| Norma et al. 2005-1                  | 0.924 (-0.006, 1.855) |
| Norma et al. 2005-2                  | 1.928 (0.643, 3.210) |
| Norma et al. 2005-3                  | 2.594 (0.474, 3.513) |
| Norma et al. 2005-4                  | 3.094 (0.620, 3.568) |
| Norma et al. 2005-5                  | 3.065 (-0.553, 6.674) |
| Pifre-Palquis et al. 2017-1          | 0.686 (-0.778, 1.157) |
| Pifre-Palquis et al. 2017-2          | 0.877 (0.307, 2.444) |
| Pifre-Palquis et al. 2017-3          | 0.426 (<0.001, 1.272) |
| Pifre-Palquis et al. 2017-4          | 0.376 (-0.081, 0.833) |
| Subgroup Guayacans (P=0.5% , P=0.515) | 0.596 (0.205, 1.221) |
| Martelo et al. 2020-1                | 0.405 (0.832, 1.135) |
| Martelo et al. 2020-2                | -0.926 (-1.700, -0.153) |
| Pifre-Palquis et al. 2019a-1         | 0.080 (-1.500, 1.660) |
| Pifre-Palquis et al. 2019b-2         | 0.003 (-1.237, 1.242) |
| Pifre-Palquis et al. 2019b-3         | -0.420 (-1.673, 0.833) |
| Pifre-Palquis et al. 2019c-4         | -1.888 (<0.001, 2.699) |
| Subgroup Lecanea racemosa (P=0.5% , P=0.689) | -1.436 (-3.299, 0.424) |
| Overall (P=0.4% , P=0.011)           | 0.073 (0.581, 1.226) |
Regardless of TANs’ supplementation, animals from studies that used rotational experimental designs (i.e., Latin squares and crossover designs) had lower DMD (SMD = −0.765; p < 0.001), while no differences were observed regarding DMD in animals from studies that used continuous experimental designs (i.e., completely randomized and randomized blocks designs; p > 0.05). OMD decreased in animals from studies that used rotating experimental designs (SMD = −0.856; p < 0.001), while no difference was observed in DMD in animals from studies that used continuous experimental designs (p > 0.05). Studies that used rotating experimental designs had lower NDFD (SMD = −0.704; p < 0.001); however, NDFD was not affected in animals from studies that used continuous experimental designs (p > 0.05). Ruminal pH was not affected by the type of experimental design used (p > 0.05). MEDMI decreased in animals from studies that used rotating experimental designs (SMD = −0.836; p < 0.001), while no differences were observed with respect to MEDMI in animals from studies that used continuous experimental designs (p > 0.05).

Regarding the level of TANs’ supplementation, animals in studies using doses greater than 12 g/kg DM showed lower DMD (SMD = −0.917; p < 0.001), while no differences were observed in DMD in animals in studies using doses lower than 12 g/kg DM (p > 0.05). OMD was lower in animals supplemented with doses of TANs higher than 12 g/kg DM (SMD = −0.976; p < 0.001), but doses lower than 12 g did not change OMD (p > 0.05). Studies using TANs’ doses higher than 12 g/kg DM had lower NDFD (SMD = −0.775; p < 0.001); however, NDFD was not affected when TANs’ doses lower than 12 g/kg DM were used (p > 0.05). The concentration of acetate in the ruminal fluid increased in animals from studies that used TANs’ doses lower than 12 g/kg DM (SMD = 0.387; p = 0.038), while there was no effect when more than 12 g TANs were used (p > 0.05). Animals in studies that
used TANs’ doses higher than 12 g/kg DM showed higher rumen propionate concentration (SMD = 0.319; p = 0.010), whereas TANs’ doses lower than 12 g/kg DM did not change rumen propionate concentration (p > 0.05). FNE increased significantly regardless of the dose of TANs used; however, the effect was greater (SMD = 1.119; p < 0.001) when doses greater than 12 g/kg DM were used compared to doses of less than 12 g/kg DM (SMD = 0.482; p < 0.01).

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

Regarding the age, animals younger than 12 months old showed lower DMI (SMD = −1.249; p < 0.05), while no differences were observed in DMI for animals older than 12 months (SMD = 0.104; p > 0.05). CPD was lower in animals younger than 12 months old (SMD = −1.090; p < 0.001), but animals older than 12 months old did not change (SMD = 0.201; p > 0.05). Ruminal pH was lower in animals older than 12 months old (SMD = −0.767; p < 0.05), while no differences were observed in ruminal pH for animals younger than 12 months old (SMD = 0.154; p > 0.05). Ruminal concentration of NH₃-N decreased in animals younger than 12 months old (SMD = −0.745; p < 0.05), while there was no effect in animals older than 12 months old (SMD = −0.030; p > 0.05). The ruminal concentration of total VFA increased in animals older than 12 months old (SMD = 0.753; p = 0.01) but decreased in animals younger than 12 months old (SMD = −1.245; p < 0.001).

4. Discussion

The environmental sustainability of beef production is a significant concern within the food production system [49]. Current literature suggests that TANs can be supplemented to improve the sustainability of both dairy and beef cattle by reducing CH₄ emissions and enhancing animal performance [1,25]. In ruminants, some studies suggest that dietary supplementation with TANs increases duodenal amino acid flux [11], reduces enteric CH₄ production [7,10], and improves the rumen microbial activity [50]. Consequently, it was expected that beef cattle supplemented with TANs in the diet would have higher growth rate. However, the present meta-analysis showed that ADG and FBW were not affected by dietary supplementation with TANs. A positive relationship exists between improved productivity and both environmental and economic sustainability [49]. This suggests that TANs do not affect growth rate or environmental or economic sustainability in beef cattle. Nevertheless, these results should be interpreted carefully considering that both variables were subject to publication bias. Similar to our results, a meta-analysis conducted by Méndez-Ortiz et al. [51] showed that CTs’ intake did not affect significantly the weight gain of growing lambs.

There is considerable interest in improved feed efficiency as a means of augmenting the economic and environmental sustainability of beef production systems [52]. It has been reported that dietary inclusion of TANs reduces ruminal protein degradation, resulting in higher efficiency of nitrogen utilization [5,25]. On the other hand, enteric CH₄ emissions represent losses of 2–12% of energy intake in ruminants [53]. In the present meta-analysis, the values observed for ruminal NH₃-N concentration and CH₄ emissions indicated a reduction in ruminal protein degradation and enteric CH₄ emissions. This could be associated with higher efficiency of protein utilization and energy consumed. However, these effects did not modify the feed efficiency. This suggests that TANs do not affect either environmental or economic sustainability in beef cattle.

Some review articles have hypothesized that the presence of TANs in the diet may negatively affect feed intake in ruminants due to their astringent nature [5,54]. However, in the present meta-analysis, no changes in DM or nutrient intake were observed in response to dietary supplementation with TANs. Such absence probably occurred because the average dose of TANs used was 14.6 g/kg DM and the negative effects of TANs on the intake seem to occur with doses higher than 50 g/kg DM [6]. Similar to our results, two
previously conducted meta-analyses reported that dietary supplementation with TANs at average concentrations of 46.3 and 9.5 g/kg DM did not affect significantly the feed intake of growing lambs and dairy cows in production, respectively [31,51]. These results together suggest that TANs can be used in beef cattle and other ruminants during their different productive stages without negative effects on feed intake.

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

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

Regarding the TANs’ source, it explained between 13 and 48% of the heterogeneity observed for CPD and NDFD. Although most of the TANs’ sources used by the studies included in our investigation reduced CPD, CPD improved when Leucaena leucocephala was used as TANs’ source. This result, together with the higher ruminal concentration of NH$_3$-N observed in the studies using L. leucocephala, suggests that TANs from this plant have low capacity for binding to rumen proteins, similar to what has been previously observed in CTs from other plants [57]. It is suggested that TANs with higher molecular weight have a greater capacity to bind to other molecules [21]. Although it has previously been reported that L. leucocephala contains CTs with higher molecular weight [58], it is suggested that the molecular weight of CTs is not the only factor influencing the binding capacity of TANs to the proteins.

Ruminants are inefficient animals for converting the ingested protein into animal product because a large part of this protein is lost as NH$_3$-N in the rumen [54]. In the present meta-analysis, dietary supplementation with TANs reduced rumen NH$_3$-N concentration, indicating a lower protein degradability in the rumen due to the presence of TANs. However, TANs did not influence ADG, FBW, or FE, probably because CPD also decreased in response to dietary supplementation with TANs. Consequently, the beef cattle seem not to better use the protein ingested even in the presence of TANs in the diet. Similar to our results, a meta-analysis by Herremans et al. [31] reported that dietary supplementation with TANs reduced NH$_3$-N ruminal concentration in dairy cows. However, it did not improve the nitrogen utilization efficiency. Furthermore, a meta-analysis of 15 in vivo and 15 in vitro studies showed that NH$_3$-N concentration decreased when increasing TANs’ levels in ruminant diets [59]. The free TANs can bind to the soluble protein in the diet and consequently reduce the NH$_3$-N ruminal concentration [25]. This is to be expected and would partially explain the results observed in this and other studies. However, NH$_3$-N ruminal concentration also appears to decrease when ruminal protozoa are reduced or elim-
inated [60]. Consequently, in our investigation, the lower NH$_3$-N ruminal concentration could be associated with the 3.7% reduction observed in the rumen protozoan population.

Supplementation with TANs did not alter the ruminal concentration of total VFA. However, it did improve the concentrations of propionate and butyrate, but this last response variable was subject to publication bias, making it difficult to interpret. The absence of significant changes in ruminal concentration of total VFA can be considered desirable when it is accompanied by a reduction in enteric CH$_4$ emissions [61], as observed in our meta-analysis. Similar to our results, Dai and Faciola [62] reported that dietary supplementation with TANs improved ruminal concentration of propionate and butyrate in large and small ruminants and also reduced CH$_4$ production. Because there is a negative correlation between propionate and CH$_4$ production due to the competition for hydrogen [63], the increase in ruminal concentration of propionate observed in our investigation could be associated with the reduction in enteric CH$_4$ emissions observed in response to TANs’ supplementation.

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

Previous studies have reported that TANs from Leucaena leucocephala can reduce the rumen protozoan population [64,66]. However, the mechanisms of action through which these and other TANs act on rumen protozoa are still unknown [67]. Although, in our meta-analysis, the rumen protozoa decreased 3.7% in response to dietary supplementation with TANs, this effect was insignificant, perhaps because only 7.6% of the included studies used L. leucocephala as a source of TANs. Similar to our results, a meta-analysis conducted by Jayanegara et al. [39] reported that inclusion of TANs in ruminant diets did not affect counts of protozoa in rumen fluid under in vivo and in vitro conditions. Similarly, Dai and Faciola [62] also did not observe significant effects of dietary supplementation with TANs on the rumen protozoan population in small and large ruminants.

Enteric CH$_4$ production represents approximately 43% of the greenhouse gases emitted in beef production worldwide [68]. To ensure sustainable livestock production, it is necessary to reduce enteric CH$_4$ emissions [25]. It has been suggested that TANs can be used to minimize the environmental impact of ruminant production because they can improve ruminal fermentation and mitigate CH$_4$ emissions [69]. Some studies have reported that TANs decrease rumen methanogenesis directly by reducing methanogenic bacteria populations [63,66,70]. However, often the effects of TANs on CH$_4$ reduction are more indirect than direct [71]. For example, Fagundes et al. [23] reported that enteric CH$_4$ emissions from beef cattle decreased in response to dietary supplementation with CTs. However, they attributed the CH$_4$ reduction to a decrease in feed intake rather than to direct effects of CTs on rumen methanogenic archaea. In addition, some review articles have suggested that enteric CH$_4$ production may vary depending on the type, dose, and source of TANs employed in the diet [14,25]. However, in our meta-analysis MEDMI was only affected by the source of TANs. This suggests that TANs could improve the environmental sustainability of beef production regardless of the type and dose of TANs used, similar to what has been previously observed in small ruminant production [15].

The period of supplementation with TANs could also contribute to the variability of its effects on methanogenesis [50]. One of the most important problems with the use of phytochemicals in ruminants is the adaptation of ruminal microorganisms to their effects after long periods of supplementation [72]. For example, some essential oils seem to be
more effective in reducing CH$_4$ production when used for short periods. However, they lose effectiveness over time [40]. In the present meta-analysis, the period of supplementation showed inconsistent effects on MEDMI. In short-term studies (less than 90 days), the reduction in MEDMI was small (SMD = −0.141) but increased (SMD = −0.791) in animals used in long-term studies (91 to 180 days). These results suggest that in beef cattle, ruminal microorganisms related to CH$_4$ production are not able to adapt to the effects of TANs, even during long periods of supplementation. Similar results were previously reported by Salami et al. [56] in lambs supplemented with different sources of CTs (Castanea sativa and Caesalpinia spinosa) and HTs (Acacia negra and Uncaria gambir) during long periods. In their investigation, they observed that all TANs’ sources had specific antimicrobial activity against methanogenic bacteria and ruminal protozoa during the whole experimental phase.

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

Although most of the studies (34%) included in the present meta-analysis used TANS from quebracho tree, the subgroup analysis revealed that the MEDMI decreased significantly only in response to the use of Leucaena leucocephala and tannic acid as TANs’ sources. According to Huang et al. [58], L. leucocephala contains high-molecular-weight CTs, which varies between 2737 and 2872 Da. On the other hand, tannic acid, although it is a typical HT [5,73], has a molecular weight of 1701 Da [74], which is higher than the weight of 939 Da reported for quebracho tree CTs [75]. TANs with a high molecular weight act better than those with a low molecular weight in suppressing ruminal protozoa populations [64], which are correlated with CH$_4$ emissions by the equation:

$$\text{methane (g/kg dry matter intake)} = -30.7 + 8.14 \times \text{protozoa (log10 cells/mL)}$$ [76]. Consequently, the use of L. leucocephala, tannic acid, and other high-molecular-weight TANs’ sources could have a greater impact on reducing enteric CH$_4$ emissions compared to other widely studied TANs (e.g., quebracho).

According to Nichols et al. [77], beef cattle production plays an important role in the N cycle as beef cattle excrete up to 80% of the consumed dietary N through urine and feces, and urinary N accounts for approximately 60–80% of the total N excretion [78]. In the present meta-analysis, no changes in NUE were observed in response to dietary supplementation with TANs. However, the observed values for UNE and FNE indicated a reduction in UNE and an increase in FNE, respectively. Similar to our results, a meta-analysis conducted by Herremans et al. [31] reported that dietary supplementation with TANs reduced UNE (−11%) and increased FNE (+10%) without affecting NUE in dairy cows. According to Singh et al. [79], the excreted N might be lost through nitrate (NO$_3^-$) leaching, emissions of N$_2$O, and emissions from ammonia volatilization. Compared with feces, urine could rapidly supply available mineral N for nitrification and denitrification through hydrolysis of urea, leading to higher N$_2$O emissions [80], which has a global warming potential over a 100-year period of 298 times greater than that of carbon dioxide [2]. Therefore, strategies based on changing the composition and concentration of urinary compounds by diet manipulation could be considered potential options to mitigate N$_2$O emissions from urine [4]. Consequently, the shift from urine to fecal N observed in this study may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N.

5. Conclusions

One of the most significant findings to emerge from this study is that the environmental impact of beef production systems can be markedly reduced when tannins are included in the diet. The results of the present meta-analysis indicate that TANs reduce enteric CH$_4$ emissions in beef cattle, particularly when they are supplied naturally as ingredients in the diet, when they are supplemented for long periods, or when Leucaena leucocephala and
tannic acid are used as sources of these secondary metabolites. In addition, the shift from urinary to fecal N observed in this study may be beneficial for environment preservation, as urinary N induces more harmful emissions than fecal N. Therefore, the addition of tannins in the diet of beef cattle could be used as a sustainable natural alternative to reduce the environmental impact of beef production without affecting the economic sustainability. However, several issues need to be addressed before specific recommendations for commercial use of TANs to reduce environmental impact.

Our meta-analysis demonstrates that TANs’ supplementation does not affect weight gain, feed intake, or feed efficiency in beef cattle, but reduces diet digestibility at doses above 12 g/kg DM. In addition, TANs’ supplementation improves ruminal fermentation characteristics by reducing ruminal NH$_3$-N concentration and increasing rumen propionate and butyrate concentration. The best result in ruminal propionate and NH$_3$-N concentration is achieved using TANs from pistachio and HTs, respectively.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/su13137410/s1. Figure S1: Flow chart of paper selection process. Figure S2: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on average daily gain (ADG); (b) funnel plot of the effect of dietary supplementation with TANs on ADG obtained using the trim-and-fill method of Duval and Tweedie. Figure S3: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on final body weight (FBW); (b) funnel plot of dietary supplementation with TANs on FBW obtained using the trim-and-fill method of Duval and Tweedie. Figure S4: (a) Funnel plot of the effect of dietary tannins’ supplementation (TANs) on organic matter intake (OMI); (b) funnel plot of the effect of dietary supplementation with TANs on OMI obtained using the trim-and-fill method of Duval and Tweedie. Figure S5: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on neutral detergent fiber intake (NDFI); (b) funnel plot of the effect of dietary supplementation with TANs on NDFI obtained using the trim-and-fill method of Duval and Tweedie. Figure S6: (a) Funnel plot of the effect of dietary supplementation with tannins (TANs) on rumen butyrate concentration; (b) funnel plot of the effect of dietary supplementation with TANs on the ruminal concentration of butyrate obtained using the trim-and-fill method of Duval and Tweedie. Figure S7: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins type on neutral detergent fiber digestibility (NDFD) in beef cattle. Figure S8: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins type on ruminal ammonia nitrogen (NH$_3$-N) concentration in beef cattle. Figure S9: Forest plot of effect size or standardized mean difference and 95% confidence interval of the chemical or botanical source of tannins on ruminal ammonia nitrogen (NH$_3$-N) concentration in beef cattle. Figure S10: Forest plot of effect size or standardized mean difference and 95% confidence interval of tannins’ inclusion method on crude protein digestibility (CPD) in beef cattle.

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## Appendix A

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

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

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

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