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A Meta-Analysis of 3-Nitrooxypropanol Dietary Supplementation on Growth Performance, Ruminal Fermentation, and Enteric Methane Emissions of Beef Cattle

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Abstract: This study aimed to evaluate the effects of dietary supplementation with 3-nitrooxypropanol (3-NOP) on growth performance, ruminal fermentation, and enteric methane emissions of beef cattle using a meta-analytic approach. The final meta-analysis database included results from 15 scientific articles. The response variables were analyzed through random effects models, where the results were reported as weighted mean differences (WMD) between the treatments without 3-NOP and those supplemented with 3-NOP. The dietary inclusion of 3-NOP decreased ($p < 0.001$) dry matter intake but did not affect ($p > 0.05$) average daily gain and increased ($p < 0.05$) feed efficiency. In the rumen, 3-NOP supplementation increased ($p < 0.01$) the pH and ruminal concentration of propionate, butyrate, valerate, isobutyrate, and isovalerate. In contrast, dietary supplementation with 3-NOP decreased ($p < 0.001$) the rumen concentration of ammonia nitrogen, total volatile fatty acids, acetate, and the acetate/propionate ratio. Furthermore, daily methane (CH_4) emission, CH_4 yield, and CH_4 emission as a percentage of gross energy ingested decreased ($p < 0.001$) in response to 3-NOP dietary supplementation. In conclusion, dietary supplementation with 3-nitrooxypropanol can be used as a nutritional strategy to improve feed efficiency and ruminal fermentation in beef cattle and, at the same time, reduce enteric methane emissions.

Keywords: dietary additive; methane enzyme inhibitor; climate change; meta-regression

Citation: Orzuna-Orzuna, J.F.; Godina-Rodríguez, J.E.; Garay-Martínez, J.R.; Granados-Rivera, L.D.; Maldonado-Jáquez, J.A.; Lara-Bueno, A. A Meta-Analysis of 3-Nitrooxypropanol Dietary Supplementation on Growth Performance, Ruminal Fermentation, and Enteric Methane Emissions of Beef Cattle. *Fermentation* **2024**, *10*, 273. https://doi.org/10.3390/fermentation10060273

Academic Editor: Qing Zhang

Received: 25 April 2024
Revised: 15 May 2024
Accepted: 21 May 2024
Published: 23 May 2024

1. Introduction

Beef cattle production systems are essential worldwide because they contribute to the supply of high-quality proteins required for human consumption [1]. However, beef cattle and other ruminants produce enteric methane (CH_4) as one of the end products of digestion [2,3]. According to Gerber et al. [4], enteric CH_4 emitted by ruminants contributes to approximately 40% of total livestock emissions and represents between 4 and 6% of global anthropogenic greenhouse gas (GHG) emissions. CH_4 is a GHG, which according to several authors [1,5], has a global warming potential 28 times greater than carbon dioxide (CO_2). Therefore, in recent years, there has been an increased interest in reducing enteric CH_4 emitted by ruminants [6,7]. Several mitigation strategies for enteric CH_4 have been proposed and evaluated, among which are dietary supplementation with feed additives, such as ionophores, essential oils, macroalgae, tannins, and 3-nitrooxypropanol

(3-NOP) [8,9]. Among these additives, 3-NOP is one of the most effective in reducing enteric CH_4 in ruminants [7].

3-NOP is a small molecule of low molecular weight (121.09 g/mol) with dual chemical functional groups: a primary alcohol and an organic nitrate ester [10]. According to Duin et al. [3], 3-NOP can inhibit enteric CH_4 emitted by ruminants through the specific inactivation of the enzyme methyl-coenzyme M reductase (MCR), which is required to catalyze the last step of methanogenesis in methanogenic archaea [8]. Various in vitro studies [11–13] suggest that the dietary inclusion of 3-NOP may be an effective strategy to reduce enteric CH_4 emissions in ruminants. Likewise, other studies found that dietary supplementation with 3-NOP successfully mitigated enteric CH_4 emissions in sheep [14] and dairy cows [6].

Particularly in beef cattle, to date, few studies have evaluated the effects of 3-NOP as a dietary additive on enteric CH_4 emissions [15–17], ruminal fermentation [18,19], and growth performance [1,5]. Furthermore, the individual interpretation of these studies limits the obtainment of scientifically sound conclusions, since the results obtained in some of them are contradictory. For example, some authors [5,15] did not observe the effects of supplementation with low doses (30 to 75 mg/kg DM) of 3-NOP on growth performance, ruminal acid concentration volatile fatty acids, and enteric CH_4 emissions from beef cattle. In contrast, other authors found that high doses (100 to 200 mg/kg DM) of 3-NOP reduced CH_4 emissions [2] and improved beef cattle's feed efficiency [16] and rumen parameters [20]. According to Yu et al. [7] and Dijkstra et al. [21], the type of diet, the dose, and the supplementation period are factors that influence the response of ruminants to 3-NOP supplementation.

Data reported in four previous meta-analyses [6,21–23] show that 3-NOP supplementation successfully decreases enteric CH_4 emissions in ruminants without affecting productive performance. However, one of these meta-analyses [6] evaluated the effects of 3-NOP only in dairy cows. Likewise, the meta-analyses by Kim et al. [22], Dijkstra et al. [21], and Jayanegara et al. [23] only included four to seven beef cattle studies in their database. Furthermore, in the meta-analyses of Kim et al. [22], Dijkstra et al. [21], and Jayanegara et al. [23], the results for growth performance, ruminal fermentation, and enteric CH_4 were obtained through combining data from beef cattle and dairy cows since there were not enough studies available with beef cattle alone at that time. High flexibility in the design of a meta-analysis, such as using a low number of studies or mixing data from different experimental units (e.g., beef cattle and dairy cows), decreases the probability that the study findings are conclusive [24]. In contrast, periodically updating meta-analyses using larger databases can increase the reliability of the results [6]. In response to the increasing number of published studies on the use of 3-NOP in beef cattle, our study aimed to evaluate the effects of dietary supplementation with 3-nitrooxypropanol on growth performance, ruminal fermentation, and the enteric methane emissions of beef cattle through a meta-analytic approach. The hypothesis of the present study establishes that the inclusion of 3-NOP in beef cattle diets will benefit growth performance and ruminal fermentation while decreasing enteric CH_4 emissions.

2. Materials and Methods

2.1. Literature Search

To formulate the research question, the Population, Intervention, Comparison, and Outcomes (PICO) [25] strategy was used. Therefore, in the current study, the population was beef cattle, the intervention was the dietary supplementation of 3-NOP, the comparison was between diets with and without the addition of 3-NOP, and the results were the means of treatments obtained in growth performance, ruminal fermentation, and enteric CH_4 emissions. Subsequently, the PRISMA protocol [26] was used in the processes of identification, selection, election, and inclusion of scientific articles that tested the effects of the inclusion of 3-NOP in diets for beef cattle (Figure 1). To complete the process of identifying scientific documents, systematic searches were carried out in the PubMed, ScienceDirect,

Scopus, and Web of Science databases. The keywords used in all databases were: (1) 3-nitrooxypropanol, (2) beef cattle, (3) growth performance, (4) ruminal fermentation, and (5) enteric methane emissions. The search results only included studies published between January 2014 and February 2024 since the databases did not contain studies on 3-NOP dietary supplementation for beef cattle published before 2014.

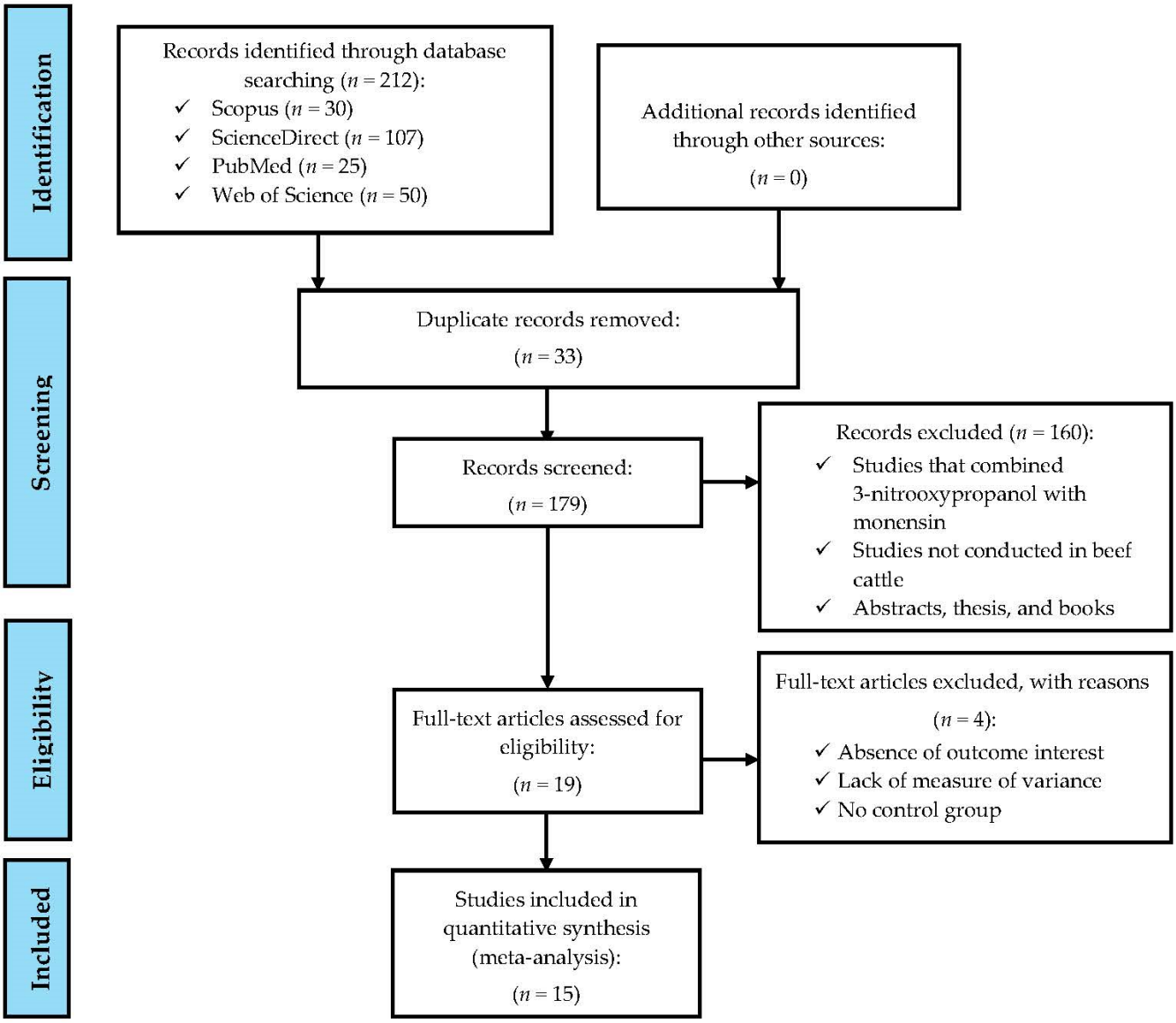

Figure 1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

2.2. Inclusion and Exclusion Criteria

Initially, 212 peer-reviewed manuscripts were identified through the searches. Duplicate documents were first removed from these manuscripts. Subsequently, from the remaining documents, those that had one or more of the following characteristics (exclusion criteria) were eliminated: (1) review articles, simulations, books, or conference proceedings; (2) studies that did not use beef cattle as experimental units; and (3) studies that did not use 3-NOP or combined 3-NOP with monensin or another additive. Finally, to be included in the final database of the meta-analysis, the remaining documents had to meet each of the following characteristics (inclusion criteria), similar to those reported in previous meta-analysis [27,28]: (1) peer-reviewed manuscripts published in the English language; (2) studies that used beef cattle as experimental units; (3) studies that measured

and reported data on growth performance, ruminal fermentation, or enteric CH_4 emissions (measured with the sulfur hexafluoride "SF_6" tracer technique, the Green-Feed system, or respirometry chambers); (4) studies that compared the effect of dietary inclusion of 3-NOP versus a control treatment (diet without 3-NOP) using the same basal diet; (5) studies that indicated the dose (mg/kg DM) of 3-NOP added to the experimental diets or had information necessary to estimate it; (6) studies that reported data on treatment means, standard error of the mean (SEM) and number of experimental units (n) of the experimental diets (diets added with 3-NOP) and control diets (diets without 3-NOP).

2.3. Data Extraction

The final meta-analysis database includes data from 15 scientific articles (Table 1). The following information was obtained from each selected manuscript: (1) dose of 3-NOP (mg/kg DM) added to the diets; (2) duration of the period of dietary supplementation with 3-NOP (days); and (3) amount of concentrate (g/kg DM) included in the experimental diets. The included response variables were grouped as follows: (1) dry matter intake (DMI), average daily gain (ADG), and feed efficiency (FE); (2) ruminal pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA), acetate, propionate, butyrate, valerate, iso-butyrate, iso-valerate, and acetate/propionate ratio; (3) daily CH_4 emission (g/d), CH_4 yield (g/kg DMI), CH_4 emission as a percentage of gross energy (GE) ingested, daily H_2 emission (g/d), and daily CO_2 emission (kg/d). From the 15 scientific articles selected, the means of treatments, SEM, and n for each response variable were extracted.

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

Reference	Days of Experiment	3-NOP-Dose (mg/kg DM)	Concentrate, g/kg DM	BW, kg ± SD
Alemu et al. [2]	28	150, 175, 200	300	282 ± 8
Alemu et al. [19]	84	100, 125, 150	906	421 ± 11
Alemu et al. [20]	33	150	100	515 ± 40.5
Almeida et al. [29]	90	50, 75, 100, 125	807	356 ± 14.4
Araújo et al. [1]	33, 96	100, 150	930	360 ± 37.3
Kim et al. [30]	21	100, 104	356, 902	451 ± 21
Kirwan et al. [5]	84	150	500	147 ± 28
Lee et al. [31]	8	100	356, 902	349 ± 9
Martínez-Fernández et al. [32]	21	329	0	492 ± 7
Romero-Pérez et al. [17]	28	35, 109, 217	400	637 ± 16.2
Romero-Pérez et al. [18]	112	280	400	549 ± 64.3
Vyas et al. [15]	28	50, 75, 100, 150, 200	350, 920	371 ± 18
Vyas et al. [16]	105	100, 200	300, 920	308 ± 22
Vyas et al. [33]	105	125, 200	350, 920	319 ± 30
Zhang et al. [34]	28	200	100	732 ± 43

BW: body weight; SD: standard deviation.

2.4. Calculations and Statistical Analysis

Statistical analyses were performed through the "metaphor" and "meta" packages [35,36], available in the statistical software R (version 4.1.2). The effect size (ES) of 3-NOP supplementation was estimated by the weighted mean difference (WMD) between the treatments added with 3-NOP and control (without 3-NOP). WMDs were used in the current study because, according to Takeshima et al. [37], they allow the results to be easily interpreted and have greater statistical power. The weighting in the WMD was performed using the inverse of the variance following the methods and procedures described by Der-Simonian and Laird [38] for random effects models.

2.5. Heterogeneity and Publication Bias

The presence of heterogeneity was evaluated through Cochran's Q statistic (chi-square test), which was considered significant when $p \leq 0.05$ [39]. Additionally, the I^2 statistic was

used to measure the percentage of variation attributed to heterogeneity [40]. According to some authors [41,42], the degree of heterogeneity obtained with the I^2 statistic can be classified as follows: no heterogeneity ($0 < I^2 \leq 25\%$), low ($25\% < I^2 \leq 50\%$), moderate ($50\% < I^2 \leq 75\%$), and high heterogeneity ($I^2 > 75\%$). On the other hand, the presence of publication bias in the meta-analysis was tested using two statistical tests: (1) Egger's regression asymmetry [43]; and (2) Begg's adjusted rank correlation [44]. Publication bias was considered when $p \leq 0.05$ in one or both tests (i.e., Egger and Begg).

2.6. Meta-Regression and Subgroup Analysis

Meta-regression analyses were performed to test the effects of 3-NOP doses, periods of 3-NOP supplementation, and the amount of concentrate included in the diets on the heterogeneity detected in the response variables. The method of moments proposed by Der-Simonian and Laird [38] was used in the meta-regression analyses since, according to Borenstein et al. [39], this method is well established for estimating the variance between studies. The response variables had to meet the following requirements to be evaluated with meta-regression analysis: (1) have $I^2 > 50\%$ and $p < 0.05$ in the Q test [39,45]; and (2) have $p > 0.05$ in the Egger regression asymmetry and Begg's adjusted rank correlation tests [43,44]. Covariates were divided as follows: (1) 3-NOP doses 35–100 and 101–280 mg/kg DM; (2) 3-NOP supplementation period $\leq$ 60 and >60 days; and (3) amount of concentrate included in the diet $\leq$ 700 and >700 g/kg DM. When a significant effect ($p < 0.05$) of any covariate was detected, the WMD of the response variable was evaluated through subgroup analysis, as suggested by other authors [27,46].

3. Results

3.1. Growth Performance

Table 2 shows that DMI decreased ($p < 0.001$) in response to 3-NOP supplementation. In contrast, the dietary inclusion of 3-NOP did not affect ($p > 0.05$) ADG and increased ($p < 0.05$) FE.

Table 2. Growth performance of beef cattle supplemented with 3-nitrooxypropanol.

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, kg/d	15 (51)	9.96 (1.72)	−0.361 (−0.524; −0.199)	<0.001	<0.001	70.07	0.379	0.644
ADG, kg/d	7 (15)	1.48 (0.17)	−0.026 (−0.055; 0.003)	0.077	0.529	0.00	0.076	0.064
FE, ADG/DMI	6 (14)	0.159 (0.026)	0.004 (0.000; 0.008)	0.040	0.109	35.20	0.311	0.645

N: number of studies; NC: number of comparisons between 3-nitrooxypropanol treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between treatments with 3-nitrooxypropanol and control (without 3-nitrooxypropanol); CI: confidence interval of WMD; *p*-Value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation in 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; FE: feed efficiency.

3.2. Ruminal Fermentation

Table 3 shows that 3-NOP supplementation increased ($p < 0.01$) ruminal pH and the ruminal concentration of propionate, butyrate, valerate, isobutyrate, and isovalerate. However, the ruminal concentration of NH_3-N, TVFA, and the acetate/propionate ratio decreased ($p < 0.05$) in response to 3-NOP supplementation.

Table 3. Ruminal fermentation of beef cattle supplemented with 3-nitrooxypropanol.

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
Ruminal pH	8 (15)	6.30 (0.22)	0.096 (0.057; 0.135)	<0.001	0.993	0.00	0.469	0.634
NH_3-N, mg/dL	10 (20)	6.63 (1.94)	−0.666 (−0.973; −0.359)	<0.001	0.395	4.98	0.095	0.112
TVFA, mM	10 (20)	122.07 (22.50)	−7.520 (−10.831; −4.210)	<0.001	0.329	10.10	0.437	0.716
Acetate, mol/100 mol	10 (20)	58.37 (7.67)	−4.741 (–5.576; −3.906)	<0.001	0.065	48.55	0.448	0.146
Propionate, mol/100 mol	10 (20)	24.38 (8.12)	2.488 (1.705; 3.272)	<0.001	0.077	37.24	0.401	0.267
Butyrate, mol/100 mol	10 (20)	11.86 (1.64)	0.987 (0.434; 1.539)	<0.001	0.060	37.56	0.426	0.856
Valerate, mol/100 mol	9 (17)	1.84 (0.50)	0.239 (0.099; 0.378)	<0.001	0.081	42.12	0.087	0.681
Isobutyrate, mol/100 mol	9 (17)	1.30 (0.53)	0.067 (0.018; 0.117)	0.008	0.138	28.43	0.071	0.064
Isovalerate, mol/100 mol	9 (17)	1.95 (0.34)	0.291 (0.157; 0.425)	<0.001	0.098	33.76	0.243	0.855
Acetate/propionate	10 (20)	2.81 (1.04)	−0.452 (−0.575; −0.329)	<0.001	0.086	46.54	0.084	0.682

N: number of studies; NC: number of comparisons between 3-nitrooxypropanol treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between treatments with 3-nitrooxypropanol and control (without 3-nitrooxypropanol); CI: confidence interval of WMD; *p*-Value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation in size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; [2]: Begg's adjusted rank correlation; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids.

3.3. Enteric Methane Emissions

Dietary supplementation with 3-NOP decreased ($p < 0.001$) daily CH_4 emission, CH_4 yield, and CH_4 emission as a percentage of ingested GE (Table 4). In contrast, H_2 emissions increased ($p < 0.001$) in response to 3-NOP supplementation. However, CO_2 emissions were not affected ($p > 0.05$) by the dietary inclusion of 3-NOP.

Table 4. Enteric methane emissions in beef cattle supplemented with 3-nitrooxypropanol.

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
CH_4, g/d	14 (48)	157.86 (41.69)	−55.052 (−62.253; −47.852)	<0.001	<0.001	70.55	0.772	0.321
CH_4, g/kg DMI	14 (48)	18.40 (4.98)	−5.445 (−6.250; −4.639)	<0.001	<0.001	66.39	0.475	0.282
CH_4, % of GEI	10 (33)	5.44 (1.76)	−1.634 (−1.874; −1.395)	<0.001	0.061	48.01	0.256	0.947
H_2, g/d	9 (28)	0.33 (0.23)	1.465 (1.163; 1.767)	<0.001	0.085	47.74	0.288	0.986
CO_2, kg/d	6 (12)	8.45 (1.95)	0.022 (−0.087; 0.131)	0.689	0.069	43.75	0.388	0.270

N: number of studies; NC: number of comparisons between 3-nitrooxypropanol treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between treatments with 3-nitrooxypropanol and control (without 3-nitrooxypropanol); CI: confidence interval of WMD; *p*-Value to χ^2 (Q) test of heterogeneity; I^2: proportion of total variation in size effect estimates that is due to heterogeneity; [1]: Egger's regression asymmetry test; [2]: Begg's adjusted rank correlation; CH_4: methane; H_2: hydrogen; GEI: gross energy intake; CO_2: carbon dioxide.

3.4. Heterogeneity and Publication Bias

Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant ($p > 0.05$) for any of the response variables analyzed (Tables 2–4), indicating that there was no publication bias. On the other hand, Table 2 shows that there was significant ($p < 0.05$) heterogeneity (Q) for DMI. In contrast, no Q ($p > 0.05$) was detected for any of the

response variables related to ruminal fermentation (Table 3). However, Q ($p < 0.05$) was observed in the daily CH_4 emission and CH_4 yield (Table 4).

Table 5 shows no significant relationship ($p > 0.05$) between DMI and the covariates included in the models used for the analysis. The 3-NOP dose explained ($p < 0.001$) 26.40 and 36.04% of the observed heterogeneity in the daily CH_4 emission and CH_4 yield, respectively. Likewise, the 3-NOP supplementation period explained ($p < 0.001$) 6.56 and 9.65% of the heterogeneity observed in the daily CH_4 emission and CH_4 yield, respectively. Furthermore, the amount of concentrate included in the diets explained ($p < 0.05$) 5.21% of the heterogeneity observed in the daily CH_4 emission.

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

Parameter	Covariates	QM	Df	*p*-Value	R^2 (%)
DMI, kg/d	3-nitrooxypropanol dose	0.023	1	0.879	0.00
	Supplementation period	1.228	1	0.475	0.00
	Concentrate level	1.840	1	0.175	0.24
CH_4, g/d	3-nitrooxypropanol dose	24.00	1	<0.001	26.40
	Supplementation period	18.17	1	<0.001	6.56
	Concentrate level	4.46	1	0.050	5.21
CH_4, g/kg DMI	3-nitrooxypropanol dose	21.38	1	<0.001	36.04
	Supplementation period	11.52	1	<0.001	9.65
	Concentrate level	0.01	1	0.917	0.00

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. DMI: dry matter intake; CH_4: methane.

3.5. Subgroup Analysis

Figure 2a shows that the daily CH_4 emission decreased ($p < 0.001$), regardless of the dose of 3-NOP included in the diet. However, the reduction in daily CH_4 emission was greater (WMD = −68.29 g/d) with high doses (101–329 mg/kg DM) of 3-NOP than with doses between 35 and 100 mg/kg DM (WMD = −34.75 g/d). Similarly, CH_4 yield decreased ($p < 0.001$) regardless of the 3-NOP dose used (Figure 2b). However, the reduction in the CH_4 yield was greater (WMD = −6.82 g/kg DM) when high doses (101–280 mg/kg DM) of 3-NOP were used than when the dietary inclusion of 3-NOP ranged from 35 to 100 mg/kg DM (WMD = −3.56 g/kg DM).

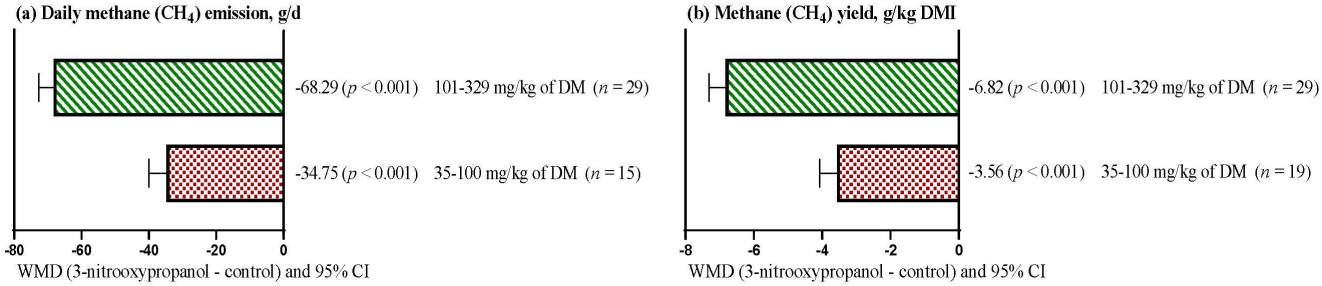

Figure 2. Subgroup analysis (subgroup = doses of 3-nitrooxypropanol (mg/kg DM) of the effect of including 3-nitrooxypropanol in beef cattle diets, WMD = weighted mean differences between 3-nitrooxypropanol treatments and control. CI: confidence interval of WMD.

Figure 3a shows that the daily CH_4 emission decreased ($p < 0.001$) regardless of the 3-NOP supplementation period. However, the reduction in daily CH_4 emission was greater (WMD = −68.29 g/d) when supplementation periods lasted more than 60 days compared to supplementation periods of up to 60 days (WMD = −39.19 g/d). Likewise, CH_4 yield decreased ($p < 0.001$) regardless of the 3-NOP supplementation period used (Figure 3b). However, the reduction in CH_4 yield was greater (WMD = −6.75 g/kg DM) when supplementation periods lasted more than 60 days than with supplementation periods of up to

60 days (WMD = −4.25 g/kg DM). On the other hand, the daily CH_4 emission decreased ($p < 0.001$) regardless of the amount of concentrate included in the diets (Figure 3c). However, the reduction in daily CH_4 emission was greater when 3-NOP was included in diets with more than 700 g/kg DM of concentrate (WMD = −61.48 g/d) than when 3-NOP was included in diets with up to 700 g/kg DM of concentrate (WMD = −47.31 g/d).

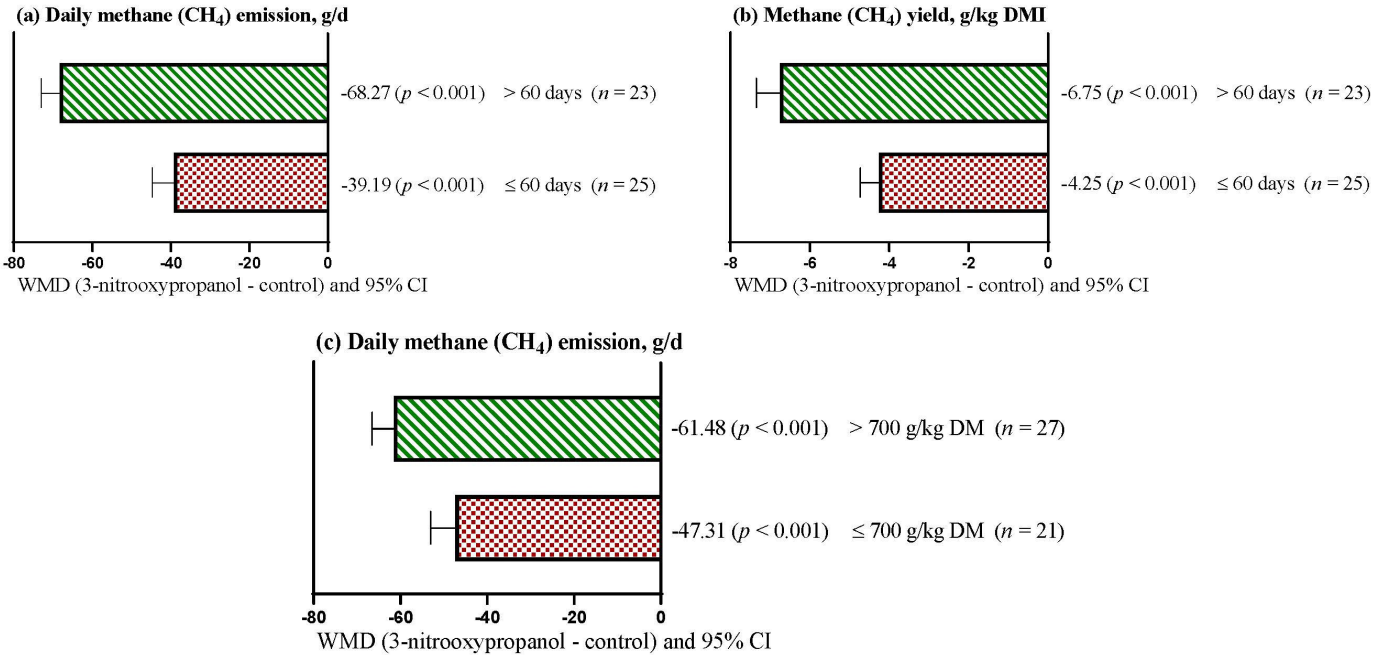

Figure 3. Subgroup analysis (subgroups = supplementation period (days) and concentrate in diet (g/kg of DM)) of the effect of 3-nitrooxypropanol on the diet of the beef cattle; WMD = weighted mean differences between 3-nitrooxypropanol treatments and control. CI: confidence interval of WMD.

4. Discussion

4.1. Growth Performance

Lower DMI was observed in response to dietary supplementation with 3-NOP. In contrast, a previous meta-analysis [23] did not detect significant changes in the DMI of ruminants supplemented with 3-NOP. However, in that meta-analysis, the database only included four beef cattle studies. Another meta-analysis [22] using seven beef cattle studies reported only a trend toward decreased DMI in these animals. The database of the present meta-analysis used 15 studies, which explains the difference in the results obtained in DMI compared to previous meta-analyses. The exact mechanism through which 3-NOP modifies DMI in ruminants has not yet been studied [22]. However, in beef cattle, previous studies [47,48] show that DMI decreases linearly when ruminal propionate increases. Therefore, the lower DMI detected in the present study could be partially explained by the increase in ruminal propionate concentration.

In the current study, 3-NOP supplementation did not affect ADG. Although an increase in ADG would be desirable in beef cattle, it should be considered that the main objective of dietary supplementation with 3-NOP is to reduce enteric CH_4 emissions rather than improve growth performance [29]. On the other hand, FE increased in response to 3-NOP supplementation. This effect could be directly related to the decrease in DMI and the lack of change in ADG. According to Johnson and Johnson [49], cattle lose up to 12% of the GE ingested due to enteric CH_4 emissions. In the present meta-analysis, the emission of CH_4 as a percentage of the ingested GE decreased by 30.0%, which could increase the metabolic energy available in the animals and partially explain the better FE observed. Likewise, supplementation with 3-NOP decreased (−10%) the ruminal NH_3-N concentration, which

is generally associated with increased microbial protein synthesis in the rumen [50]. In ruminants, greater microbial protein synthesis could increase the supply of amino acids to the small intestine and result in greater absorption and the metabolic availability of amino acids [51,52], which would partially explain the increase in FE.

4.2. Ruminal Fermentation

Supplementation with 3-NOP increased ruminal pH, which could be explained by the observed reduction in ruminal TVFA concentration [5]. Likewise, the average ruminal pH values detected in the current study were within the range (5.8–6.5) reported as normal in beef cattle [53]. This result suggests that 3-NOP does not alter microbial populations [51] or physiological functions of the rumen, such as absorption and motility [53]. On the other hand, a lower ruminal concentration of NH_3-N was observed in response to 3-NOP supplementation. The higher rumen concentration of butyrate observed with 3-NOP supplementation could partially explain the reduction in NH_3-N since, according to Rémond et al. [54], ruminal NH_3-N absorption is stimulated when ruminal butyrate concentration increases. In dairy cows, dietary supplementation with 3-NOP decreases (−33.9%) the relative rumen abundance of the microbial genus *Clostridium* [55], within which bacteria with high proteolytic and deaminative activity are found [56,57]. A similar effect of 3-NOP consumption in beef cattle would partially explain the reduction in ruminal NH_3-N concentration in the current study.

In dairy cows, a previous meta-analysis [22] reported similar changes to those obtained in the current study for the ruminal concentration of TVFA, acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, and acetate/propionate ratio. The reduction in ruminal TVFA concentration observed in the present study could be explained by the lower DMI in beef cattle supplemented with 3-NOP, as suggested by Kirwan et al. [5]. On the other hand, according to several authors [17,18,58], ruminal propionate synthesis is considered the main alternative pathway to eliminate H_2 when methanogenesis is inhibited. Therefore, the increase in ruminal propionate concentration detected in the present study could be closely related to the reduction in daily CH_4 emission. In beef cattle, dietary supplementation with 3-NOP increases the relative abundance of the microbial genus *Succiniclasticum*, which plays an important role in rumen propionate production through succinate utilization [59]. A high ruminal concentration of propionate is positive because this volatile fatty acid is the main source of energy in ruminants [51,58].

Lopes et al. [55] reported that dietary supplementation with 3-NOP decreases (−20.7%) the abundance of *Ruminococcus* bacteria and increases (+33.3%) the abundance of *Butyrivibrio* bacteria in the ruminal fluid of dairy cows. Acetate-producing bacteria are found in the *Ruminococcus* genus [1], while the *Butyrivibrio* genus contains butyrate-producing microorganisms [55]. Therefore, the similar effects of 3-NOP intake on the ruminal microbiota of beef cattle could explain the reduction in acetate and the increase in butyrate observed in the present meta-analysis. Likewise, the increase in the ruminal concentration of butyrate and valerate observed in the present meta-analysis can be explained by the lower emission of CH_4 since, according to several authors [5,58,60], the formation of butyrate and valerate can act as alternative H_2 sinks when methanogenesis is decreased or inhibited. On the other hand, Gruninger et al. [60] detected that 3-NOP increases (+22.5%) the ruminal relative abundance of *Bacteroidetes* microorganisms in beef cattle. This effect of 3-NOP could partially explain the increases observed in the ruminal concentration of isobutyrate and isovalerate in the current study since, according to Zhao et al. [61], the presence of *Bacteroidetes* in the rumen of cattle is positively correlated ($r > 0.60$) with the ruminal concentration of isobutyrate and isovalerate.

4.3. Enteric Methane Emissions

According to Gerber et al. [4], enteric CH_4 contributes a high proportion (43%) of the GHGs emitted in beef production systems worldwide. Some studies [28,62] indicate that reducing enteric CH_4 can improve environmental sustainability in beef cattle production

systems. In the current study, daily CH_4 emission (−34.9%), CH_4 yield (−29.6%), and CH_4 emission as a percentage of ingested GE (−30.0%) decreased in response to dietary supplementation with 3-NOP. These effects suggest that dietary supplementation with 3-NOP could help improve environmental sustainability in beef production systems. Furthermore, the simultaneous reduction in the three types of CH_4 emission indicates that 3-NOP has a constant anti-methanogenic effect in beef cattle. Similarly, a recent meta-analysis [6] reported that dietary supplementation with 3-NOP decreases daily CH_4 emission, CH_4 yield, and CH_4 intensity by 32.7%, 30.9%, and 32.6%, respectively. Likewise, Martínez-Fernández et al. [14] detected 15.7% lower daily CH_4 emission and 23.7% lower CH_4 yield in sheep supplemented with 3-NOP. According to Duin et al. [3], 3-NOP acts through inhibiting the activity of the enzyme methyl-coenzyme M reductase (MCR), which is necessary for the formation of CH_4 because it catalyzes the last step of methanogenesis [8]. Therefore, the lower CH_4 emissions observed in the current study can be explained by this 3-NOP's mechanism of action. Furthermore, 3-NOP can inhibit methanogenic archaea's growth without negatively affecting other non-methanogenic rumen bacteria [3].

Subgroup analyses revealed that the reduction in daily CH_4 emission and CH_4 yield was 49.8 and 47.8% greater, respectively, with high doses (101–329 mg/kg DM) of 3-NOP than with low doses (35–100 mg/kg DM) of 3-NOP. This effect was expected since a previous study in cattle [21] estimated that, for every 10 mg/kg DM increase in the dose of 3-NOP, the emission of enteric CH_4 decreases by 2.56%. This variation in the effects of 3-NOP doses on CH_4 emission could be related to the differential effect that the 3-NOP dose has on the rumen microbiota [7]. For example, Romero-Pérez et al. [17] reported that low doses (≤109 mg/kg DM) of 3-NOP do not affect the rumen abundance of methanogens in beef cattle. In contrast, other studies [17,18,32] show that high doses (>200 mg/kg DM) of 3-NOP decrease between 35 and 60% rumen methanogens in beef cattle. Rumen methanogens have a positive correlation with daily CH_4 emission and CH_4 yield in beef cattle [63]. Therefore, a lower population of rumen methanogens may result in a greater reduction in enteric CH_4 emissions.

Yu et al. [7] mention that more research is necessary to identify whether the effects of 3-NOP on enteric CH_4 emissions are maintained in the long term. In the current study, some subgroup analyses showed that daily CH_4 emission and CH_4 yield decreased, regardless of the 3-NOP supplementation period. These effects suggest that 3-NOP could be used to mitigate enteric CH_4 in beef cattle for long periods without the apparent risk of adaptation of ruminal microorganisms to its effects. Similar to our results, previous studies also reported a persistent long-term (>10 weeks) reduction in enteric CH_4 emissions in dairy cows [64,65] and calves [66] supplemented with 3-NOP. In the present meta-analysis, another subgroup analysis showed that the reduction in daily CH_4 emission was 23.05% greater when high-concentrate diets (>700 g/kg DM) were used, compared to low-concentrate diets (≤700 g/kg DM). These effects could be related to the variation in the amount of NDF between high and low-concentrate diets. In support of this hypothesis, it has been reported that the effect of 3-NOP on the daily emission of CH_4 decreases by 1.64% for each increase of 10 g/kg of DM in the NDF content of the diet [21], and the level of NDF in ruminant diets decreases linearly when the proportion of concentrate in the diet increases [67].

In the rumen, CH_4 is the main sink for H_2 [58]. According to Beauchemin et al. [9], the H_2 available in the rumen can be used by methanogenic archaea as a substrate for the formation of CH_4. Therefore, the increase in daily H_2 emission observed in the current study may be directly related to the detected reduction in daily CH_4 emission. Furthermore, previous studies [17,18,20] detected between a 21.6 and 82.5% greater abundance of rumen protozoans in beef cattle supplemented with 3-NOP. Ruminal protozoans (e.g., *Epidinium*, *Entodinium*, *Isotricha*, and *Dasytricha*) have hydrogenosomes [68], which are organelles that produce and release hydrogen into the ruminal environment [69]. Therefore, an increase in the abundance of rumen protozoans in beef cattle supplemented with 3-NOP could partially explain the greater daily H_2 emission detected in the present study. Although

the emission of H_2 also causes energy loss in ruminants, on average, this energy is much lower than the energy lost due to the enteric emission of CH_4 [58]. On the other hand, in the present study, dietary supplementation with 3-NOP did not affect the daily CO_2 emission. Similarly, other authors [64,70] also did not detect significant effects of dietary supplementation with 3-NOP (between 40 and 200 mg/kg DM) on CO_2 emission (g/d, g/kg DMI or g/kg milk yield) in dairy cows.

5. Conclusions

The dietary supplementation with 3-nitrooxypropanol can be used as a nutritional strategy to improve feed efficiency and ruminal fermentation in beef cattle and, at the same time, reduce enteric methane emissions. The greatest reduction in enteric methane emissions is obtained with high doses (101–329 mg/kg DM) of 3-nitrooxypropanol, using long periods (>60 days) of supplementation with 3-nitrooxypropanol and with diets high in concentrate (>700 g/kg DM). However, more long-term studies are necessary to corroborate the anti-methanogenic effect of 3-nitrooxypropanol over long periods.

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

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

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

References

1. Araújo, T.L.; Rabelo, C.H.; Cardoso, A.S.; Carvalho, V.V.; Acedo, T.S.; Tamassia, L.F.; Vasconcelos, G.S.F.M.; Duval, S.M.; Kindermann, M.; Gouvea, V.N.; et al. Feeding 3-nitrooxypropanol reduces methane emissions by feedlot cattle on tropical conditions. *J. Anim. Sci.* **2023**, *101*, skad225. [CrossRef]
2. Alemu, A.; Shreck, A.; Booker, C.; McGinn, S.; Pekrul, L.; Kindermann, M.; Beauchemin, K. Use of 3-nitrooxypropanol in a commercial feedlot to decrease enteric methane emissions from cattle fed a corn-based finishing diet. *J. Amim. Sci.* **2021**, *99*, skaa394. [CrossRef]
3. Duin, E.C.; Wagner, T.; Shima, S.; Prakash, D.; Cronin, B.; Yáñez-Ruiz, D.R.; Duval, S.; Rümbeli, R.; Stemmler, R.T.; Thauer, R.K. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 6172–6177.
4. Gerber, P.J.; Steinfeld, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities*; FAO: Rome, Italy, 2013.
5. Kirwan, S.F.; Tamassia, L.F.; Walker, N.D.; Karagiannis, A.; Kindermann, M.; Waters, S.M. Effects of dietary supplementation with 3-nitrooxypropanol on enteric methane production, rumen fermentation, and performance in young growing beef cattle offered a 50:50 forage: Concentrate diet. *J. Anim. Sci.* **2023**, *102*, skad399. [CrossRef]
6. Kebreab, E.; Bannink, A.; Pressman, E.M.; Walker, N.; Karagiannis, A.; van Gastelen, S.; Dijkstra, J. A meta-analysis of the effects of 3-nitrooxyptropanol on methane production, yield, and intensity in dairy cattle. *J. Dairy Sci.* **2023**, *106*, 927–936. [CrossRef]
7. Yu, G.; Beauchemin, K.A.; Dong, R. A review of 3-nitrooxypropanol for enteric methane mitigation from ruminant livestock. *Animals* **2021**, *11*, 3540. [CrossRef]
8. Beauchemin, K.A.; Ungerfeld, E.M.; Abdalla, A.L.; Alvarez, C.; Arndt, C.; Becquet, P.; Benchaar, C.; Berndt, A.; Mauricio, R.M.; McAllister, T.A.; et al. Invited Review: Current Enteric Methane Mitigation Options. *J. Dairy Sci.* **2022**, *105*, 9297–9326. [CrossRef]
9. Beauchemin, K.A.; Ungerfeld, E.M.; Eckard, R.J.; Wang, M. Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Anim. Int. J. Anim. Biosci.* **2020**, *14*, S2–S16. [CrossRef]

10. Thiel, A.; Rümbeli, R.; Mair, P.; Yeman, H.; Beilstein, P. 3-NOP: ADME studies in rats and ruminating animals. *Food Chem. Toxicol.* **2019**, *125*, 528–539. [CrossRef] [PubMed]
11. Alvarez-Hess, P.S.; Moate, P.J.; Williams, S.R.O.; Jacobs, J.L.; Beauchemin, K.A.; Hannah, M.C.; Durmic, Z.; Eckard, R.J. Effect of combining wheat grain with nitrate, fat or 3-nitrooxypropanol on in vitro methane production. *Anim. Feed Sci. Technol.* **2019**, *256*, 114237. [CrossRef]
12. Romero-Pérez, A.; Okine, E.K.; Guan, L.L.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. Effects of 3-nitrooxypropanol and monensin on methane production using a forage-based diet in Rusitec fermenters. *Anim. Feed Sci. Technol.* **2016**, *220*, 67–72. [CrossRef]
13. Romero-Pérez, A.; Okine, E.K.; Guan, L.L.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. Effects of 3-nitrooxypropanol on methane production using the rumen simulation technique (Rusitec). *Anim. Feed Sci. Technol.* **2015**, *209*, 98–109. [CrossRef]
14. Martínez-Fernández, G.; Abecia, L.; Arco, A.; Cantalapiedra-Hijar, G.; Martín-García, A.I.; Molina-Alcaide, E.; Kindermann, M.; Duval, S.; Yáñez-Ruiz, D.R. Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. *J. Dairy Sci.* **2014**, *97*, 3790–3799. [CrossRef] [PubMed]
15. Vyas, D.; McGinn, S.; Duval, S.; Kindermann, M.; Beauchemin, K. Optimal dose of 3-nitrooxypropanol for decreasing enteric methane emissions from beef cattle fed high-forage and high-grain diets. *Anim. Prod. Sci.* **2016**, *58*, 1049–1055. [CrossRef]
16. Vyas, D.; McGinn, S.M.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. *J. Anim. Sci.* **2016**, *94*, 2024–2034. [CrossRef]
17. Romero-Perez, A.; Okine, E.K.; McGinn, S.M.; Guan, L.L.; Oba, M.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. *J. Anim. Sci.* **2014**, *92*, 4682–4693. [CrossRef] [PubMed]
18. Romero-Perez, A.; Okine, E.K.; McGinn, S.M.; Guan, L.L.; Oba, M.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. Sustained reduction in methane production from long-term addition of 3-nitrooxypropanol to a beef cattle diet. *J. Anim. Sci.* **2015**, *93*, 1780–1791. [CrossRef] [PubMed]
19. Alemu, A.W.; Pekrul, L.K.; Shreck, A.L.; Booker, C.W.; McGinn, S.M.; Kindermann, M.; Beauchemin, K.A. 3-Nitrooxypropanol decreased enteric methane production from growing beef cattle in a commercial feedlot: Implications for sustainable beef cattle production. *Front. Anim. Sci.* **2021**, *2*, 641590. [CrossRef]
20. Alemu, A.W.; Gruninger, R.J.; Zhang, X.M.; O'Hara, E.; Kindermann, M.; Beauchemin, K.A. 3-Nitrooxypropanol supplementation of a forage diet decreased enteric methane emissions from beef cattle without affecting feed intake and apparent total-tract digestibility. *J. Anim. Sci.* **2023**, *101*, skad001. [CrossRef]
21. Dijkstra, J.; Bannink, A.; France, J.; Kebreab, E.; van Gastelen, S. Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. *J. Dairy Sci.* **2018**, *101*, 9041–9047. [CrossRef]
22. Kim, H.; Lee, H.G.; Baek, Y.C.; Lee, S.; Seo, J. The effects of dietary supplementation with 3-nitrooxypropanol on enteric methane emissions, rumen fermentation, and production performance in ruminants: A meta-analysis. *J. Anim. Sci. Technol.* **2020**, *62*, 31–42. [CrossRef]
23. Jayanegara, A.; Sarwono, K.A.; Kondo, M.; Matsui, H.; Ridla, M.; Laconi, E.B.; Nahrowi. Use of 3-nitrooxypropanol as feed additive for mitigating enteric methane emissions from ruminants: A meta-analysis. *Ital. J. Anim. Sci.* **2018**, *17*, 650–656. [CrossRef]
24. Ioannidis, J.P.A. Why most published research findings are false. *PLoS Med.* **2005**, *2*, e124. [CrossRef] [PubMed]
25. Santos, C.M.D.C.; Pimenta, C.A.D.M.; Nobre, M.R.C. The PICO strategy for the research question construction and evidence search. *Rev. Latino-Am. Enferm.* **2007**, *15*, 508–511. [CrossRef]
26. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Group, P. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. [CrossRef] [PubMed]
27. Dorantes-Iturbide, G.; Orzuna-Orzuna, J.F.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Lee-Rangel, H.A. Essential oils as a dietary additive for small ruminants: A meta-analysis on performance, rumen parameters, serum metabolites, and product quality. *Vet. Sci.* **2022**, *9*, 475. [CrossRef] [PubMed]
28. Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Hernández-García, P.A. Effects of dietary tannins' supplementation on growth performance, rumen fermentation, and enteric methane emissions in beef cattle: A meta-analysis. *Sustainability* **2021**, *13*, 7410. [CrossRef]
29. Almeida, A.K.; Cowley, F.; McMeniman, J.P.; Karagiannis, A.; Walker, N.; Tamassia, L.F.; McGrath, J.J.; Hegarty, R.S. Effect of 3-nitrooxypropanol on enteric methane emissions of feedlot cattle fed with a tempered barley-based diet with canola oil. *J. Anim. Sci.* **2023**, *101*, skad237. [CrossRef] [PubMed]
30. Kim, S.H.; Lee, C.; Pechtl, H.A.; Hettick, J.M.; Campler, M.R.; Pairis-Garcia, M.D.; Beauchemin, K.A.; Celi, P.; Duval, S.M. Effects of 3-nitrooxypropanol on enteric methane production, rumen fermentation, and feeding behavior in beef cattle fed a high-forage or high-grain diet. *J. Anim. Sci.* **2019**, *97*, 2687–2699. [CrossRef]
31. Lee, C.; Kim, S.-H.; Beauchemin, K.; Celi, P.; Duval, S. Short-term eating preference of beef cattle fed high forage or high grain diets supplemented with 3-nitrooxypropanol. *Animals* **2020**, *10*, 64. [CrossRef]

32. Martinez-Fernandez, G.; Duval, S.; Kindermann, M.; Schirra, H.J.; Denman, S.E.; McSweeney, C.S. 3-NOP vs. Halogenated compound: Methane production, ruminal fermentation and microbial community response in forage fed cattle. *Front. Microbiol.* **2018**, *9*, 1582. [CrossRef]
33. Vyas, D.; Alemu, A.W.; McGinn, S.M.; Duval, S.M.; Kindermann, M.; Beauchemin, K.A. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. *J. Anim. Sci.* **2018**, *96*, 2923–2938. [CrossRef] [PubMed]
34. Zhang, X.; Smith, M.; Gruninger, R.; Kung, L.; Vyas, D.; McGinn, S.; Kindermann, M.; Wang, M.; Tan, Z.; Beauchemin, K. Combined effects of 3-nitrooxypropanol and canola oil supplementation on methane emissions, rumen fermentation and biohydrogenation, and total tract digestibility in beef cattle. *J. Amim. Sci.* **2021**, *99*, skab081. [CrossRef] [PubMed]
35. Schwarzer, G.; Carpenter, J.R.; Rücker, G. *Meta-Analysis with R*; Springer: Berlin/Heidelberg, Germany, 2015; ISBN 9783319214153.
36. Viechtbauer, W. Conducting Meta-Analyses in R with the metafor Package. *J. Stat. Soft.* **2010**, *36*, 1–48. [CrossRef]
37. Takeshima, N.; Sozu, T.; Tajika, A.; Ogawa, Y.; Hayasaka, Y.; Furukawa, T.A. Which is more generalizable, powerful and interpretable in meta-analyses, mean difference or standardized mean difference? *BMC Med. Res. Methodol.* **2014**, *14*, 30. [CrossRef]
38. Der-Simonian, R.; Laird, N. Meta-analysis in clinical trials. *Control. Clin. Trials* **1986**, *7*, 177–188. [CrossRef]
39. Borenstein, M.; Hedges, L.V.; Higgins, J.P.T.; Rothstein, H.R. *Introduction to Meta-Analysis*, 1st ed.; John Wiley and Sons, Ltd.: Chichester, UK, 2009; p. 413.
40. Littell, J.H.; Corcoran, J.; Pillai, V. *Systematic Reviews and Meta-Analysis*, 1st ed.; Oxford University Press: Oxford, UK, 2008; pp. 111–132.
41. Borenstein, M.; Higgins, J.P.T.; Hedges, L.V.; Rothstein, H.R. Basics of meta-analysis: I_2 is not an absolute measure of heterogeneity. *Res. Synth. Methods* **2017**, *8*, 5–18. [CrossRef]
42. Higgins, J.P.T.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analysis. *BMJ* **2003**, *327*, 557–560. [CrossRef]
43. Egger, M.; Smith, G.D.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **1997**, *315*, 629–634. [CrossRef]
44. Begg, C.B.; Mazumdar, M. Operating characteristics of a rank correlation test for publication Bias. *Biometrics* **1994**, *50*, 1088–1101. [CrossRef]
45. Egger, M.; Smith, G.D.; Altman, D.G. *Systematic Reviews in Health Care*, 2nd ed.; MBJ Publishing Group: London, UK, 2001; pp. 109–121.
46. Hernández-García, P.A.; Orzuna-Orzuna, J.F.; Godina-Rodríguez, J.E.; Chay-Canul, A.J.; Silva, G.V. A meta-analysis of essential oils as a dietary additive for weaned piglets: Growth performance, antioxidant status, immune response, and intestinal morphology. *Rese. Vet. Sci.* **2024**, *170*, 105181. [CrossRef]
47. Rathert-Williams, A.R.; McConnell, H.L.; Salisbury, C.M.; Lindholm-Perry, A.K.; Lalman, D.L.; Pezeshki, A.; Foote, A.P. Effects of adding ruminal propionate on dry matter intake and glucose metabolism in steers fed a finishing ration. *J. Anim. Sci.* **2023**, *101*, skad072. [CrossRef]
48. Rathert-Williams, A.R.; Salisbury, C.M.; Lindholm-Perry, A.K.; Pezeshki, A.; Lalman, D.L.; Foote, A.P. Effects of increasing calcium propionate in a finishing diet on dry matter intake and glucose metabolism in steers. *J. Anim. Sci.* **2021**, *99*, skab314. [CrossRef]
49. Johnson, K.A.; Johnson, D.E. Methane emissions from cattle. *J. Anim. Sci.* **1995**, *73*, 2483–2492. [CrossRef]
50. Calsamiglia, S.; Ferret, A.; Reynolds, C.K.; Kristensen, N.B.; Van Vuuren, A.M. Strategies for optimizing nitrogen use by ruminants. *Animal* **2010**, *4*, 1184–1196. [CrossRef]
51. Pathak, A.K. Various factors affecting microbial protein synthesis in the rumen. *Vet. World.* **2008**, *1*, 186.
52. Bach, A.; Calsamiglia, S.; Stern, M. Nitrogen metabolism in the rumen. *J. Dairy Sci.* **2005**, *88*, E9–E21. [CrossRef]
53. Nagaraja, T.G.; Titgemeyer, E.C. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *J. Dairy Sci.* **2007**, *90*, E17–E38. [CrossRef]
54. Rémond, D.; Chaise, J.P.; Delval, E.; Poncet, C. Net transfer of urea and ammonia across the ruminal wall of sheep. *J. Anim. Sci.* **1993**, *71*, 2785–2792. [CrossRef]
55. Lopes, J.C.; de Matos, L.F.; Harper, M.T.; Giallongo, F.; Oh, J.; Gruen, D.; Ono, S.; Kindermann, M.; Duval, S.; Hristov, A.N. Effect of 3-nitrooxipropanol on methane and hydrogen emissions, methane isotopic signature, and ruminal fermentation in dairy cows. *J. Dairy Sci.* **2016**, *99*, 5335–5344. [CrossRef]
56. McSweeney, C.S.; Palmer, B.; Bunch, R.; Krause, D.O. Isolation and characterization of proteolytic ruminal bacteria from sheep and goats fed the tannin-containing shrub legume *Calliandra calothyrsus*. *Appl. Environ. Microbiol.* **1999**, *65*, 3075–3083. [CrossRef]
57. Krause, D.O.; Russell, J.B. An rRNA approach for assessing the role of obligate amino acid fermenting bacteria in ruminal amino acid deamination. *Appl. Environ. Microbiol.* **1996**, *62*, 815–821. [CrossRef]
58. Ungerfeld, E.M. Inhibition of rumen methanogenesis and ruminant productivity: A meta-analysis. *Front. Vet. Sci.* **2018**, *5*, 113. [CrossRef]
59. Wang, K.; Xiong, B.; Zhao, X. Could propionate formation be used to reduce enteric methane emission in ruminants? *Sci. Total Environ.* **2023**, *855*, 158867. [CrossRef]

60. Gruninger, R.J.; Zhang, X.M.; Smith, M.L.; Kung, L.; Vyas, D.; McGinn, S.M.; Kindermann, M.; Wang, M.; Tan, Z.L.; Beauchemin, K.A. Application of 3-Nitrooxypropanol and Canola Oil to Mitigate Enteric Methane Emissions of Beef Cattle Results in Distinctly Different Effects on the Rumen Microbial Community. *Anim. Microbiome* **2022**, *4*, 35. [CrossRef]
61. Zhao, C.; Shen, B.; Huang, Y.; Kong, Y.; Tan, P.; Zhou, Y.; Yang, J.; Xu, C.; Wang, J. Effects of chromium propionate and calcium propionate on lactation performance and rumen microbiota in postpartum heat-stressed holstein dairy cows. *Microorganisms* **2023**, *11*, 1625. [CrossRef]
62. Aboagye, I.A.; Beauchemin, K.A. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: A review. *Animals* **2019**, *9*, 856. [CrossRef]
63. Smith, P.E.; Kelly, A.K.; Kenny, D.A.; Waters, S.M. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emissions. *Front. Microbiol.* **2022**, *13*, 855565. [CrossRef]
64. Melgar, A.; Lage, C.F.A.; Nedelkov, K.; Räisänen, S.E.; Stefenoni, H.; Fetter, M.E.; Chen, X.; Oh, J.; Duval, S.; Kindermann, M.; et al. Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. *J. Dairy Sci.* **2021**, *104*, 357–366. [CrossRef]
65. Hristov, A.N.; Oh, J.; Giallongo, F.; Frederick, T.W.; Harper, M.T.; Weeks, H.L.; Branco, A.F.; Moate, P.J.; Deighton, M.H.; Williams, S.R.O. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 10663–10668. [CrossRef]
66. Meale, S.J.; Popova, M.; Saro, C.; Martin, C.; Bernard, A.; Lagree, M.; Yáñez-Ruiz, D.R.; Boudra, H.; Duval, S.; Morgavi, D.P. Early life dietary intervention in dairy calves results in a long-term reduction in methane emissions. *Sci. Rep.* **2021**, *11*, 3003. [CrossRef]
67. Hernández, P.A.; Mendoza, G.D.; Castro, A.; Lara, A.; Plata, F.X.; Martínez, J.A.; Ferraro, S. Effects of grain level on lamb performance, ruminal metabolism and leptin mRNA expression in perirenal adipose tissue. *Anim. Prod. Sci.* **2017**, *57*, 2001–2006. [CrossRef]
68. Finlay, B.J.; Esteban, G.; Clarke, K.J.; Williams, A.G.; Embley, T.; Hirt, R.P. Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiol. Lett.* **1994**, *117*, 157–162. [CrossRef]
69. Tan, C.; Ramírez-Restrepo, C.A.; Shah, A.M.; Hu, R.; Bell, M.; Wang, Z.; McSweeney, C. The Community Structure and Microbial Linkage of Rumen Protozoa and Methanogens in Response to the Addition of Tea Seed Saponins in the Diet of Beef Cattle. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 80. [CrossRef]
70. Melgar, A.; Welter, K.; Nedelkov, K.; Martins, C.; Harper, M.; Oh, J.; Räisänen, S.; Chen, X.; Cueva, S.; Duval, S.; et al. Dose-response effect of 3-nitrooxypropanol on enteric methane emissions in dairy cows. *J. Dairy Sci.* **2020**, *103*, 6145–6156. [CrossRef]