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Article

Meta-Analysis of the Effect of Malic Acid or Malate Supplementation on Ruminal Parameters, Nutrient Digestibility and Blood Characteristics of Cattle

Leonardo Tombesi da Rocha [1,*], Tiago Antonio Del Valle [1], Fernando Reimann Skonieski [2], Stela Naetzold Pereira [1], Paola Selau de Oliveira [1], Francine Basso Facco [1] and Julio Viégas [1]

[1] Federal University of Santa Matia, Animal Science Department; Avenida Roraima 1000, CEP 97105-900, Santa Maria – RS, Brazil

[2] Federal University of Technology – Parana *Campus* Dois Vizinhos; Estrada para Boa Esperança km 04, CEP 85660-000, Dois Vizinhos – PR, Brazil

* Correspondence: leonardo_ltr@live.com

Simple Summary: Improving nutrient use and metabolic balance in cattle is essential for sustainable livestock production. Malic acid, a natural compound, has been studied as an alternative to conventional feed additives. This study combined data from several experiments to evaluate the effects of malic acid and malate on digestion and blood indicators in cattle. Malate improved ruminal pH stability, supporting fiber digestion. Both compounds increased beneficial fermentation products, especially propionate, and improved blood glucose while reducing signs of fat mobilization. These findings suggest that malic acid and malate can enhance digestive efficiency and energy balance in cattle, offering a natural strategy to improve performance and reduce reliance on synthetic additives.

Abstract: The aim of this study was to determine, through meta-analysis, the effects of malic acid/malate supplementation on ruminal and blood parameters and diet digestibility in cattle. The effect of malic acid/malate addition was evaluated using the effect size (ES) method. Two sensitivity analyses were performed: subgroup analysis and meta-regression. The subgroups were defined as follows: "acid," representing animals supplemented with malic acid, and "salt," representing animals supplemented with malate. Diet-related covariates were included in the meta-regression analysis. Overall, malic acid (MAC) supplementation did not significantly affect rumen pH (ES = 0.310, P = 0.17), but subgroup analysis showed that malate increased pH (ES = 1.420, P < 0.01). MAC increased rumen propionate (ES = 0.560, P < 0.01) and total volatile fatty acids (VFAs; ES = 0.508, P = 0.03), while reducing the acetate-to-propionate ratio (P < 0.01). Starch and NDF intake were significant covariates affecting pH and VFA-related variables. MAC improved total-tract digestibility of dry matter (DM; ES = 0.547, P ≤ 0.05), crude protein (CP; ES = 0.422, P ≤ 0.05), and acid detergent fiber (ADF; ES = 0.635, P ≤ 0.05). It increased glucose levels (ES = 0.170, P = 0.05) and reduced NEFA (ES = -0.404, P = 0.03). In conclusion, MAC effects were influenced by its chemical form, improving rumen pH, CP, NDF, and ADF digestibility. MAC increased VFAs, particularly propionate, lowered the acetate-to-propionate ratio, raised blood glucose, and reduced NEFA. Diet-related covariates were important to explain the between-study heterogeneity.

Keywords: blood metabolites; dietary covariates; meta-regression; organic acid; ruminal pH; volatile fatty acids

1. Introduction

Feed additives have been used to improve ruminants productively for decades [1]. However, one of the most popular feed additives (ionophores) has faced restrictions due to the potential limited safety [2]. In this way, several additives are evaluated as ionophores replacers. Among these

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alternative additives, malic acid (MAC) highlights as an organic acid naturally found in animals and plants' organisms.

Malic acid is a citric acid cycle intermediate, and its addition increases in vitro rumen lactate uptake, volatile fatty acids (VFA) production, and diet digestibility, reducing methane emission [3–5]. Besides, MAC increases in vivo VFA rumen concentration [6,7] through increased propionate and butyrate content [8]. In addition, animals fed MAC show higher rumen pH [9] and improved nutrient digestibility [10]. However, these effects have been not observed in all studies [7,11,12].

The diversity of results among studies may be linked to differences in MAC presentation (acid or salt) and potential interaction with the substrates, such as dietary starch level [5]. In this sense, meta-analysis could be used to allows the results of several experiments into a single effect estimate to determine the real effect of the MAC on the variables of interest in addition to determining and quantifying the influence of covariates on the meta-analyzed result.

Therefore, the objective of this study was to determine, through meta-analysis, the effects of supplementation with MAC on ruminal and blood parameters and digestibility of dietary fractions in cattle.

2. Materials and Methods

2.1. Database

The manuscripts search was carried out in the search engines "Web of Science", "Science Direct", and "Google Scholar". The Boolean moderators used, alone or in combination, were: "organic acids", "malic acid", "malate", and "bovine". Searches based on title and abstract of articles, master's dissertations, and PhD thesis. The PICO (population/problem, intervention/exposure, comparison, outcome) method was considered to build the database [13]. Population was cattle; intervention was supplementation with MAC acid or salt; control was cattle fed without MAC; and results were rumen parameters, nutrients digestibility, and blood parameters.

The studies needed to be original and show mean and dispersion for each variable. As the analysis requires the standard deviation associated with each variable, if these were not provided directly, they were calculated using the measures presented in the paper, such as standard error of the mean, coefficients of variation and others. Only studies that presented results for a control and treatment group (MAC supplementation) were considered. Independent experiments in the same study were included as a new comparison. Similarly, malate doses in the same trial were included as a new comparison. The following information was recorded from each trial: study reference, adaptation period, year, experimental design, MAC dose, forage to concentrate ratio, diet chemical composition, dry matter intake, animals breed, and initial body weight. Only studies reporting diet chemical composition or providing information to estimate it were included (Table 1). Rumen parameters, nutrient digestibility, and blood parameters results were recorded. The final database had 47 comparisons from 19 studies.

Table 1. Chemical form, cereal and main forage, supplement dose and calculated composition of the total ration mixed in experiments with cattle supplemented with malic acid or malate.

Author	Form	Main Cereal	Main forrage	Dose (g/day)	CP (%)	NDF (%)	ADF (%)	Starch (%)	EE (%)
Kung Jr. et al. 1982 A	Acid	Corn	Corn silage	70.00	11.18	24.71	13.95	35.77	1.99
Kung Jr. et al. 1982 B	Acid	Corn	Corn silage	105.00	11.18	24.71	13.95	35.77	1.99
Kung Jr. et al. 1982 C	Acid	Corn	Corn silage	140.00	11.18	24.71	13.95	35.77	1.99
Kung Jr. et al. 1982 D	Acid	Corn	Corn silage	42.00	8.76	25.54	13.76	46.78	2.77
Kung Jr. et al. 1982 E	Acid	Corn	Corn silage	84.00	8.76	25.54	13.76	46.78	2.77
Martin et al. 1999 A	Saltt	Corn	Cottonseed hulls	27.00	11.39	19.10	9.93	49.60	2.95
Martin et al. 1999 B	Saltt	Corn	Cottonseed hulls	54.00	11.39	19.10	9.93	49.60	2.95
Martin et al. 1999 C	Saltt	Corn	Cottonseed hulls	80.00	11.39	19.10	9.93	49.60	2.95
Sniffen et al. 2006	Saltt	Corn	Corn silage	50.00	18.20	31.80	21.40	29.40	2.70
Khampa et al. 2006 A	Saltt	Cassava	Rice straw	9.00	8.61	41.14	23.86	34.90	3.51
Khampa et al. 2006 B	Saltt	Cassava	Rice straw	18.00	8.61	41.14	23.86	34.90	3.51
Khampa et al. 2006 C	Saltt	Cassava	Rice straw	27.00	8.61	41.14	23.86	34.90	3.51

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Devant et al. 2007	Saltt	-	-	84.00	14.31	32.84	16.93	30.16	3.16
Foley et al. 2009 A	Acid	Barley	Silage	34.00	15.60	23.10	13.80	28.10	2.50
Foley et al. 2009 B	Acid	Barley	Silage	65.40	15.60	23.10	13.80	28.10	2.50
Foley et al. 2009 C	Acid	Barley	Silage	32.38	15.57	23.09	13.82	28.12	2.47
Foley et al. 2009 D	Acid	Barley	Silage	64.85	15.57	23.09	13.82	28.12	2.47
Foley et al. 2009 E	Acid	Barley	Silage	98.25	15.57	23.09	13.82	28.12	2.47
Wang et al. 2009 A	Acid	Corn	Corn silage	70.00	16.50	42.40	27.10	31.70	1.50
Wang et al. 2009 B	Acid	Corn	Corn silage	140.00	16.50	42.40	27.10	31.70	1.50
Wang et al. 2009 C	Acid	Corn	Corn silage	210.00	16.50	42.40	27.10	31.70	1.50
Liu et al. 2009 A	Acid	Corn	Corn straw	70.20	8.29	55.82	21.85	14.78	1.72
Liu et al. 2009 B	Acid	Corn	Corn straw	140.40	8.29	55.82	21.85	14.78	1.72
Liu et al. 2009 C	Acid	Corn	Corn straw	210.60	8.29	55.82	21.85	14.78	1.72
Hernández et al. 2011 A	Salt	Barley	Barley straw	30.80	13.83	37.30	16.62	28.51	3.87
Hernández et al. 2011 B	Acid	Barley	Barley straw	26.80	13.83	37.30	16.62	28.51	3.87
Hernández et al. 2011 C	Salt	Barley	Barley straw	28.40	13.83	37.30	16.62	28.51	3.87
Carrasco et al. 2012 A	Acid	Barley	Barley straw	9.38	16.61	21.59	8.35	37.04	9.76
Carrasco et al. 2012 B	Salt	Barley	Barley straw	9.12	16.61	21.59	8.35	37.04	9.76
Vyas et al. 2015 A	Acid	Barley	Barley silage	89.00	9.74	16.86	6.57	45.32	1.57
Vyas et al. 2015 B	Acid	Barley	Barley silage	177.00	9.74	16.86	6.57	45.32	1.57
Malekkhahi et al. 2016 A	Salt	Corn	Corn silage	80.00	17.69	27.64	16.66	29.90	2.23
Malekkhahi et al. 2016 B	Salt	Corn	Corn silage	80.00	20.93	32.50	18.25	45.53	2.74
El-Zaiat et al. 2019	Acid	Corn	Corn silage	30.00	17.16	32.29	19.06	36.70	5.60

CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, Ether extract; Capital letters indicate different experiments/comparisons within a study.

2.2. Statistical Analysis

The effect of supplementation was evaluated using the effect size method. The effect size was calculated as the difference between the treatment group (MAC acid or MAC Salt) and the control group divided by the pooled standard deviation of each trial. The average effect of MAC supplementation was calculated using the "DerSimonian and Laird" random effects model [14]. Heterogeneity across trials was checked using Cochran's Q test, according to Higgins et al. [15].

Two analyses were performed to discuss the results: subgroup comparison and meta-regression. Subgroup analysis was performed by dividing the studies into two groups using MAC form: acid vs. salt. Meta-regression was used to explore the linear effects of covariates as variability sources. The following covariates were evaluated: NDF intake (g/kg BW), ADF intake (g/kg BW), starch intake (g/kg BW) and MAC intake (g/kg BW). It is important to point out that only those variables that had at least 10 comparisons and significant heterogeneity were subjected to meta-regression analysis [15]. Forest plots were used to present the average effect size and confidence interval. The "leave one out" analysis was used as a sensitivity test. All analyses were performed using the OpenMetaAnalyst statistical package.

3. Results

3.1. Rumen Parameters

MAC addition did not affect cattle rumen pH (Overall ES= 0.310, P = 0.17). In addition, subgroup analysis showed that MAC acid also had no effect (P = 0.12) on pH (Figure 1). However, MAC salt increased rumen pH (Subgroup ES= 1.420, P < 0.01). No effect of supplementation (P > 0.05) was observed for ammonia nitrogen (NH_3N) (Table 2).

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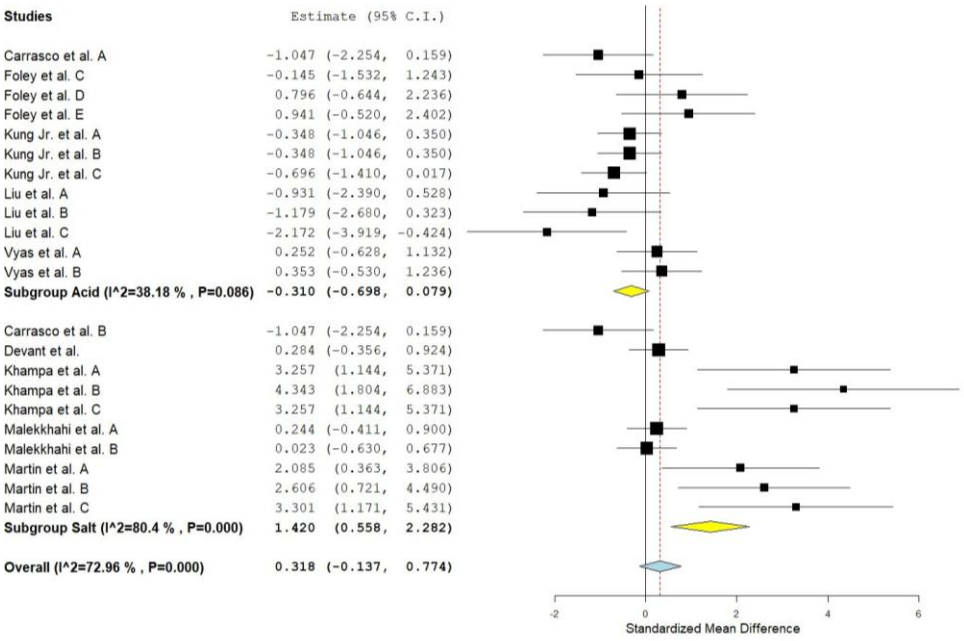

Figure 1. Forest plot of the effect of supplementation with malic acid or malate on the pH of cattle. When the diamond was presented to the left of the central line (standardized mean) without touching it, the effect was considered to be negative, favoring control. When presented to the right of the center line, the effect was considered positive, in favor of the supplement.

Table 2. Summary of meta-analysis (effect size) of malate or malic acid supplementation on ruminal parameters of cattle.

Variable	Form	n	ES(CI)	ES p-value	I^2	Het p-value
pH	Salt	11	1.420 (0.558; 2.282)	0.00	80.40	<0.01
	Acid	12	-0.310 (-0.698; 0.079)	0.12	38.18	0.09
	Overall	23	0.310 (-0.137; 0.774)	0.17	72.95	<0.01
Acetate	Salt	11	-0.592 (-1.381; 0.196)	0.14	72.26	<0.01
	Acid	14	0.167 (-0.386; 0.720)	0.55	78.08	<0.01
	Overall	25	-0.120 (-0.584; 0.345)	0.61	76.34	<0.01
Butyrate	Salt	11	-0.356 (-1.040; 0.328)	0.31	72.84	<0.01
	Acid	14	-0.058 (-0.717; 0.601)	0.86	78.39	<0.01
	Overall	25	-0.178 (-0.653; 0.297)	0.46	76.69	<0.01
Propionate	Salt	11	0.756 (-0.075; 1.588)	0.08	80.10	<0.01
	Acid	14	0.472 (0.066; 0.879)	0.02	48.17	0.02
	Overall	25	0.560 (0.160; 0.959)	0.01	67.31	<0.01
Lactate	Salt	6	0.337 (-0.517; 1.191)	0.44	67.60	0.01
	Acid	6	-0.621 (-1.512; 0.270)	0.17	74.94	<0.01
	Overall	12	-0.113 (-0.711; 0.485)	0.71	70.76	<0.01
ACT:PRP	Salt	9	-1.327 (-2.683; 0.030)	0.06	82.04	<0.01
	Acid	6	-1.109 (-2.470; 0.252)	0.11	83.70	<0.01
	Overall	15	-1.130 (-2.028; -0.232)	0.01	81.68	<0.01
NH_3N	Salt	8	0.161 (-0.170; 0.492)	0.34	0.99	0.42
	Acid	12	-0.089 (-0.560; 0.381)	0.71	47.12	0.04
	Overall	20	0.079 (-0.227; 0.385)	0.61	36.62	0.05
Total VFA	Salt	11	0.547 (-0.249; 1.343)	0.18	78.21	<0.01
	Acid	14	0.518 (-0.034; 1.071)	0.07	69.90	<0.01
	Overall	25	0.508 (0.055; 0.961)	0.03	73.78	<0.01

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N = number of comparisons; ES = effect size; CI = confidence interval; VFA = volatile fatty acids; NH_3N = ammonia nitrogen; ACT:PRP = acetate:propionate ratio.

The heterogeneity between studies (I^2) associated with these variables was significant, being 36.62 and 72.95% for NH_3N and pH, respectively. Among the covariates tested in the meta-regression, NDF intake decreases ($P \leq 0.01$) the ES of MAC supplementation for rumen pH and ammonia-N concentration (Table 3).

Table 3. Summary of meta-analysis (effect size) of malate or malic acid supplementation on blood parameters and diet digestibility of cattle.

Trait	Form	Std	ES(CI)	p-value	I^2	Het p-value
Blood parameters						
	Salt	7	0.163 (-0.132; 0.457)	0.28	0.00	0.88
Glucose	Acid	9	0.173 (-0.034; 0.379)	0.10	0.49	0.43
	Overall	16	0.170 (0.002; 0.338)	0.05	0.00	0.78
	Salt	7	0.028 (-0.385; 0.441)	0.89	45.12	0.11
Urea	Acid	8	-0.109 (-0.413; 0.194)	0.48	53.35	0.04
	Overall	15	-0.033 (-0.279; 0.212)	0.79	47.24	0.03
	Salt	7	-0.060 (-0.956; 0.836)	0.90	82.63	<0.01
Lactate	Acid	2	-1.661 (-2.690; -0.361)	0.01	57.23	0.13
	Overall	9	-0.490 (-1.316; 0.337)	0.25	83.31	<0.01
	Salt	2	-0.024 (-0.597; 0.550)	0.94	0.00	0.94
NEFA	Acid	7	-0.626 (-1.065; -0.187)	0.01	0.00	0.47
	Overall	9	-0.404 (-0.759; -0.049)	0.03	3.56	0.40
	Salt	2	0.532 (-0.769; 1.832)	0.42	78.81	0.03
ß-hidroxibutirato	Acid	7	-0.260 (-1.172; 0.652)	0.58	75.68	0.01
	Overall	9	-0.018 (-0.742; 0.706)	0.96	75.20	<0.01
Digestibility						
Dry matter	Salt	5	-0.084 (-0.575; 0.407)	0.74	0.00	0.95
	Acid	8	0.940 (0.229; 1.651)	0.01	73.01	0.01
	Overall	13	0.547 (0.027; 1.067)	0.04	78.74	<0.01
Organic matter	Salt	4	0.056 (-0.435; 0.546)	0.82	0.00	0.99
	Acid	5	0.694 (-0.217; 1.604)	0.14	53.15	0.07
	Overall	9	0.308 (-0.148; 0.764)	0.19	21.24	0.25
Protein	Salt	5	1.168 (0.217; 2.118)	0.02	52.70	0.10
	Acid	8	0.215 (-0.197; 0.627)	0.31	0.00	0.97
	Overall	13	0.422 (0.099; 0.745)	0.01	0.00	0.47
NDF	Salt	5	1.537 (0.277; 2.797)	0.02	77.39	0.00
	Acid	6	-0.085 (-0.576; 0.406)	0.73	0.00	0.94
	Overall	11	0.699 (-0.007; 1.406)	0.05	67.29	0.01
ADF	Salt	4	0.547 (0.042; 1.051)	0.03	0.00	0.45
	Acid	8	0.654 (-0.078; 1.387)	0.08	60.62	0.01
	Overall	12	0.635 (0.148; 1.121)	0.01	46.49	0.03

N number of comparisons; ES effect size; CI confidence interval; NEFA non esterified fatty acids; NDF neutral detergent fiber; ADF acids detergent fiber.

In general, MAC salt or acid had no effect ($P > 0.05$) on rumen acetate, butyrate, and lactate (Table 4).

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Table 4. Meta-regression of the effect of supplementation with malic acid or malate on ruminal and blood parameters and digestibility of dietary fractions determined with cattle.

Variables	Covariables, g/kg BW			
	NDF	ADF	Starch	Organic acid
Rumen parameters				
pH	2.063 -0.051x*	0.908 -0.142	0.584 -0.004x	0.343+ 0.548x
Acetate	0.126 -0.008x	0.464 -0.173x	-1.726 +0.039x**	-0.455+ 2.690x
Butyrate	-1.475 +0.038x	-1.155 +0.252x	-0.649 +0.009x	-1.121 +7.116x
Propionate	0.239 +0.009x	0.357 +0.055x	-0.518+0.028x**	0.871 -2.894x
Lactate	1.250 -0.041x*	0.684 -0.242x	-1.462 +0.031xT	-0.547 +3.758x
Acetate:propionate	1.887 -0.118x*	3.057 -1.463x**	-7.483 +0.156x**	-3.000 +10.725x
NH_3N	1.337 -0.033x**	0.854 -0.176x	-0.032 +0.002x	0.262 -1.384x
Total VFA	-0.034 +0.019x	0.632-0.013x	-1.033+0.042x*	1.258 -5.318x
Blood parameters				
Urea	-0.074 +0.001x	-0.053 +0.005x	0.370 -0.007x	0.007 -0.190x
Digestibility				
Dry matter	-0.374 +0.023xT	0.418 +0.021x	0.673 -0.004x	1.067 -6.511x
Protein	0.613 -0.004x	0.336 +0.016x	0.483 -0.002x	0.560 -1.665x
NDF	0.135 +0.013x	1.309 -0.171xT	1.375 -0.025x*	1.530 -13.733xT
ADF	0.550 -0.002x	0.695 -0.039x	1.167 -0.016x	0.795 -3.606x

N number of comparisons; ES effect size; CI confidence interval; NEFA non esterified fatty acids; NDF neutral detergent fiber; ADF acids detergent fiber. *P<0.05; **P<0.01; T Tendency; NDF neutral detergent fiber; ADF acid detergent fiber; VFA volatile fatty acids.

Rumen propionate proportion (Overall ES= 0.560, P > 0.01) and total VFA concentration (Overall ES= 0.508, P = 0.03, Figure 2) were increased by MAC supplementation. Considering the subgroups, MAC acid increased (P = 0.02) rumen propionate, whereas MAC salt tended to increase (P = 0.08) in the same variable. MAC supplementation reduced the acetate to propionate ratio (Overall ES= -1,130, P > 0,01). The between studies heterogeneity was significant and greater than 65% for all variables related to VFAs and lactate in rumen. The meta-regression showed that the main covariates that affect the ES of MAC supplementation for acetate, propionate (Figure 3) and lactate were starch, starch, and NDF intake, respectively. Acetate to propionate ratio covariates were (P ≤ 0.05) NDF and starch intake. None of the covariates were useful in explaining the variation in MAC effect on butyrate proportion.

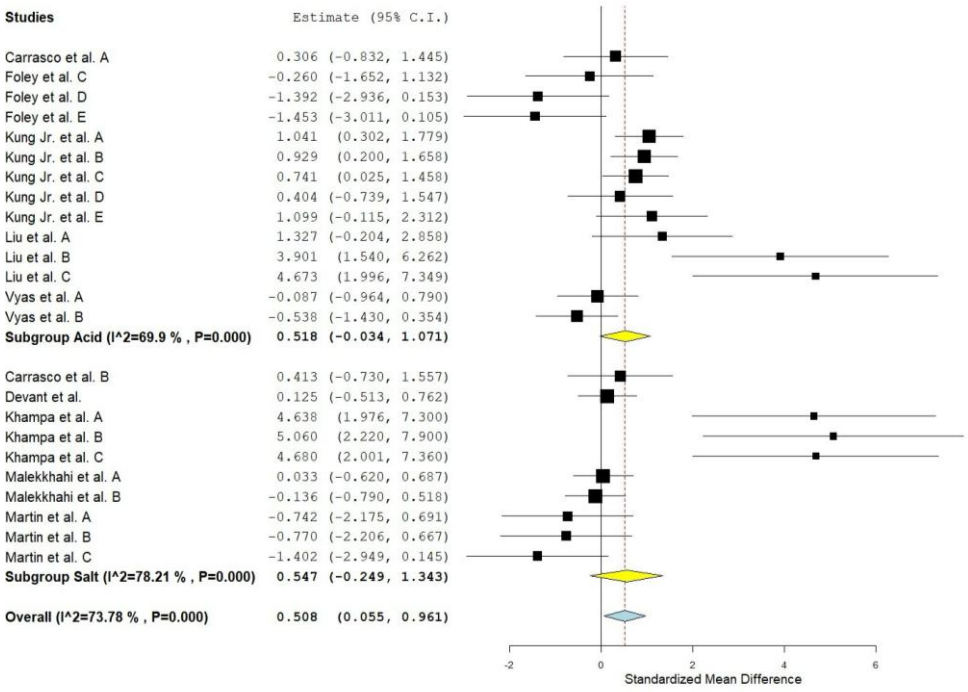

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Figure 2. Forest plot of the effect of supplementation with malic acid or malate on total volatile fatty acids in cattle. When the diamond was presented to the left of the central line (standardized mean) without touching it, the effect was considered to be negative, favoring control. When presented to the right of the center line, the effect was considered positive, in favor of the supplement.

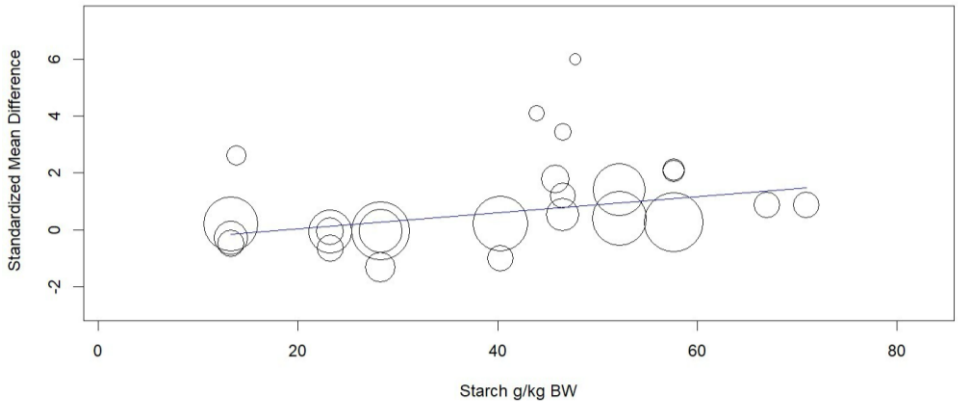

Figure 3. Meta-regression of the effect of starch intake (g/kg BW) on the standardized mean difference of malate or malic acid supplementation on propionate in the rumen of cattle.

3.2. Digestibility

In general, MAC increased ($P \leq 0.05$) macronutrient digestibility with ES of 0.547 for DM, 0.422 for CP, and 0.635 for ADF in total-tract apparent digestibility. However, MAC did not affect ($P > 0.05$) OM and NDF apparent digestibility. Dry matter digestibility increased ($P \leq 0.05$) in animals fed MAC acid and was not affected ($P > 0.05$) by MAC salt. However, MAC acid had no effect ($P > 0.05$) and MAC salts increased ($P \leq 0.05$) CP and NDF digestibility. The MAC acid tended ($P = 0.08$) to increase ADF and MAC salts increased ($P = 0.03$) ADF digestibility. Heterogeneity was significant ($P < 0.05$) for most variables related to digestibility, with the exception for CP and OM. The NDF intake tended to decrease the ES of MAC on DM digestibility. Starch intake was the main covariate explaining the variation in MAC's effect on NDF digestibility.

3.3. Blood Parameters

There was an overall effect of MAC on blood glucose concentration (ES= 0.170, $P = 0.05$). In addition, MAC decreased blood non-esterified fatty acids (NEFA) (Overall ES= -0.404, $P = 0.03$). On the other hand, MAC showed no effect ($P > 0.05$) on blood ß-hydroxybutyrate and lactate. However, MAC acid decreased lactate in blood (Subgroup ES= -1.661, $P > 0.01$). Despite the significant heterogeneity for most of the blood parameters, the small number of studies was a limiting factor for carrying out the meta-regression analysis.

4. Discussion

4.1. Rumen Parameters

We hypothesized that true effects associated with malic acid supplementation, particularly on rumen pH, lactate uptake and propionate production, could be identified and better explored through meta-analysis. These primary effects would lead to secondary ones on the diet digestibility and on blood metabolites. Additionally, we expected that qualitative and quantitative covariates related to diet composition and MAC presentation would be useful to explain the between study variation. In this context, our hypotheses were at least partially confirmed. Significant effects were observed for some of the main variables where the interference from MAC supplementation was

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expected. Subgroup analysis indicated that the chemical form of the supplement may be decisive for the control of rumen acidity. Additionally, the meta-regression indicated that there are dietary covariates that significantly influence the effect size of MAC supplementation on some outcomes.

For rumen pH, the effects observed for the MAC presentation make sense if we consider the chemical nature of the supplements. In vitro studies indicate free malic acid and its disodium salt have similar effects, except for the reduction in pH caused by free malic acid [3]. The effect of acidic MAC observed in our study, although small (ES= -0.310) and not significant, indicates that its use negatively impacts rumen pH. On the other hand, the ES of 1,420 observed in the salt subgroup is considered large [16]. Effect sizes greater than 0.8 are considered large according to the Cohen scale. However, it is important to point out that the scale is subjective and the context in which it is being applied must be considered. Additionally, this ES indicates that the mean pH of the control group and the malate group are separated by 1.42 standard deviations. Considering a standard deviation of 0.18 for pH (average from studies in the database), supplementation with the salt form of MAC would imply an increase in pH of 0.26 units. One of the premises that led malic acid to be tested in ruminant diets was its ability, demonstrated in vitro, to increase lactate uptake [4]. The presence of this organic acid favors the growth of *Selenomonas ruminantium*, these bacteria uses lactate as a source of carbon and energy, which would imply maintaining rumen pH [5]. Additionally, MAC may act on pH through a second mechanism, which is the production of CO_2 by *S. ruminantium* [9]. The I^2 values indicated that 80.4% of variability occurred due to differences between studies. Values of I^2 higher than 30% represent substantial heterogeneity, which may be investigated [15]. It was observed that NDF intake reduces the mean difference observed between treatments. It is possible that NDF intake ends up shadowing the effect of MAC on ruminal pH, as the presence of NDF implies longer rumination time and, consequently, greater buffering of ruminal pH [17].

Although there was an increase in rumen pH in animals supplemented MAC, it was not possible to confirm the effects of supplementation on lactate uptake in the rumen. Although the direction of the effect indicates a likely reduction (Overall ES= -0.113), especially when using the free acid (ES= -0.621), the result was not significant. The meta-regression analysis indicated that NDF intake decreases the ES, that is, in studies where NDF intake is higher, the effect MAC on lactate is smaller. High NDF intake implies low lactate levels in the rumen, impairing the growth of *S. ruminantium* and, consequently, the MAC effect. Additionally, for studies with high fiber intake, depending on the type and form of forage used, it is possible that MAC supplementation is occurring close to or above the limit at which its effect reaches a plateau. Malic acid can represent 2.2 to 4.5% of the dry matter of grasses and 2.9 to 7.5% of legumes, with this amount decreasing with the plant maturity [18]. Furthermore, preserved forages such as hay and silage have a lower content of this component. At this point, it is important to highlight that covariates such as fresh forage, dry forage and conserved forage intake as well as the forage:concentrate ratio were tested as continuous (meta-regression) or categorical (subgroup) covariates but were not significant (data not shown).

The effect size (0.508) obtained for total VFA concentration is considered moderate according to Cohen's [16]. This is an expected response and occurred mainly due to the greater production of propionate, as there were no changes in the proportion of acetate and butyrate in the rumen. Concomitantly, the acetate:propionate ratio was higher for the control group, which confirms the higher proportion of propionate (Overall ES= 0.560) in the supplemented animals. If we consider an average standard deviation of 4.84 for the molar proportion of propionate, this ES may represent a difference of 2.71 percentage points between the means of animals supplemented or not with MAC. The higher propionate production occurs because *S. ruminantium* bacteria are able to use lactate as a carbon source provided that oxaloacetate precursors such as malate are present [19]. This acid can follow the reverse cycle of the succinate-propionate pathway and provide the oxaloacetate for lactate fermentation to propionate [5]. The heterogeneity values showed that there is high variability that is not associated with chance. The response indicated by the meta-regression seems in line with what is known about the mechanisms of action of MAC, since starch intake favors the growth of lactate-

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producing bacteria which, associated with malic acid, becomes a substrate for the production of VFAs by *S. ruminantium* [20].

The analysis of the NH_3N indicated that supplementation with MAC, regardless of the form, results in negligible effect sizes (Overall ES= 0.079). This variable can be a marker of the amount of N available for synthesis and/or absorption in the rumen [21]. This N, when used to increase the microbial population in the rumen, would culminate in greater bacterial fermentation which, ultimately, could increase the digestibility of ruminant diet fractions. Did the meta-regression point out the NDF intake as a possible interfering factor in the effect of MAC over NH_3N. Increased fiber intake stimulates an increase in cellulolytic bacterial populations, while those with proteolytic and amylolytic characteristics decrease [22]. Furthermore, the NDF intake can reduce the concentration of sugars in the rumen [23], which is one of the substrates for *S. ruminantium*, a malate-utilizing bacteria.

4.2. Digestibility

The results obtained for fiber and protein digestibility may be a secondary effect of MAC on the control of acidity in the rumen, since the drop in ruminal pH reduces the degradability of fibrous fractions and protein [21,24]. The association of this effect with pH would also explain why the effects on NDF, ADF and protein digestibility were only detected when the MAC salt (malate) was used, since the acid form was not shown to have an effect on rumen pH. Additionally, MAC has the ability to remove H_2 from the rumen, stimulating an increase in the population of cellulolytic bacteria, which ends up impacting the total digestibility of fibrous fractions [25]. Furthermore, the effect observed on protein digestibility may have occurred due to the increase in the activity of proteolytic enzymes and/or a decrease in the duodenal pH necessary for effective proteolytic activity, promoted by malic acid [26,27]. The increase in DM digestibility due to supplementation with organic acid may occur due to an increase in enzymatic activity, increased secretions and association with the growth of beneficial bacterial populations [26]. On the other hand, MAC showed no effect on OM digestibility. As the variables are statistically independent, it occurred due to high variability and the smaller number of studies to carry out the meta-analysis for OM than DM digestibility. Despite the high heterogeneity, the meta-regression was not able to adequately explain the source of variation, with the exception of NDF digestibility. The analysis indicated that the intake of starch and also of protein (g/kg of BW) reduces the MAC ES on NDF digestibility. It is possible that starch intake reduces the MAC effect on NDF digestibility because the rapid fermentation of starch decreases rumen pH, creating a less favorable environment for cellulolytic bacteria that are responsible for fiber digestion [28].

4.3. Blood Parameters

Despite being considered small (ES=0.170), a significant effect on serum glucose level was detected due to MAC supplementation. Changes in this variable are related to the increase in propionate in the rumen and absorption by the epithelium, resulting in greater hepatic glucose synthesis [29]. Although our study observed greater protein digestibility for the supplemented group, plasma urea was not influenced by the supplementation with MAC. The concentration of urea N in plasma is used as an indicator to evaluate the protein status or protein nutrition of ruminants [30]. Despite the high heterogeneity for plasma urea, none of the covariates tested were adequate to explain the between studies variance. Animals supplemented with MAC also had lower levels of NEFA (ES= -0.404), which indicates less mobilization of body fat. This is an important answer because the level of NEFA in plasma correlates with the negative energy balance in early lactation cows, which allows this variable to be used as an indicator of energy balance [31]. It is important to point out that the small number of studies on most blood parameters may result in less precise estimates of the overall or subgroups effects and heterogeneity associated with these variables [15].

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5. Conclusions

The effect of malic acid on pH was dependent on the chemical form, with positive effects being observed with the salt form of the product (malate). Supplementation did not affect lactate uptake in the rumen. Furthermore, malic acid increased the concentration of VFAs, especially propionate, resulting in a reduction in the acetate:propionate ratio. Digestibility of protein, NDF and ADF was improved with supplementation, indicating a stronger association with malate than free acid. Malic acid supplementation also resulted in an increase in glucose levels and a reduction in NEFA in the blood. Covariates such as NDF or starch intake were important in explaining the variability in effects observed in cattle supplemented with malic acid.

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