Meta-analysis of the effect of glycerin inclusion in dairy cattle diet on milk fatty acid profile

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ABSTRACT: The use of glycerin in diets for dairy cows initially emerged as an alternative for the prevention and control of ketosis. However, despite some controversy, there are still several studies associating glycerin with increases in daily milk yield, with possible changes in its constituents. Therefore, the objective of this study was to evaluate, using a meta-analysis approach, the effect of glycerin inclusion in dairy cow diets on milk fatty acid. Twenty-two peer-reviewed publications with 66 treatment means were included in data set. The effect of glycerin inclusion in diet (treatment) were evaluated using random-effect models to examine the weighted mean differences (WMD) between a control diet (without glycerin in the diet) and the treatment diet. Heterogeneity was explored by meta-regression and subgroup analysis performed for: genetic type; days in milk; experimental period; glycerin in diet; glycerin type and concentrate in diet. Inclusion of glycerin in the diet increased the digestibility of dry matter and protein, as well as ruminal propionate. It did not affect dry matter intake ($P = 0.351$) and milk yield ($P = 0.730$). The effect of glycerin inclusion on the milk fat yield is dependent on the genetic group, in which Holstein (WMD = −0.04 kg/d; $P = 0.010$) and Holstein-crossbreed (WMD = −0.10 kg/d; $P < 0.0001$) cows produced less fat in milk compared to Jersey cows, when glycerin was included in the diets. Glycine inclusions of up to 100 g/kg in the diet of dairy cows did not negatively affect milk production and composition. However, inclusions above 150 g/kg of glycerin in the diet reduced the concentration of fat, and of unsaturated, monounsaturated, polyunsaturated fatty acids and conjugated linoleic acid (CLA C18: 2 cis-9 and trans-11) in milk. The results reported in our meta-analysis does not demonstrate the effectiveness of glycerin in improving the composition of milk and a group of fatty acids of importance for human health such as C18: 2 cis-9, trans-11 CLA.

Keyword: glycerol, fatty acid, fat milk, biodiesel

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INTRODUCTION

The growing demand for the production and consumption of alternative fuels to those of fossil origins incentivizes the production of biodiesel.

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Along with the increased production of biodiesel, there was an increase in the production of glycerin, which is its main by-product. According to Johnson and Taconi (2007), for each 100 kg of biodiesel produced, 10 kg of glycerin is generated as a by-product.

Crude glycerin consists of more than 80% glycerol, which is an excellent substrate for gluconeogenesis and energy regeneration for animals (Ezequiel et al., 2015). Around half of the glycerol consumed can be absorbed directly through the rumen wall into the bloodstream, making it available for hepatic gluconeogenesis (Rémond et al., 1993). For this reason, initially glycerin represented an alternative for the prevention and control of ketosis in dairy cows (Fisher et al., 1973, DeFrain et al., 2004). However, with these studies, changes in the milk constituents were also observed, with increases mainly in the protein contents (Bajramaj et al., 2016). Besides, due to reductions in the concentrations of saturated fatty acids (Eiras et al., 2014) and in the omega n-6/n-3 ratio (Silva et al., 2017) in meat of confined cattle, glycerin may also be associated with the possible improvement in the milk fatty acid profile, due to the reduction of lipolysis and ruminal biohydrogenation (El-Nor et al., 2010).

The reduction in the biohydrogenation process results in an increase in the concentration of unsaturated fatty acids in the rumen (Krueger et al., 2010), as well as the passage of these, with biohydrogenation intermediates, for absorption in the intestines (Edwards et al., 2011). In this context, our hypothesis is that the glycerin inclusion in dairy cows diets increases the concentration of unsaturated fatty acids in milk, without changing daily production. Therefore, there is the possibility of using glycerin to improve the constitution of milk, which in addition to enabling cost reduction, would bring valorization of milk at the time of commercialization. In this sense, the goal of the present study was to evaluate, using a meta-analysis approach, the effect of glycerin inclusion in dairy cow diets on milk fatty acids profile.

MATERIALS AND METHODS

Data Set

A comprehensive literature search was conducted using four search engines: Web of Science (https://login.webofknowledge.com), PubMed (https://www.ncbi.nlm.nih.gov/pubmed), Science Direct (http://www.sciencedirect.com/) and Journal of Dairy Science. Around 359 publications were retrieved using the following search terms: "glycerin," "dairy cows," "glycerin," and "ruminant." The papers that were retrieved, only those that satisfied the predetermined inclusion criteria were included in the meta-analysis. For inclusion into the meta-analysis, studies needed to have the following (standardized criteria): 1) was conducted using lactating dairy cows; 2) the control treatment did not include of glycerin in diet. A flowchart detailing the process of study identification and selection for analysis is shown in Figure 1.

DATA EXTRACTION

Based on inclusion criteria, 22 peer reviewed publications were rated by first author, publication reference, genetic group, forage in the diet (g/kg of dry matter), amount of glycerin in diet (g glycerin/kg of dry matter), glycerin type, number of cows, experimental design, type of diet fed (pasture-based diets, total mixed ration [TMR] and partial TMR), experimental period, diet composition, number of replications used and measurements of mean dispersion (SEM and SD). The following variable responses were extracted for both, the control and the glycerin treatments: nutrient intake, feed efficiency, total tract diet digestibility, milk production, and milk composition and nitrogen metabolism. The complete data set is available in an Excel file in Supplemental File S1.

STATISTICAL ANALYSIS

Weighted Mean Difference and Publication Bias

A meta-analysis was conducted using R Statistical Software Program (Metafor package, version 3.4.2; Viechtbauer, 2010). The Forest Graph (forest plot) was created using STATA software (Version 14.2; StataCorp LP, College Station, TX). The effects of glycerin in lactating dairy cows' diets were evaluated using random-effect models to examine the weighted mean difference (WMD) between control treatment (diets with no glycerin inclusion) and the glycerin treatment (diets with glycerin inclusion). Treatments mean were weighted by the inverse of the variance, according to the method proposed by DerSimonian and Laird (1986) for random effects model. In studies from which the standard error was less than half of the mean standard error, the standard error was set to half of the mean standard error across all studies to prevent over weighting (Firkins et al., 2001).

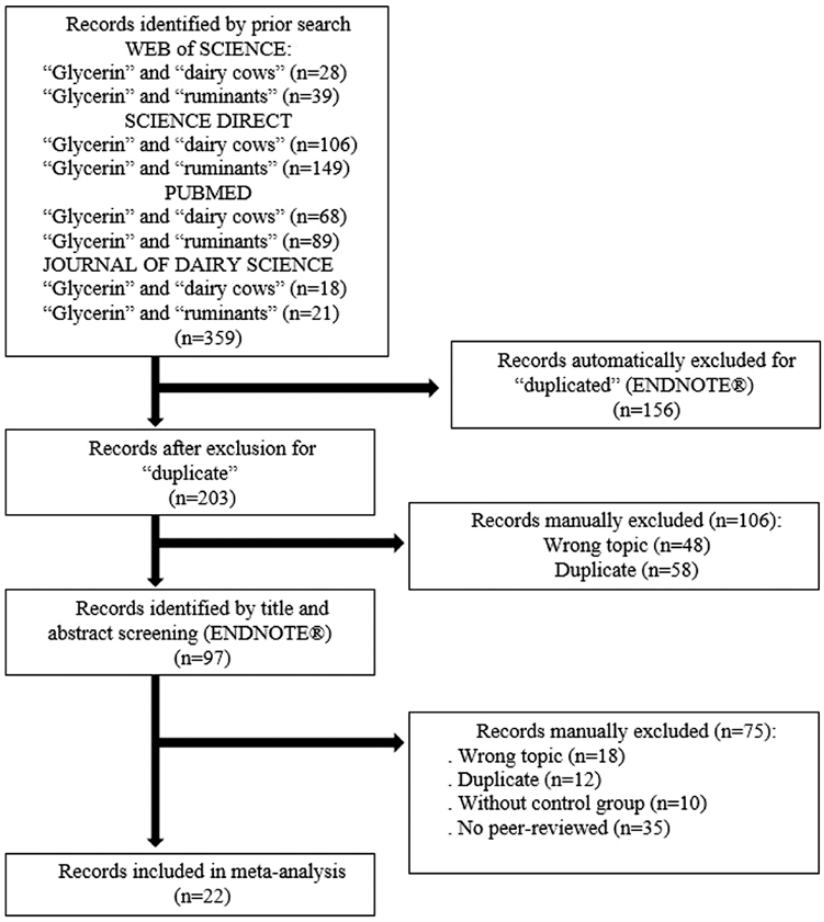

Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of glycerin in dairy cow diet.

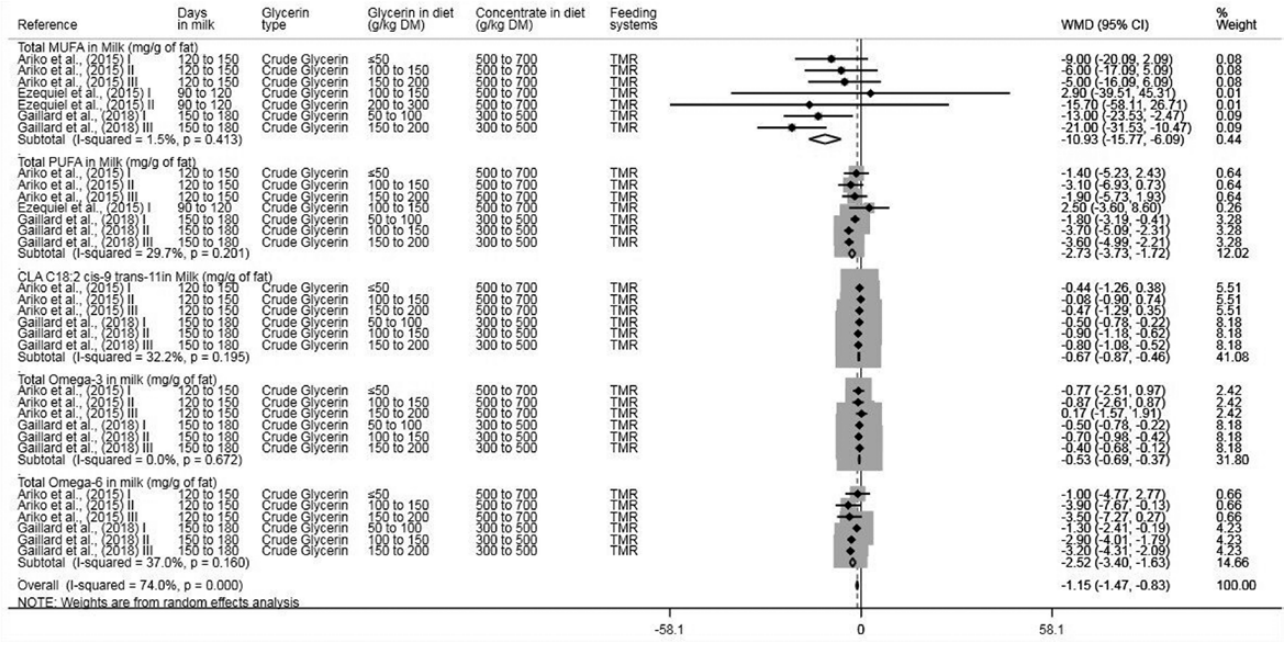

Figure 2. Forest plot showing the effect of the use of Glycerin on the diet of dairy cows on milk fatty acid profile. The *x*-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an increase. Each diamond represents the mean size effect for that study, and the size of the diamond reflects the relative weighting of the study to the overall size effect estimate with larger diamonds representing greater weight. The lines connected to the diamond represents the upper and lower 95% confidence interval for the size effect. The dotted vertical line represents the overall size effect estimate. The diamond at the bottom represents the mean response across the studies, and the solid vertical line represents a mean difference of zero or no effect.

Between-study variability (i.e., heterogeneity of the treatment effect) was evaluated using both the chi-square (***Q***) test of heterogeneity and I^2 statistics, which measures the percentage of variation due to heterogeneity (Higgins et al., 2003). Negative I^2 values were assigned as zero values. An I^2 value less than 25% indicated low heterogeneity, whereas values between 35% and 50% denoted moderate heterogeneity and those above 50% denoted high heterogeneity (Higgins et al., 2003).

Publication bias was evaluated using the funnel plot (Light and Pillemer, 1984) and asymmetry test (indicative of publication bias) which was carried according to the Egger regression asymmetry test, among the WMD and SE (Egger et al., 1997). Significance was declared at $P \leq 0.05$.

Meta-Regression and Subgroup Analysis

The meta-regression analysis was conducted to identify which study-level characteristics (covariates) influence on heterogeneity and select which one to perform the subgroup analysis. A mixed model was applied to adjust the data in the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by

$$\theta_i = \beta + \beta_i\, x_{ij} + \ldots \beta_{ip} x_{ip} + \mu_i$$

Where Θi = the true effect treatment in the ith explanatory variable; β = the overall true effect treatment; xij = the value of the jth covariate (j = 1, 2, …p) for the ith explanatory variable; βi = change in the true effect size for unit increase in the jth covariate; and $\mu i \sim N\,(0\ t^2)$. Here, t^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

To measure of between-study variance (Tau-squared = T^2). The moment estimator calculation of (T^2) is that used in Der-Simonian and Laird random effects meta-analysis but is less suitable when covariates are included (Thompson and Sharp, 1999). Was used the restricted maximum likelihood estimate (REML) approach to estimate (T^2) because it is less likely to underestimate or produce biased estimates of variance (Thompson and Sharp, 1999; Viechtbauer, 2005).Tests of null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008).

Meta-regression criteria were: 1) P-value ≤ 0.05, for the heterogeneity test; 2) P-value ≥ 0.05, for the funnel plot; 3) no observations with values for studentized residual out of the range −2.5 to 2.5 (outliers); 4) performed on all variables "Fatty acid in milk" in Tables 3 and 5) high heterogeneity (I^2 > 50%).

The subgroup analysis criteria were: 1) WMD was evaluated by subgroup analysis when the categorical covariates were significant at $P \leq 0.10$ (analysis meta-regression); 2) For variables that presented WMD with values of $P < 0.05$ in Tables 1–3.

The covariates were divided as following: genetic type (Jersey, Swedish, Holstein × Gyr and Holstein); days in milk (≤60, 60–90, 90–120, 120–150, 150–180, and 180–10 d); Experimental period (≤60, 60–90, 90–120, 120–150, 150–180, and 180–210 d). The level of concentrate in diet 300–500 and 500–700 g/kg DM). The type of glycerin (crude glycerin and refined glycerin). The levels of glycerin in the diet (≤50, 50–100, 100–150, 150–200, and 200–300 g/kg DM).

For inclusion in the dataset, standard error of difference, standard deviation and coefficient of variation were transformed to standard error of the mean as described byRoma-Garcia et al. (2016).

RESULTS

Based on the inclusion criteria, 22 peer-reviewed publications with 66 treatment means were used to evaluate the effect of glycerin inclusion in the diet for dairy cows on the production performance and milk composition. The predominant genetic group was Holstein (84.8% studies), followed by Holstein × Gyr (6.08%), Jersey (4.54%), and Swedish Red (4.54%). The lactation period in the studies were ≤60, 60–90, 90–120, 120–150, 150–180, 180–210 d in milk and not presented, representing 22.7%, 15.2%, 24.2%, 13.6%, 9.1%, 4.5%, and 10.7%, studies, respectively. The experimental period in the studies were ≤60 (25.8%), 60–90 (9.1%), 90–120 (24.2%), 120–150 (13.6%), 150–180 (9.1%), 180–210 (6.1%) and not informed, representing 12.2% of the studies.

Of all the studies analyzed, none used feed additives in their diets. The feeding systems used in the studies were the TMR (90.9%), pasture (6.06%) and not presented (3.03%). The level of concentrate (referring only to studies that used the TMR feeding system) used in the studies were 300–500, 500–700 g/kg of DM and not presented, representing 45.2%, 40.3%, and 14.5% of the studies respectively. The type of glycerin used were crude glycerin (92.4% studies) and refined glycerin (7.57% studies). The levels of glycerin in the diet were ≤50, 50–100, 100–150, 150–200, and 200–300 g/kg DM

Table 1. Effect of the glycerin inclusion in dairy cow diets on the intake, digestibility and body weight change

Item	Control[a] mean (SD)	N[b]	Glycerin		Heterogeneity[d]		Funnel test[e]
			[c]$WMD_{Random\ effect}$ (95% CI)	P	P	I^2 (%)	P
Intake, kg/d							
Dry matter	19.4 (3.55)	49	−0.11 (−0.34, 0.12)	0.350	0.332	7.06	0.911
Organic matter	17.6 (3.85)	10	−0.55 (−0.93, −0.17)	0.011	0.763	0.00	0.384
Crude protein	2.34 (0.65)	5	−0.09 (−0.18, −0.00)	0.040	0.231	28.4	0.063
NDF	7.66 (0.62)	10	−0.65 (−0.94, −0.35)	<0.0001	0.140	33.3	0.542
Digestibility, g/kg of DM							
Dry matter	645 (73.5)	20	14.6 (7.63, 21.52)	<0.0001	0.010	45.7	0.301
Organic matter	668 (60.8)	13	6.59 (0.70,12.48)	0.031	0.601	0.00	0.289
Crude protein	678 (53.3)	16	16.2 (5.97, 26.48)	0.011	<0.0001	69.2	0.918
Ethereal extract	580 (115)	6	125 (111, 140)	<0.0001	0.632	0.00	0.936
NDF	462 (120)	19	−19.2 (−28.5, −9.82)	<0.0001	0.223	19.1	0.153
Feed efficiency, kg/kg	1.32 (0.42)	20	0.01 (−0.01, 0.04)	0.352	0.814	0.00	0.692
Body weight, kg/d							
Change	−0.12 (0.85)	27	0.06 (0.04, 0.08)	<0.0001	0.821	0.00	0.571

Feed efficiency = kg milk yield/kg DMI.

NDF = neutral detergent fiber.

[a]Control treatment (without glycerin).

[b]N = number of comparisons of control and treatments (glycerin) (complete data set is available in Supplementary File S1).

[c]WMD = weighted mean differences between control and with treatments.

[d]I^2 = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

[e]Egger's regression asymmetry test.

Table 2. Effect of the glycerin inclusion in dairy cow diets on the ruminal and blood parameters

Item	Control[a] mean (SD)	N[b]	Glycerin		Heterogeneity[d]		Funnel test[e]
			[c]$WMD_{Random\ effect}$ (95% CI)	P	P	I^2 (%)	P
Ruminal parameters							
pH	6.65 (0.45)	16	−0.01 (−0.04, 0.04)	0.931	0.332	10.7	0.422
NH_3-N, mg/dL	12.9 (7.89)	11	−1.85 (−3.53, −0.16)	0.032	<0.0001	86.1	0.883
Short-chain fatty acid, mol/100 mol							
Acetate	61.5 (2.77)	19	−7.30 (−9.57, −5.03)	<0.0001	<0.0001	94.7	0.204
Propionate	21.2 (1.88)	18	2.94 (1.97, 3.91)	<0.0001	<0.0001	74.7	0.135
Butyrate	14.1 (1.51)	19	3.27 (2.09, 4.44)	<0.0001	<0.0001	94.5	0.261
Blood parameters, mg/dL							
Blood urea nitrogen	28.4 (12.6)	17	−1.68 (−2.59, −0.77)	0.011	0.012	63.9	0.111
Glucose	59.5 (11.7)	45	1.51 (0.40, 2.61)	0.012	<0.0001	67.4	0.091
Non-esterified fatty	20.4 (11.8)	11	−1.14 (−1.94, −0.34)	0.010	0.061	42.9	0.611
β-OH-Butyrate	9.40 (5.13)	14	1.37 (0.84, 1.90)	<0.0001	0.671	0.00	0.132

N = nitrogen.

[a]Control treatment (without glycerin).

[b]N = number of comparisons of control and treatments (glycerin) (complete data set is available in Supplementary File S1).

[c]WMD = weighted mean differences between control and with treatments.

[e]I^2 = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

[e]Egger´s regression asymmetry test.

representing 26.7%, 20.0%, 24.4%, 13.3%, and 15.6% of the studies, respectively.

Intake and digestibility of DM and nutrients. There was no effect of the inclusion of glycerin on dry matter intake ($P < 0.05$, Table 1). However, glycerin inclusion reduced the intake of organic matter ($P = 0.01$), crude protein ($P = 0.04$), NDF ($P < 0.0001$), and NDF digestibility ($P < 0.0001$).

Table 3. Effect of the glycerin inclusion in dairy cow diets on the milk yield, composition and fatty acid profile in milk

Item	Control[a] mean (SD)	N[b]	Glycerin		Heterogeneity[d]		Funnel test[e]
			[c]$WMD_{Random\ Effect}$ (95% CI)	P	P	I^2 (%)	P
Milk yield, kg/d	29.2 (7.58)	58	0.06 (−0.26, 0.37)	0.731	0.950	0.00	0.081
Milk fat yield, kg/d	1.15 (0.30)	37	−0.04 (−0.06, −0.02)	0.011	0.011	37.5	0.132
Milk protein yield, kg/d	0.95 (0.22)	35	−0.01 (−0.02, 0.01)	0.202	0.919	0.00	0.173
Milk lactose yield, kg/d	1.29 (0.39)	26	−0.03 (−0.05, −0.01)	0.033	0.866	0.00	0.741
Milk fat, g/kg	38.7 (6.07)	55	−0.82 (−1.18, −0.46)	<0.0001	0.425	2.63	0.453
Milk protein, g/kg	33.1 (4.85)	55	0.18 (0.00, 0.36)	0.043	0.844	0.00	0.883
Milk lactose, g/kg	47.3 (2.15)	47	−0.12 (−0.28, 0.04)	0.144	0.993	0.00	0.801
Milk SNF, g/kg	90.5 (8.12)	5	−0.12 (−2.04, 1.80)	0.915	0.592	0.00	0.282
MUN, mg/dL	16.6 (4.00)	19	−0.42 (−1.02, 0.18)	0.176	0.011	50.3	0.191
Fatty acid in milk fat, mg/g of fat in milk							
CLA, C18:2 Cis-9 Trans-11	4.68 (0.68)	6	−0.67 (−0.87, −0.46)	<0.0001	0.199	32.2	0.290
Total SFA	714 (24.9)	8	10.9 (0.99,20.98)	0.031	0.018	69.9	0.291
Total UFA	282 (39.4)	5	−21.2 (−29.8, −12.5)	<0.0001	0.216	31.5	0.102
Total MUFA	220 (43.6)	7	−10.9 (−15.8, −6.09)	<0.0001	0.415	1.50	0.153
Total PUFA	32.2 (9.40)	7	−2.73 (−3.73, −1.72)	<0.0001	0.204	29.7	0.744
Omega-3	7.17 (1.06)	6	−0.53 (−0.69, −0.37)	<0.0001	0.672	0.00	0.635
Omega-6	21.2 (2.19)	6	−2.52 (−3.40, −1.63)	<0.0001	0.161	37.0	0.946

CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acids; MUN = milk urea nitrogen; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SNF = solid not fat; UFA = unsaturated fatty acids.

[a]Control treatment (without glycerin).

[b]N = number of comparisons of control and with treatments (glycerin) (complete data set is available in Supplementary File S1).

[c]WMD = weighted mean differences between control and with treatments.

[d]I^2 = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

[e]Egger's regression asymmetry test.

The glycerin inclusion increased the digestibility of dry matter ($P < 0.0001$), organic matter ($P = 0.03$), crude protein ($P = 0.01$), ether extract ($P < 0.0001$).

Performance, composition, and fatty acid profile of milk. The inclusion of glycerin had no effect on milk production, milk protein production, feed efficiency, lactose concentration, milk urea nitrogen (MUN), and solid non-fat (SNF, $P < 0.05$). However, there were increases in body weight change ($P < 0.0001$; Table 1), protein concentration ($P = 0.04$) and total saturated fatty acids in milk ($P = 0.03$; Figure 2).

With the inclusion of glycerin in the diet, there was a reduction in fat production and concentration ($P < 0.0001$ and $P = 0.01$) and lactose production ($P = 0.03$; Figure 1). There was also a reduction in the concentration of conjugated linoleic acid (CLA C18: 2 cis-9 trans-11; $P < 0.0001$), total unsaturated ($P < 0.0001$), monounsaturated ($P < 0.0001$) and polyunsaturated fatty acids ($P < 0.0001$), the omega-3 ($P < 0.0001$), and omega-6 families ($P < 0.0001$; Table 3; Figure 2).

Rumen and blood parameters. The glycerin inclusion in the diet reduced the concentrations of ammonia ($P = 0.03$), rumen acetate ($P < 0.0001$), blood urea nitrogen (BUN; $P = 0.01$), and non-esterified fatty acid (NEFA; $P = 0.01$, Table 2). In contrast, the concentrations of ruminal propionate and butyrate ($P < 0.0001$), glucose ($P = 0.01$) and β-OH-Butyrate (BHB; $P < 0.0001$) increased. Glycerin inclusion did not affect rumen pH ($P = 0.33$).

Meta-regression analysis and funnel plot asymmetry. High heterogeneity (I^2 statistic >50%) was found for crude protein digestibility, rumen ammonia, acetate, propionate and butyrate concentration, BUN, glucose, MUN, total SFA (Tables 1–3). There was no evidence of publication bias ($P > 0.05$) from the funnel plot asymmetry test for any of the variables evaluated.

Based on the meta-regression analysis, in Tables 4 and 5, covariates effects are presented; genetic type; days in milk; experimental period in days (ED); glycerin diet; glycerin type; and concentrate in the diet (CON). The covariates "days in milk and glycerin in the diet (g/kg DM)" were the most consistent factors in influencing the response to glycerin use in the diet, since they account for the variability

Table 4. Meta-regression of the effect glycerin inclusion in dairy cow diets on weighted mean differences (WMD) for digestibility, ruminal and blood, and milk production variable

	Meta-regression parameters (*P*)							
Dependent variable (Y, WMD)	Intercept	Genetic type	Days in milk (day)	ED (day)	Glycerin diet	Glycerin type	CON in diet	*N*[a]
DMd, g/kg	35.2 (0.05)	−7.62 (0.65)	−63.8 (0.04)	–	−18.3 (0.01)	32.4 (0.10)	–	20
CPd, g/kg	28.1 (0.40)	9.33 (0.64)	−23.6 (0.29)	−2.03 (0.92)	5.69 (0.81)	–	–	16
N-NH_3, mg/dL	−6.83 (0.01)	–	6.86 (0.01)	–	3.98 (0.01)	–	–	11
Acetate, mol/100 mol	−16.2 (0.01)	–	−1.15 (0.73)	4.21 (0.47)	6.07 (0.02)	–	–	19
Propionate, mol/100 mol	8.10 (0.01)	–	−2.99 (0.07)	−1.38 (0.80)	−7.40 (0.01)	–	–	18
Butyrate, mol/100`mol	3.74 (0.06)	–	5.35 (0.01)	−3.67 (0.06)	1.57 (0.03)	–	–	19
BUN, mg/dL	−4.29 (0.34)	0.31 (0.80)	2.71 (0.01)	0.34 (0.92)	0.22 (0.89)	–	–	17
Glucose, mg/dL	7.53 (0.22)	−2.17 (0.70)	7.93 (0.01)	−10.8 (0.002)	−7.13 (<.00)	2.52 (0.13)	7.02 (0.02)	45
NEFA, mg/dL	−5.96 (0.08)	1.12 (0.20)	2.28 (0.58)	–	0.73 (0.82)	–	–	11
Milk fat yield, kg/d	−0.25 (0.84)	0.30 (0.08)	−0.07 (0.69)	−0.19 (0.88)	0.82 (0.12)	−0.08 (0.20)	−0.52 (0.21)	37
MUN, mg/dL	−1.19 (0.68)	0.45 (0.86)	0.87 (0.39)	–	−0.80 (0.52)	–	−0.40 (0.80)	19

BUN = blood urea nitrogen; CON = concentrate in diet (g/kg); CPd = crude protein digestibility; DMd = dry matter digestibility; ED = experiment in day; MUN = milk urea nitrogen; NEFA = non esterified fatty acids.

[a]*N* = number of comparisons between treatment with (glycerin) and control (without crude glycerin) (complete data set is available in Supplementary file S1).

Table 5. Meta-regression of the effect glycerin inclusion in dairy cow diets on weighted mean differences (WMD) for milk fatty acid variables

	Meta-regression parameters (*P*)							
Dependent variable (Y, WMD)	Intercept	Genetic type	Days in milk (day)	ED (day)	Glycerin diet	Glycerin Type	CON in diet	*N*[a]
CLA C18:2 cis-9 trans-11	−0.82 (0.01)	–	0.57 (0.06)	–	0.32 (0.09)	–	–	6
Total SFA, mg/g	46.2 (0.19)	–	−28.5 (<0.001)	–	−11.7 (0.74)	–	–	8
Total UFA, mg/g	−8.30 (0.78)	–	–	–	−5.70 (0.87)	–	–	5
Total MUFA, mg/g	−15.7 (0.47)	–	–	–	6.70 (0.76)	–	–	7
Total PUFA, mg/g	−3.54 (0.01)	–	6.26 (0.04)	–	1.74 (0.07)	–	–	7
Total Omega-3, mg/g	−0.39 (0.01)	–	0.20 (0.75)	–	−0.31 (0.10)	–	–	6
Total Omega-6, mg/g	−3.17 (0.01)	–	−0.65 (0.64)	–	1.87 (0.01)	–	–	6

CLA = conjugated linoleic acid; CON = concentrate in diet (g/kg); ED = experiment in day; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; UFA = unsaturated fatty acids.

[a]*N* = number of comparisons between treatment with (glycerin) and control (without crude glycerin) (complete data set is available in Supplementary file S1).

in the digestibility of dry matter, ammonia, acetate, ruminal propionate, and butyrate, as well as BUN, milk fat yield, and total SFA values.

Subgroup analysis. Subgroup analysis was applied to assess the effect of covariates associated with the inclusion of glycerin on the response variables. When evaluating the "genetic type," it was observed that the inclusion of glycerin in the diet for Holstein and Hosltein × Gyr cows reduced the milk fat yield (WMD = −0.04 kg/d; P = 0.01; and WMD = −0.10 kg/d; P < 0.0001, respectively; Figure 3A). For the other genetic groups, no significant effects were detected.

For the covariate "days in milk," the DM digestibility increased (Figure 4A) in the periods of 90–120 (WMD = 11.0 g/kg; P = 0.04), 150–180 (WMD = 30.3 g/kg; P < 0.0001) and 180–210 days in milk (WMD = 18.7g/kg; P = 0.01), for the other days in milk, there was no significant effect. Periods between 180 and 210 d in milk reduced the rumen ammonia concentration (WMD = −4.70 mg/dL; P = 0.01; Figure 4B), as well as days in milk between ≤60, 120–150, and 180–210, showing only an increase for the ruminal butyrate (Figure 4D) concentration. The same behavior was verified for the concentration of propionate (Figure 4C), in which its increase was observed for up to 210 d in milk.

The lactation period also affected BUN values, in which periods of 60–90, 90–120, and 180–210 d in milk reduced BUN concentrations, while 120–150 d in milk increased BUN values (WMD = 5.20 mg/dL; P = 0.03; Figure 4E).

For blood glucose, days in milk 90–120, 150–180, and 180–210 d, showed an increase in values (Figure 4F). Days in milk 150–180 increased the total SFA values in milk fat (WMD = 22.7 mg/g; P < 0.0001; Figure 4G). The same period reduced the concentration of CLA (WMD = −0.73 mg/g; P < 0.0001; Figure 4H). Days in milk between 120–150 and 150–180 days reduced the concentration of total polyunsaturated fatty acid (PUFA; WMD = −2.13 mg/g; P = 0.05; and WMD = −3.03 mg/g; P < 0.0001; Figure 4I, respectively).

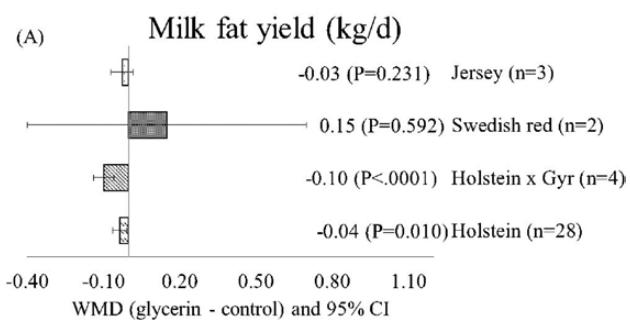

Figure 3. Subgroup analysis (Subgroup = Genetic type) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

The covariate "experimental period" (Figure 5A) in periods between 60 and 90 d accounted for the increase in blood glucose (WMD = 2.91 mg/dL; P < 0.0001) in the animals receiving glycerin in the diet.

Levels of inclusion of glycerin in the diet (Covariate = glycerin in the diet) of 100–150 and 200–300 g/kg DM increased dry matter digestibility (WMD = 18.3 g/kg; P = 0.01; and WMD = 22.6 g/kg; P = 0.01; Figure 6A, respectively). Inclusions between 50 and 100 g/kg DM of glycerin reduced the ruminal ammonia values (WMD = −2.49 mg/dL; P < 0.0001; Figure 6B). Inclusions of up to 150 g/kg DM glycerin reduced the concentration of rumen acetate (Figure 6C), however, for the same level of inclusion there was an increase in the values of propionate (Figure 6D) and butyrate (Figure 6E) in the rumen. However, the inclusion of ≤50 g/kg DM glycerin in the diet reduced the concentration of glucose (WMD = −1.43 mg/dL; P = 0.01; Figure 6F), for the other levels of inclusion there was no effect (P > 0.05). For inclusion levels ≥100 g/kg DM glycerin in the diet, a reduction in milk fat yield was observed. In contrast, the inclusion of glycerin above 100 g/kg DM had no effect (P > 0.05; Figure 6H) on the total concentration of monounsaturated fatty acid (MUFA) in milk. The inclusion of glycerin above 100 g/kg DM reduced the total PUFA (Figure 6I), omega-3 (Figure 6K), and omega-6 (Figure 6L). While, inclusions above 150 g/kg DM reduced the content of CLA (Figure 6J) in milk.

When assessing the type of glycerin, crude glycerin increased dry matter digestibility (WMD = 14.5 g/kg; P < 0.0001; Figure 7A), and blood glucose (WMD = 1.68 mg/dL; P = 0.01; Figure 7B), however, there was no effect (P > 0.05) with the use of refined glycerin on the same variables.

The proportion of concentrate in the diet influenced the responses in diets with glycerin, in which levels of 300–500 g/kg DM concentrate in the diet increased the concentration of glucose in the blood (WMD = 2.45 mg/dL; P = 0.01; Figure 8A). However, concentrate levels of 300–500 and 500–700 g/kg DM, reduced the total MUFA (WMD = −17.0 mg/g; P < 0.0001; and WMD = −6.66 mg/g; P = 0.04, Figure 8B, respectively).

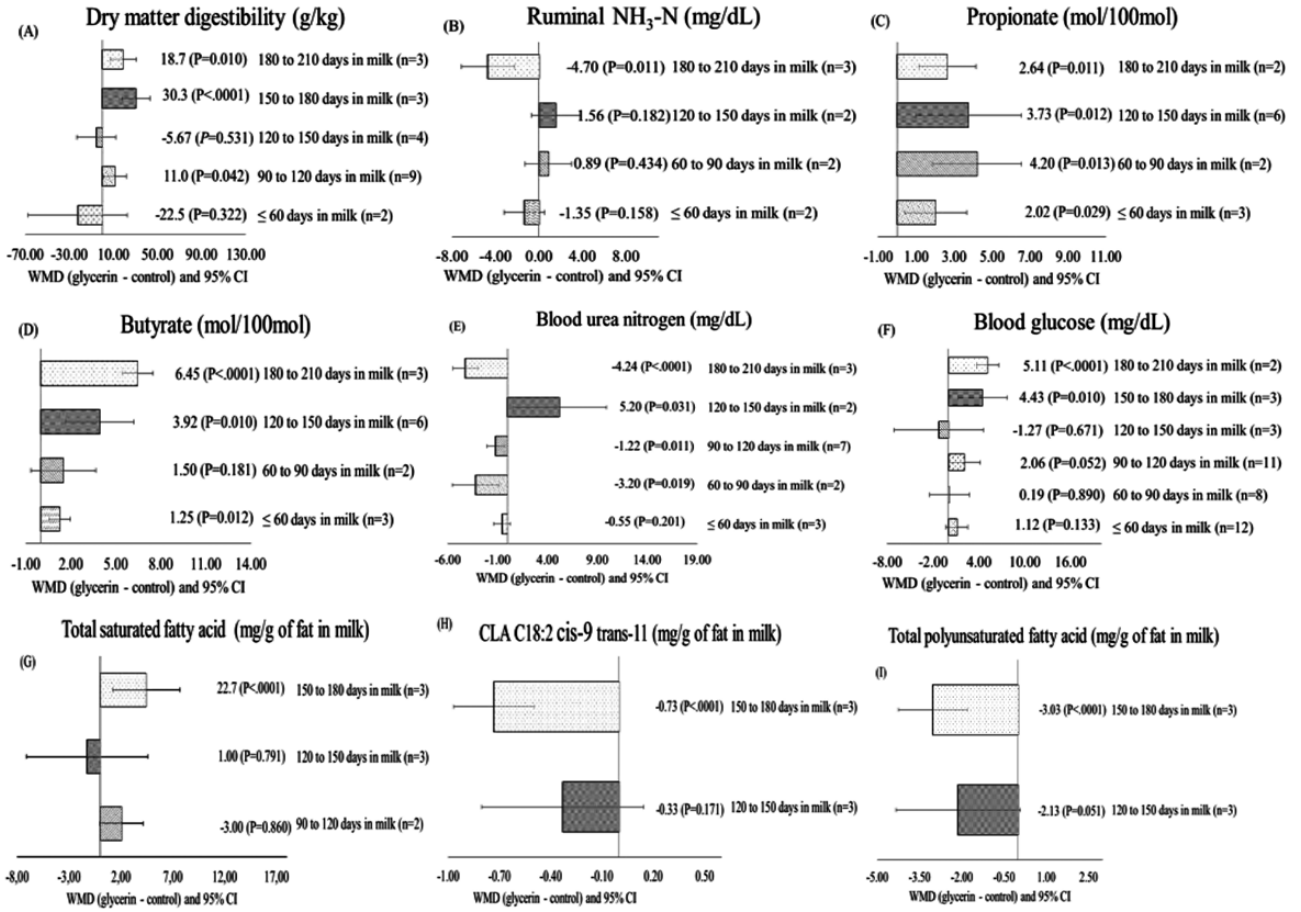

Figure 4. Subgroup analysis (Subgroup = Days in Milk) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

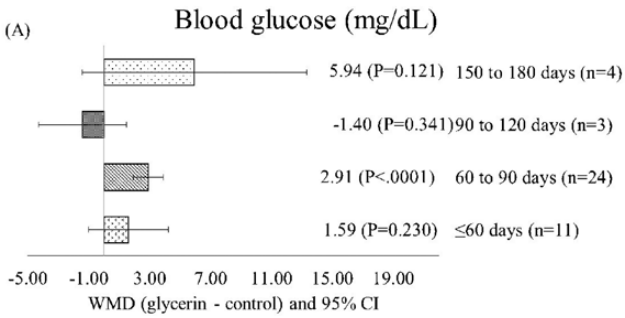

Figure 5. Subgroup analysis (Subgroup = Experiment period) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

DISCUSSION

In view of the present results, our hypothesis that the inclusion of glycerin in the diet for dairy cows increases the concentration of unsaturated fatty acids in milk was not supported, as the inclusion of glycerin from 150 g/kg DM reduced the total UFA, MUFA, PUFA, CLA, omega-3, and omega-6 in milk. According to Palmquist and Mattos (2011), fatty acids in milk have three origins: the diet, from the activity of the ruminal microbiota and those of endogenous origin, from the synthesis in the mammary gland and the incorporation of reserve lipids. Thus, with the inclusion of glycerin in the diets, there was a reduction in the consumption of fatty acids, since glycerin has a low concentration of these acids in its composition (Eiras et al., 2014). From our results, it was observed that glycerin acts in the three origins, because it has the lowest participation of these fatty acids in its composition resulting in less consumption of unsaturated fatty acids when is used in the place of grains (e.g., corn). As well as it did not affect the biohydrogenation process. However, it changed the synthesis activity of mammary gland.

Likewise, the increase in total SFA observed in milk is due to the composition of this by-product, whose main component is glycerol, a gluconeogenic precursor (Krueger et al., 2010), which may have caused an energy surplus in animals, reducing the use of reserve fatty acids and favoring greater synthesis of fatty acids in the mammary gland, increasing the incorporation of short- and medium-chain and odd-saturated fatty acids in milk. The inclusion

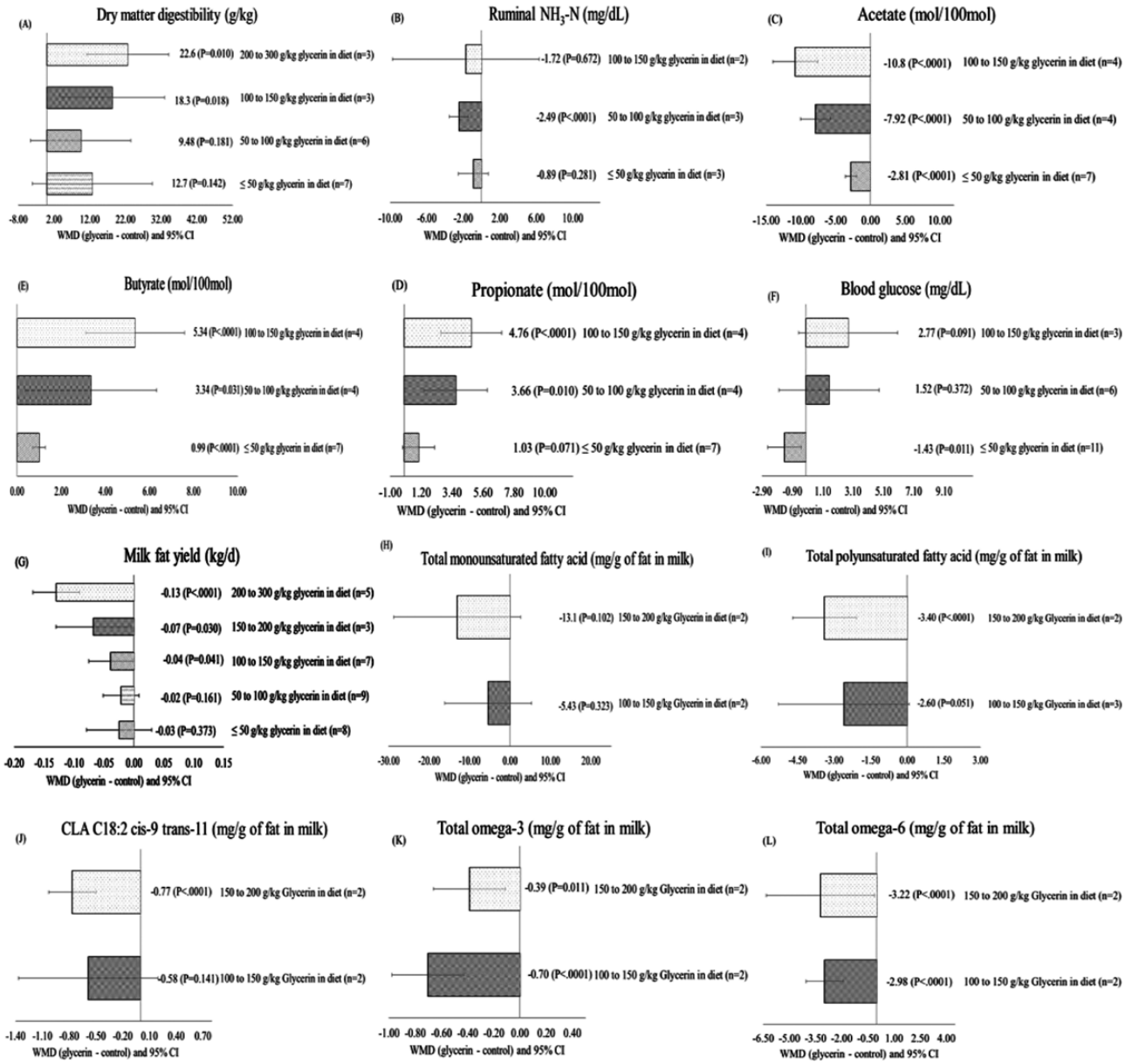

Figure 6. Subgroup analysis (Subgroup = Glycerin in diet [g/kg]) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

of glycerin in the diet increased the concentration of glucose in plasma (+2.5%) and rumen propionate (+13.9%), thus suggesting that there was a greater synthesis of glucose via gluconeogenesis, contributing to the energy surplus in animals, resulting in a reduction in NEFA (−5.6%), which is considered an indicator of mobilization of body lipids.

Regarding the reduction in the mobilization of body reserves, without changing the dry matter intake, with the inclusion of glycerin throughout lactation, it may be due to the lower weight loss in animals receiving glycerin in the diet. Confirming the ability of glycerin to reduce the effects of the negative energy balance period in dairy cows.

The change in blood parameters (glucose and NEFA) may be associated with an increased digestibility of DM, OM, CP, and EE of diets with glycerin, which showed increases of 2.26%, 1.0%, 2.39%, and 21.7%, respectively, showing once again that there was greater availability of energy for the animal.

The increase in plasma glucose can reduce the uptake of acetate and long-chain fatty acids (LCFA), which suggests inhibition of lipoprotein lipase (LPL) in the mammary gland (Cant et al., 2002). The lower uptake of reserve fatty acids by the mammary gland may have contributed to the increase in the concentration of BHB (+14.5%) in the plasma. The increase in BHB concentration was also found by Cant et al. (2002), when

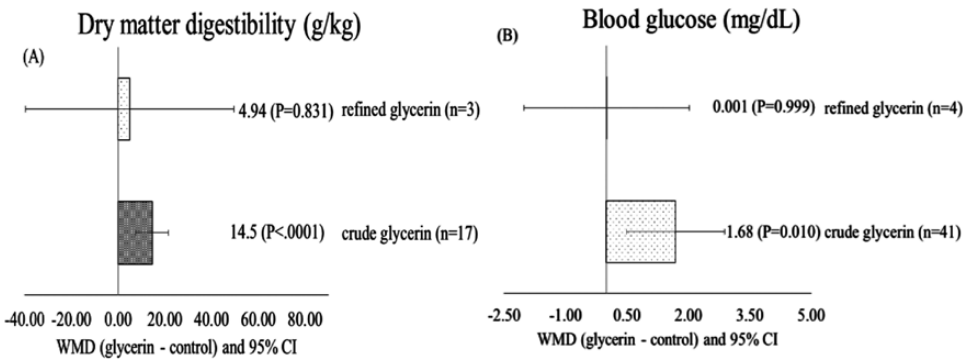

Figure 7. Subgroup analysis (Subgroup = glycerin type) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

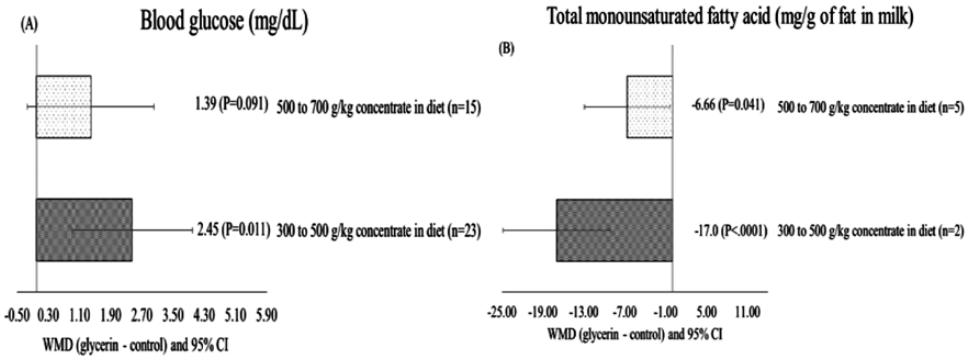

Figure 8. Subgroup analysis (Subgroup = Concentrate in diet [g/kg]) of the effect of the use of glycerin on the diet of dairy cows on productive performance, WMD = weighted mean differences between glycerin and control treatments.

infusing glucose into the duodenum. Nevertheless, this cannot be explained only by the increased concentration of ruminal butyrate with the inclusion of glycerin, as observed in our meta-analysis, but may also be due to the lower uptake of reserve fatty acids by the mammary gland and its greater availability for conversion to ketone bodies in the liver.

The hypothesis of energy surplus and its effect on the content of unsaturated fatty acids can be favored both by the changes that glycerin causes in blood parameters, and by the change in the size of the fatty acids chains that constitute milk fat, as was observed by Ariko et al. (2015) and Gaillard et al. (2018), which by including glycerin in the diet for dairy cows, found an increase in the concentration of C6–C10 and C5–C17 fatty acids, while C14–C16 reduced. In the present meta-analysis, it was not possible to individually evaluate fatty acids, however, the reduction of C14–C16 with the inclusion of glycerin found by the authors mentioned above, may explain the reduction in the total MUFA found herein, since C14–C16 fatty acids are used by the enzyme stearoyl-COA desaturase 1 (SCD1) for the synthesis of monounsaturated fats in the mammary gland (Buitenhuis et al., 2019).

The reduction in the content of polyunsaturated fatty acids with the inclusion of glycerin (Total PUFA, Omega-3, and Omega-6), probably demonstrates the lower intake of these when replacing the grains with glycerin, as well as the stability in the rumen, as was observed in this meta-analysis, in which the inclusion of glycerin did not affect ruminal pH. The average pH observed in the studies was higher than 6.2, adequate for the maximum growth and activity of most ruminal microorganisms (Stewart et al., 1997). Thus, biohydrogenation occurs completely, which reduces the concentration of CLA C18: 2 cis-9 trans-11 in milk, as observed in this study, as well as the total PUFA and omega n-3 and n-6. Fuentes et al. (2011) investigated the effect of ruminal pH on lipolysis and in vitro biohydrogenation of omega-3 and 6 (continuous culture system), and observed that the drop in pH from 6.4 to 5.6, reduced the lipolysis of omega-3 and 6 by 8.16% and 12.3%, while biohydrogenation decreased by 65.8% and 52.5%, respectively. Another factor that can be associated is the change in the microbial population of the rumen, mainly on the population of *B. fibrisolvens* and *C. proteoclasticum*, which play an important role in the process of

biohydrogenation and in the formation of TRANS fatty acids in the rumen (Maia et al., 2007; Paillard et al., 2007).

The reduction in the concentration of fat in milk with the inclusion of glycerin in the diets was because of the reduction of lipogenic precursors in the mammary gland, such as acetate and NEFA, as well as by the alteration in blood metabolites such as glucose. The acetate being the main lipogenic precursor in the mammary gland, as well as NEFA, which are absorbed and incorporated into milk fat (Bauman and Griinari, 2003). The observed in this study, there was an 11.9% reduction in rumen acetate, which may be related to changes in the rumen microbiota with the inclusion of glycerin in the diet, as observed by El-Nor et al. (2010).

Another factor that may be associated with the reduction of milk fat is the increase in ruminal propionate production raises the hepatic gluconeogenesis rate, thus increasing the blood insulin circulation, resulting in a change induced by the insulin and the use of precursors for fat synthesis, both in tissues of the mammary gland, as well as in other tissues with lipogenic activity (Harfoot and Hazlewood, 1997; Bauman and Griinari, 2003). Thus, as observed in the present study, the increased production of ruminal propionate may be associated with an increase in blood glucose by +2.53%, which in addition to causing an increase in the concentration of circulating insulin, acts in the regulation of the rate of lipogenesis (stimulating) and lipolysis (inhibiting) in the adipose tissue (Bauman et al., 1973; Bauman, 2000). This effect of insulin on other tissues may be associated with a reduction of −5.60% in NEFA in animals that received glycerin.

The higher concentration of glucose in plasma, in addition to affecting the availability of precursors to the mammary gland, may also be associated with inhibitory effects on the activity of the LPL enzyme in the mammary gland, as reported by Cant et al. (2002), in which there was a reduction in the uptake of lipogenic precursors by the mammary gland such as acetate, NEFA and LCFA.

Our results suggest that the inclusion of glycerin in the diets triggers different behavior according to each genetic group, mainly regarding the production of fat in milk. The Holstein animals and their crossbreeds showed reductions in the concentration of milk fat yield with the inclusion of glycerin in the diets. As stated by Brown et al. (2012), Jersey animals have greater stability in the concentration of IGF-1 between lactations, demonstrating the efficiency in the use of nutrients as well as their directing to the mammary gland, allowing the concentration of solids in milk compared to Holstein cows. Thus, we suggest the evaluation of different levels of inclusion of glycerin in different genetic groups for future work, thus enabling a better understanding and use of this by-product.

The reduction in lactose production in milk may be associated with an increase in the concentration of glucose in plasma, as verified by Rulquin et al. (2004), who conducted the infusion of glucose into the duodenum and registered an increase in the plasma glucose concentration, thus detecting a reduced synthesis of lactose in the mammary gland. The elevation in blood glucose causes a reduction in the number of active glucose transporters in the mammary gland (Mepham, 1988), reducing the glucose intake, thus affecting the synthesis of lactose in the mammary gland. Even though was no difference in lactose concentration in milk, lactose yield was reduced, as there was fluctuation in the blood glucose level (Figure 3) according to days in milk.

The increase in protein concentration in milk with the inclusion of glycerin in the diet may be associated with greater production of propionate in the rumen. This is because the increase in propionate in the rumen and its availability to the mammary gland reduces the use of gluconeogenic amino acids for glucose synthesis or oxidation for energy production, making it available for protein synthesis in the mammary gland (Rulquin et al., 2004), as glycerin inclusion increased dietary protein digestibility by reducing the concentration of ruminal ammonia and BUN.

The reduction in BUN was observed after 60 d in milk with a reduction during lactation, an effect also reported by McDonald et al. (2007). Nevertheless, the reduction of ruminal ammonia was found between 180 and 210 d in milk. However, the reduction of ammonia was −14.3% with the inclusion of glycerin, observed from inclusions above 50 g/kg DM in the diet. These results demonstrate that the inclusion of glycerin in the diet improves the use of dietary nitrogen and enhances the production of microbial protein and availability of essential amino acids for production of milk and constituents. Omazic et al. (2014) showed that glycerin is rapidly fermented by microorganisms, and is a source of readily available energy for their growth. The supply of readily available energy, associated with the reduction of ruminal ammonia, is a response to the better energy: protein synchrony in the rumen (Hristov et al., 2005). In this context, glycerin presents high ruminal fermentation, favoring

the use of preformed amino acids of the diet and/or NNP, which can originate from nitrogen recycling, thus reducing BUN and/or the degradation of soluble protein in the diets.

CONCLUSION

Glycine inclusions of up to 100 g/kg in the diet of dairy cows did not negatively affect milk production and composition. However, inclusions above 150 g/kg of glycerin in the diet reduced the concentration of fat, and of unsaturated, monounsaturated, polyunsaturated fatty acids and conjugated linoleic acid (CLA C18: 2 cis-9 and trans-11) in milk.

Our meta-analysis does not demonstrate the effectiveness of glycerin in improving the composition of milk and a group of fatty acids of importance for human health such as C18: 2 cis-9, trans-11 CLA.

SUPPLEMENTARY DATA

Supplementary data are available at *Translational Animal Science* online.

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Conflicts of interest statement. The authors declare that they have no competing interests.

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