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Glycerine as a feed supplement for beef and dairy cattle: A meta-analysis on performance, rumen fermentation, blood metabolites and product characteristics

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Abstract

This study aimed to comprehensively evaluate the effects of glycerine supplementation at various concentrations on performance, rumen fermentation, blood metabolites and product characteristics of beef and dairy cattle in vivo by using a quantitative meta-analysis approach. Meta-analysis was performed by integrating a total of 52 studies from 39 articles and 182 treatments into a database. Data were constructed into an intact database and did not distinguish between beef and dairy cattle, except for the parameters of production performance and product characteristics. Data summarized were analysed by using a statistical meta-analysis that employed a fixed effect of glycerine supplementation level and a random effect of various studies for both beef and dairy cattle. Significance of an effect was stated at the probability level of $p < .05$, and $p < .1$ was considered as a tendency of significant. Results revealed that there was a linear decrease on dry matter intake ($p < .01$) and daily gain ($p < .05$) of beef cattle with the increasing levels of glycerine supplementation. Glycerine supplementation did not decrease milk production of lactating dairy cows. Molar proportion of acetate in the rumen was decreased ($p < .001$), whereas propionate and butyrate proportions were increased (both at $p < .001$) by glycerine supplementation. Generally, glycerine did not change nutrient digestibility except that it reduced fibre digestibility ($p < .001$). Glycerine supplementation linearly lowered triglyceride and NEFA concentrations (both at $p < .05$) in the blood serum, but not other blood metabolites. Glycerine tended to linearly increase ($p < .1$) carcass percentage in beef cattle. Increasing dietary glycerine levels decreased milk fat ($p < .01$) but elevated milk protein ($p < .001$). Glycerine tended to increase milk lactose ($p < .1$) by following a quadratic pattern. The proportion of MUFA was increased quadratically by glycerine supplementation ($p < .05$), whereas glycerine tended to decrease SFA by following a quadratic pattern ($p < .1$).

KEYWORDS
beef, dairy, glycerine, in vivo, meta-analysis

1 | INTRODUCTION

Glycerine is recognized as a safe ingredient to use as an energy source for animal feed. It is primarily produced as a main by-product of biodiesel production. Its usage through supplementation and/or substitution in the ration is able to reduce feed cost due to the high feed price and uncertain availability of energy sources. Moreover, glycerine usage as an animal feed is a solution to help preventing environmental pollution due to the accumulation of biodiesel by-products in abundant quantities, reaching 0.92 kg per 10 L of biodiesel produced (Donkin et al., 2009). Previously, glycerine was used to treat ketosis for lactating cows during the negative energy balance period (Fisher et al., 1973), and then it has been widely explored as an energy source for ruminants (Carvalho et al., 2011; Wang, et al., 2009), non-ruminants (Kerr et al., 2007) and poultry (Henz et al., 2014). Both in vitro and in vivo studies had been performed to evaluate the use of glycerine as an energy supplement in ruminant rations. A meta-analysis of in vitro studies on glycerine supplementation had been conducted, and it revealed that glycerine did not cause detrimental effects on rumen fermentation and environmentally friendly by reducing ruminal methane emission (Syahniar et al., 2016).

In contrast to in vitro studies that limited to rumen fermentation parameters, in vivo studies provide more comprehensive parameters to evaluate the glycerine supplementation. In vivo studies of the glycerine supplementation and/or substitution as an energy source had been evaluated on performance, rumen fermentation, blood metabolites and product characteristics specifically for beef (Bartoň et al., 2013; Benedeti et al., 2016; Fávaro et al., 2015) and dairy cattle (Ariko et al., 2015; Chung, Raungprim, et al., 2007; Ezequiel et al., 2015). However, the varied results of glycerine supplementation in ruminants indicated inconsistency whether it is able to increase or merely energy substitution or even interfere with the performance, metabolism and product quality of beef and dairy cattle. In a recent review, Kholif (2019) described that the effects of glycerine administration in the diet of lactating animals have been varied and are not consistent. Further, those in vivo studies of glycerine supplementation in ruminant diets have not been quantitatively summarized yet to date. Therefore, the aim of this study was to comprehensively evaluate the effects of glycerine supplementation at various concentrations on performance, rumen fermentation, blood metabolites and product characteristics of beef and dairy cattle in vivo by using a quantitative meta-analysis approach.

2 | MATERIALS AND METHODS

2.1 | Database development

A database was developed from published experiments that reported glycerine supplementation at various concentrations in the diets of beef and dairy cattle. Scopus, Science Direct and EBSCO were employed for searching the literatures by using two or more combination of keywords consisted of "glycerine", "glycerol", "ruminant", "beef" and "dairy". Criteria for an article to be included in the database were as follows: (a) article was published in a peer-reviewed journal, not originated from any conference proceedings or other non-peer-reviewed sources, (b) glycerine was added into a certain basal diet, (c) performed directly on beef or dairy cattle in vivo as the experimental animals and (d) measured their performance, rumen fermentation, blood metabolites and/or product characteristics. Any in vitro experiments related to the topic were excluded from the present study. Schematic representation of literature searching and study selection process in the current meta-analysis is presented in Figure 1. The publications found were in total 39 articles that comprised of 52 studies and 182 treatments (Table 1). Glycerine used was a crude glycerine as the main by-product of the biodiesel industry. The variables included in this present study were production performance, rumen fermentation, nutrient digestibility, blood metabolites and product characteristics. Data were constructed into an intact database and did not distinguish between beef and dairy cattle. For exceptions, the variables which separated automatically between beef and dairy cattle were production performance and product characteristics.

The statistical summary of all variables is presented in Table 2. Production performance-related variables were dry matter intake (DMI), average daily gain (ADG), gain to feed ratio (G:F), milk production, milk to feed ratio (M:F) and fat corrected milk (FCM). Rumen fermentation variables were pH, total volatile fatty acid (VFA), acetate, propionate, butyrate and ammonia (NH_3), whereas digestibility of nutrients included was DM, organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Blood metabolites included were glucose, triglyceride, non-esterified fatty acid (NEFA), aspartate aminotransferase (AST), hydroxybutyric acid (HBA) and blood urea nitrogen (BUN). Product characteristics for beef cattle were carcass, fat thickness, longissimus muscle, thawing loss and cooking loss, while those for dairy cattle were milk fat, milk protein, milk lactose, milk solids and milk urea nitrogen (MUN). Other variables of product characteristics were concentrations of various fatty acids, that is, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Apart from these, some other variables were not possible to be integrated due to their insufficient number of data points. Any variables that were reported in less than three studies or <10 data points were excluded from further analyses.

2.2 | Statistical analysis

Publication bias was detected by using a funnel plot. This was performed by plotting the change of performance (a measure of effect size) on *X*-axis and number of replicates (a measure of precision) on *Y*-axis. Performance in this funnel plot was ADG and milk production

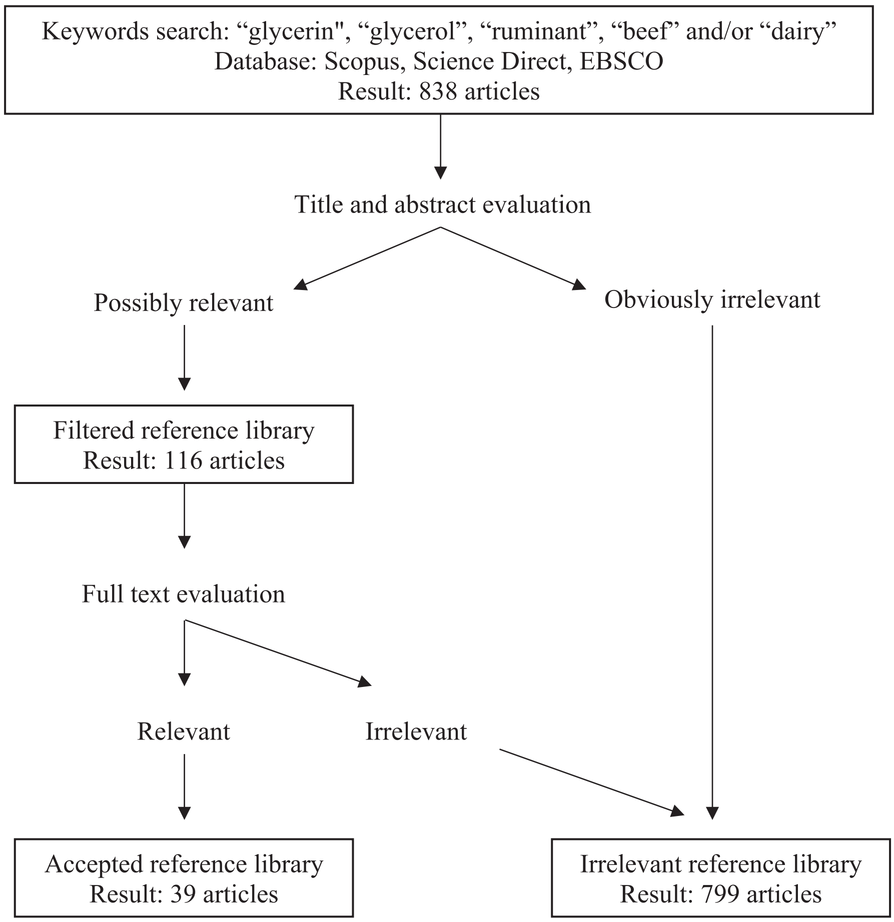

FIGURE 1 Schematic representation of literature searching and study selection process in the meta-analysis of dietary glycerin effects on performance, rumen fermentation, blood metabolites and product characteristics of beef and dairy cattle

for beef and dairy cattle respectively. Further, Egger's test was conducted to statistically analysed the presence of publication bias. Data summarized were analysed by using a statistical meta-analysis approach (Jayanegara et al., 2014; Sauvant et al., 2008). The present study assumed a fixed effect of glycerine supplementation level and a random effect of various studies for both beef and dairy cattle. All the statistical meta-analyses were performed by employing SAS software 9.1.3 on the mixed model procedure (PROC MIXED) with the following model:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij},$$

where Y_{ij} = dependent variable, B_0 = overall intercept across all studies (fixed effect), B_1 = linear regression coefficient of Y on X (fixed effect), B_2 = quadratic regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable (glycerine supplementation level), s_i = random effect of study i, b_i = random effect of study i on the regression coefficient of Y on X in study i and e_{ij} = the unexplained residual error. Significance of an effect was stated at the probability level of $p < .05$, and $p < .1$ was considered as a tendency of significance. In case that the quadratic model above was not significant, the model was changed into its corresponding linear model. The variable of the study was declared in the class statement as it did not contain any quantitative information. The regression equations are also presented with p-value, root mean square error (RMSE) and the coefficient of determination (R^2).

3 | RESULTS

3.1 | Production performance, rumen fermentation and nutrient digestibility

Publication bias was unlikely to exist in the present meta-analysis as indicated by the symmetry of the funnel plot and the insignificance of Egger's test (Figure 2). There was a linear decrease ($p < .01$) on DMI with the increasing level of glycerine supplementation (Table 3). Similarly, an increase of dietary glycerine lowered ADG value of beef cattle ($p < .05$), but did not affect gain to feed ratio. In contrast to the production performance of beef cattle, glycerine supplementation did not decrease milk production of lactating dairy cows as well as their FCM and milk to feed ratio.

Glycerine supplementation tended ($p < .1$) to decrease rumen pH with a quadratic pattern (Table 4). Increasing dietary glycerine level reduced total VFA concentration in the rumen ($p < .01$) but it did not alter the ammonia concentration. The molar proportion of acetate was decreased ($p < .001$), whereas propionate and butyrate proportions were increased (both at $p < .001$) by glycerine supplementation. Generally, glycerine did not change nutrient

TABLE 1 In vivo studies included in the meta-analysis of glycerine as a feed supplement for beef and dairy cattle

No.	References	Animal type	Glycerine purity (%)	Glycerine level (g/kg DM)	Production performance	Rumen fermentation	Nutrient digestibility	Blood metabolites	Product characteristics
1	Bartoň et al. (2013)	Beef	–	0–93.5	√	√	×	√	√
2	Wang, Liu, et al. (2009)	Beef	–	0–3.3	×	√	√	×	×
3	Mach et al. (2009)	Beef	–	0–121	√	√	×	√	√
4	Françozo et al. (2013)	Beef	81.2	0–120	√	×	×	×	√
5	Leão et al. (2013)	Beef	80.35	0–240	×	×	×	×	√
6	Leão et al. (2013)	Beef	80.35	0–240	×	×	×	×	√
7	Parsons et al. (2009)	Beef	–	0–160	√	×	×	×	√
8	Benedeti et al. (2016)	Beef	82.8	0–150	√	×	×	×	√
9	Buttrey et al. (2015)	Beef	–	0–100	√	×	×	×	√
10	Chanjula et al. (2016)	Beef	88.91	0–210	√	×	×	×	√
11	Egea et al. (2014)	Beef	87.5	0–40	√	×	×	×	√
12	Eiras et al. (2014)	Beef	81.2	0–180	√	×	×	×	√
13	Fávaro et al. (2015)	Beef	83	0–200	×	×	√	√	×
14	Favaro et al. (2016)	Beef	83	0–150	√	×	×	×	×
15	Favaro et al. (2016)	Beef	83	0–150	√	×	×	×	√
16	Hales, Kraich, et al. (2013)	Beef	79.7	0–150	√	×	×	×	×
17	Hales, Kraich, et al. (2013)	Beef	–	0–100	×	×	√	×	×
18	Hales, Kraich, et al. (2013)	Beef	–	0–100	√	×	×	×	×
19	Hales, Kraich, et al. (2013)	Beef	–	0–75	√	×	×	×	×
20	Hales, Kraich, et al. (2013)	Beef	–	0–75	√	×	×	×	×
21	Hales et al. (2015)	Beef	82.7	0–150	×	×	√	×	×
22	Carvalho et al. (2014)	Beef	83.12	0–180	×	×	×	×	√
23	Ciriaco et al. (2016)	Beef	–	0–160	×	√	×	×	×
24	Ladeira et al. (2016)	Beef	83.1	0–180	√	×	×	√	√
25	Lage et al. (2014)	Beef	80.34	0–100	×	×	×	×	√
26	Lage et al. (2014)	Beef	80.34	0–100	×	×	×	×	√
27	Ramos and Kerley (2012)	Beef	–	0–200	√	×	×	×	×
28	Ramos and Kerley (2012)	Beef	–	0–150	√	×	×	×	×

(Continues)

TABLE 1 (Continued)

No.	References	Animal type	Glycerine purity (%)	Glycerine level (g/kg DM)	Production performance	Rumen fermentation	Nutrient digestibility	Blood metabolites	Product characteristics
29	Ramos and Kerley (2012)	Beef	–	0–200	√	×	×	×	×
30	San Vito et al. (2015)	Beef	80.34	0–280	×	×	×	×	√
31	van Cleef et al. (2014)	Beef	86	0–300	√	×	√	×	√
32	van Cleef et al. (2015)	Beef	86	0–300	×	√	×	×	×
33	Moriel et al. (2011)	Beef	–	0–150	√	×	×	√	×
34	Ariko et al. (2015)	Dairy	88.9	0–156	×	√	×	×	×
35	Boyd et al. (2013)	Dairy	80–85	0–15.7	√	√	√	√	√
36	Carvalho et al. (2011)	Dairy	99.5	0–115	×	×	×	√	×
37	Carvalho et al. (2011)	Dairy	99.5	0–108	√	√	×	√	√
38	Chung et al. (2007)	Dairy	65	0–11.3	√	×	×	√	√
39	DeFrain et al. (2004)	Dairy	–	0–76.1	×	√	×	√	×
40	DeFrain et al. (2004)	Dairy	–	0–54.4	√	√	×	√	√
41	Donkin et al. (2009)	Dairy	99.5	0–150	√	×	√	√	√
42	Ezequiel et al. (2015)	Dairy	83	0–300	√	×	×	√	√
43	Harzia et al. (2013)	Dairy	82.6	0–156	√	×	×	×	√
44	Kafilzadeh et al. (2015)	Dairy	–	0–17.2	√	×	√	√	√
45	Kass et al. (2012)	Dairy	82.6	0–156	√	√	×	√	√
46	Paiva et al. (2016)	Dairy	80.6	0–120	√	√	√	√	√
47	Shin et al. (2012)	Dairy	80.3	0–100	√	×	√	√	√
48	Shin et al. (2012)	Dairy	80.3	0–100	√	×	√	√	√
49	Shin et al. (2012)	Dairy	80.3	0–100	√	√	×	×	√
50	Shin et al. (2012)	Dairy	80.3	0–100	√	√	×	×	√
51	Wang, Liu, et al. (2009)	Dairy	99.8	0–300	√	×	×	√	√
52	Wilbert et al. (2013)	Dairy	81.44	0–120	√	×	√	√	√

TABLE 2 Descriptive statistics summary of the database

Parameter	Unit	*n*	Mean	*SD*	Minimum	Maximum
Glycerine	g/kg	182	75.9	74.2	0	300
Production performance						
DMI	kg/day	144	13.2	6.13	5.5	31.6
ADG	kg/day	75	1.40	0.246	0.92	1.92
G:F	kg/kg	73	0.159	0.032	0.110	0.249
Milk production	kg/day	49	29.8	7.42	16.0	44.6
M:F	kg/kg	45	1.46	0.321	0.95	2.15
FCM	kg/day	16	31.6	5.33	24.0	42.0
Rumen fermentation						
pH		40	6.46	0.374	5.68	7.30
Total VFA	mM	47	97.7	25.2	46.7	149
C_2	% molar	47	60.1	8.80	46.8	86.6
C_3	% molar	47	23.6	5.75	12.2	38.6
C_4	% molar	47	14.7	5.02	7.60	30.5
NH_3	mM	46	7.85	4.18	1.68	16.9
Nutrient digestibility						
DMD	%	39	66.1	7.32	53.4	80.7
OMD	%	38	69.6	7.46	57.4	81.6
CPD	%	35	65.4	5.75	56.1	77.2
EED	%	20	81.0	7.06	67.4	89.1
NDFD	%	45	50.6	12.3	20.2	66.7
ADFD	%	17	39.0	6.21	29.8	47.7
Blood metabolites						
Glucose	mM	64	3.83	0.654	2.82	5.45
Triglyceride	µM	11	115	37.6	59.9	180
NEFA	µM	29	278	167	68.0	639
AST	µkat/l	10	53.2	38.9	1.28	95.4
HBA	µM	27	567	404	1.30	1,160
BUN	mg/dl	26	29.1	13.5	10.6	51.2
Product characteristics						
Carcass	%	67	56.8	3.50	52.2	64.2
Fat thickness	mm	53	7.16	4.38	2.66	19.2
Longissimus	cm^2	60	77.3	11.6	60.2	104
Thawing loss	%	24	9.71	4.66	2.48	16.5
Cooking loss	%	41	26.1	5.88	14.5	38.2
Milk fat	%	49	3.73	0.606	2.71	4.67
Milk protein	%	43	3.22	0.326	2.75	3.76
Milk lactose	%	36	4.71	0.164	4.41	5.26
Milk solid	%	13	12.2	0.513	11.3	12.9
MUN	mg/dl	24	16.3	4.77	10.2	23.5
MUFA	%	32	43.1	6.92	25.9	52.0
PUFA	%	32	7.18	2.54	3.68	12.6
SFA	%	32	49.3	6.51	42.1	68.1
UFA/SFA		32	1.03	0.238	0.47	1.32
PUFA/SFA		16	0.142	0.049	0.079	0.240

Note: Abbreviations: ADFD, acid detergent fibre digestibility; ADG, average daily gain; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C_2, acetate; C_3, propionate; C_4, butyrate; CH_4, methane; CPD, crude protein digestibility; DMD, dry matter digestibility; DMI, dry matter intake; EED, ether extract digestibility; FCM, fat corrected milk; G:F, gain to feed ratio; HBA, hydroxybutyric acid; M:F, milk to feed ratio; MUFA, monounsaturated fatty acid; MUN, milk urea nitrogen; NDFD, neutral detergent fibre digestibility; NEFA, non-esterified fatty acid; NH_3, ammonia; OMD, organic matter digestibility; PUFA, polyunsaturated fatty acid; *SD*, standard deviation; SFA, saturated fatty acid; UFA, unsaturated fatty acid; VFA, volatile fatty acid.

FIGURE 2 Funnel plot (change of performance versus number of replicates) of the meta-analysis of dietary glycerine effects on beef (○) and dairy cattle (Δ). Performance in this plot was average daily gain and milk production for beef and dairy cattle respectively. P-value of Egger's test = 0.579

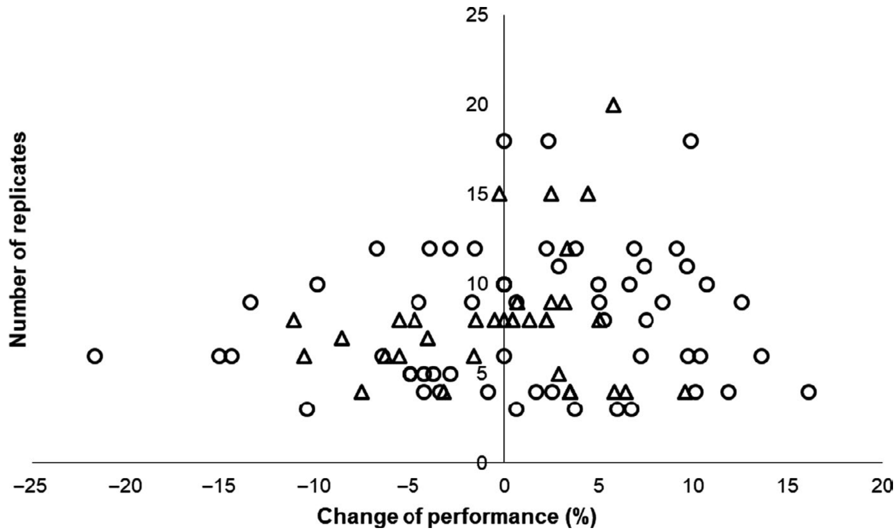

TABLE 3 Effects of glycerine (GLY) supplementation (in kg/kg dry matter) on production performance of beef and dairy cattle

Response		Independent		Parameter estimates				Model statistics		
Parameter	Unit	variable	*n*	Intercept	*SE* int	Slope	SE slope	*p*-Value	RMSE	R^2
DMI	kg/day	GLY	144	14.0	0.989	−2.49	0.847	.004	0.616	0.104
ADG	kg/day	GLY	75	1.46	0.055	−0.348	0.164	.039	0.086	0.095
G:F	kg/kg	GLY	73	0.161	0.007	−0.008	0.015	.584	0.008	0.006
Milk production	kg/day	GLY	49	30.8	1.90	−0.002	0.002	.319	0.885	0.034
M:F	kg/kg	GLY	45	1.49	0.086	0.146	0.130	.271	0.056	0.068
FCM	kg/day	GLY	16	31.6	2.24	2.14	4.86	.672	0.785	0.034

Note: Abbreviations: ADG, average daily gain; DMI, dry matter intake; FCM, fat corrected milk; G:F, gain to feed ratio; M:F, milk to feed ratio; *n*, number of data; R^2, coefficient of determination; RMSE, root mean square error; *SE*, standard error.

digestibility except for fibre components. Glycerine decreased NDF digestibility ($p < .001$) and tended to reduce ADF digestibility ($p < .1$).

3.2 | Blood metabolites and product characteristics

Glycerine supplementation linearly lowered triglyceride and NEFA concentrations (both at $p < .05$) in the blood serum (Table 5). Other blood metabolites such as glucose, AST, HBA and BUN were not affected by dietary glycerine.

With regard to product characteristics, glycerine supplementation tended to linearly increase ($p < .1$) carcass percentage in beef cattle (Table 6). Fat thickness, longissimus muscle area, thawing loss and cooking loss were not influenced by glycerine supplementation. For the milk product characteristics of dairy cows, increasing dietary glycerine level decreased milk fat ($p < .01$) but elevated milk protein ($p < .001$). Glycerine tended to increase milk lactose and tended to reduce milk solid (both at $p < .1$) by following quadratic patterns. Proportion of MUFA was increased quadratically by glycerine supplementation ($p < .05$), whereas glycerine tended to decrease SFA by following a quadratic pattern ($p < .1$). Glycerine did not alter MUN and PUFA profiles in both meat and milk.

4 | DISCUSSION

Although glycerine may potentially enhance feed palatability because of its sweet taste (Kholif, 2019), a lower DMI in response to the increased level of glycerine supplementation is apparently as an implication of the high energy density contained in the molecule (Chanjula et al., 2016). The energy value of glycerine is approximately 3,000–7,000 Kcal/kg or 12–29 MJ/kg (Jung & Batal, 2011), which is categorized as a medium to a high level of energy density. Such high variation of glycerine energy value may be related to the variation in the purity of the substance. The DMI of cattle with high energy density diets is thought to be controlled by the energetic demands and metabolic factors (NRC, 2000). Such elevated dietary energy density due to glycerine inclusion may induce oxidation reaction in the liver by upregulating Krebs cycle and increasing ATP concentration, and as a consequence, satiety feeling is stimulated and DMI is reduced (Allen et al., 2009). Apart from that possible reason, DMI may be lowered because of glycerine impurities such as methanol

TABLE 4 Effects of glycerine (GLY) supplementation (in kg/kg dry matter) on rumen fermentation and nutrient digestibility of beef and dairy cattle

				Parameter estimates				Model statistics		
Response parameter	Unit	Independent variable	*n*	Intercept	*SE* int	Slope	*SE* slope	*p*-Value	RMSE	R^2
Rumen fermentation										
pH		GLY	40	6.53	0.116	−1.67	0.669	.019		
		GLY^2				5.45	2.82	.064	0.109	0.364
Total VFA	mM	GLY	47	97.2	7.01	−34.7	11.9	.006	4.708	0.305
C_2	% molar	GLY	47	64.5	2.00	−84.7	8.54	<.001	3.419	0.850
C_3	% molar	GLY	47	21.6	1.35	33.8	5.00	<.001	1.998	0.656
C_4	% molar	GLY	47	12.4	0.896	32.0	3.93	<.001	1.576	0.725
NH_3	mM	GLY	46	7.94	1.17	−5.99	3.82	.127	1.575	0.125
Nutrient digestibility										
DMD	%	GLY	39	65.8	2.38	2.05	4.56	.657	1.770	0.007
OMD	%	GLY	38	69.9	2.50	−0.651	4.73	.892	1.712	0.001
CPD	%	GLY	35	65.5	1.91	−0.808	6.60	.904	2.450	0.002
EED	%	GLY	20	79.0	2.83	15.7	10.0	.140	3.341	0.130
NDFD	%	GLY	45	52.5	3.67	−36.0	8.05	<.001	3.181	0.514
ADFD	%	GLY	17	41.2	2.39	−22.7	11.4	.070	3.405	0.191

Note: Abbreviations: ADFD, acid detergent fibre digestibility; C2, acetate; C4, butyrate; CH4, methane; CPD, crude protein digestibility; DMD, dry matter digestibility; EED, ether extract digestibility; *n*, number of data; NDFD, neutral detergent fibre digestibility; NH_3, ammonia; OMD, organic matter digestibility; R^2, coefficient of determination; RMSE, root mean square error; SE, standard error; VFA, volatile fatty acid.

TABLE 5 Effects of glycerine (GLY) supplementation (in kg/kg dry matter) on blood metabolites of beef and dairy cattle

				Parameter estimates				Model statistics		
Response parameter	Unit	Independent variable	*n*	Intercept	*SE* int	Slope	*SE* slope	*p*-Value	RMSE	R^2
Glucose	mM	GLY	64	3.75	0.146	0.430	0.457	.352	0.229	0.024
Triglyceride	μM	GLY	11	138	21.1	−165	67.1	.043	18.5	0.620
NEFA	μM	GLY	29	306	1,015	−174	82.2	.049	30.7	0.191
AST	μkat/l	GLY	10	49.0	25.8	1.57	18.2	.934	4.727	0.001
HBA	μM	GLY	27	539	124	154	180	.405	55.8	0.053
BUN	mg/dl	GLY	26	27.0	4.97	−4.30	7.98	.597	2.546	0.030

Note: Abbreviations: AST, aspartate aminotransferase; BUN, blood urea nitrogen; HBA, hydroxybutyric acid; n, number of data; NEFA, non-esterified fatty acid; R^2, coefficient of determination; RMSE, root mean square error; *SE*, standard error.

and catalysts, or from the increasing concentration of sodium or other salts (Ezequiel et al., 2015). The purity of glycerine used in this meta-analysis study ranged from 65% to 99%. The use of pure glycerine as an animal feed is less possible due to its expensive price; it is rather used for pharmaceutical and cosmetic industries. In contrast, unpurified glycerine has a relatively low economic value, and therefore, it has a competitive advantage to be used as a dietary energy supplement for animals.

Lower ADG of beef cattle due to glycerine supplementation is apparently as a response to the lower DMI. However, interestingly, such glycerine supplementation does not reduce milk production of lactating dairy cows despite the lower DMI. Since the energy density of glycerine is high, it may compensate such lower DMI and therefore preventing a reduction in milk production. Lower DMI is also associated with the lower total VFA concentration in the rumen since nutrient is degraded and fermented by rumen microbes into various VFA components such as acetate, propionate, butyrate and others. Further, a lower concentration of total VFA may also be possible due to a direct absorption of glycerine through rumen wall (Werner Omazic et al., 2013) or lower gastrointestinal tract (Remond et al., 1993). The present meta-analysis study also reveals the propiogenic property of glycerine in the rumen that promotes a higher proportion of propionate at the expense of acetate. This is in agreement with the previous meta-analysis study on glycerine supplementation under in vitro rumen environment (Czerkawski, 1970; Remond et al., 1993; Syahniar et al., 2016). Most of 14C labelled glycerine had

TABLE 6 Effects of glycerine (GLY) supplementation (in kg/kg dry matter) on product characteristics of beef and dairy cattle

Response parameter	Unit	Independent variable	*n*	Parameter estimates				Model statistics		
				Intercept	*SE* int	Slope	*SE* slope	*p*-Value	RMSE	R^2
Beef cattle										
CARCASS	%	GLY	67	56.5	0.779	2.00	1.12	.081	0.635	0.091
Fat thickness	mm	GLY	53	7.21	1.091	−1.98	2.68	.466	1.413	0.020
Longissimus	cm^2	GLY	60	76.6	3.015	6.18	5.13	.235	2.847	0.053
Thawing loss	%	GLY	24	9.81	1.96	−0.019	3.01	.995	1.21	0.001
Cooking loss	%	GLY	41	25.5	1.94	2.05	3.52	.565	1.63	0.014
Dairy cattle										
Milk fat	%	GLY	49	3.71	0.152	−0.661	0.237	.009	0.105	0.259
Milk protein	%	GLY	43	3.16	0.083	0.380	0.103	<.001	0.044	0.416
Milk lactose	%	GLY	36	4.74	0.057	0.239	0.269	.384		
		GLY^2				−1.83	1.041	.091	0.045	0.205
Milk solid	%	GLY	13	12.3	0.284	−2.40	0.906	.033		
		GLY^2				7.90	3.46	.056	0.101	0.869
MUN	mg/dl	GLY	24	15.9	1.58	−1.74	2.35	.469	0.715	0.050
MUFA	%	GLY	32	42.6	2.25	21.0	6.85	.006		
		GLY^2				−74.0	27.6	.014	1.14	0.586
PUFA	%	GLY	32	6.84	0.795	0.358	2.25	.875	0.938	0.002
SFA	%	GLY	32	50.1	2.11	−18.7	8.54	.041		
		GLY^2				66.1	34.4	.069	1.42	0.363
UFA/SFA		GLY	32	0.995	0.076	0.803	0.384	.049		
		GLY^2				−2.77	1.55	.089	0.064	0.321
PUFA/SFA		GLY	16	0.140	0.023	0.020	0.111	.861	0.025	0.022

Abbreviations: MUFA, monounsaturated fatty acid; MUN, milk urea nitrogen; *n*, number of data; PUFA, polyunsaturated fatty acid; R^2, coefficient of determination; RMSE, root mean square error; *SE*, standard error; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

been found in the form of propionate (Bergner et al., 1995), and it has been argued that glycerine is selectively used by rumen microbes to generate propionate rather than acetate (Ramos & Kerley, 2012). With regard to rumen pH, rapid fermentation of glycerine in the rumen may explain the decrease of rumen pH despite the concomitant reduction of total VFA concentration. Glycerine has been used to partially substitute a feed ingredient rich in starch such as corn, barley and wheat grains due to its high energy value as previously described above. It had been observed that ruminal degradation and fermentation of glycerine was more rapid than that of wheat starch (Schröder & Südekum, 1999). Further, shortly after entering the rumen, glycerol was rapidly fermented and mostly disappeared within 24 hr (Trabue et al., 2007).

Changes of individual VFA pattern (typically due to dietary changes) are often associated with shifts in the abundance of microbial species present in the rumen (Bergner et al., 1995; Shin et al., 2012). Glycerine had been observed to cause a negative effect on the activity of cellulolytic bacteria in the rumen, that is, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* and *Ruminococcus albus* as well as anaerobic fungi like *Neocallimastix frontalis* (Roger et al., 1992; Elias San Vito et al., 2016). This is apparently one of the primary causes of the reduced fibre (NDF and ADF) digestibility observed in the current meta-analysis. A number of mechanisms by which glycerine inhibits the cellulolytic activity of rumen microbes have been proposed, that is lowering accessibility of cellulolytic microbes to substrate, inhibiting the adhesion of rumen microbes particularly fungi to substrate, negatively affecting the permeability of microbial cell wall, inhibiting the secretion of cellulase and hampering the action of cellulase enzyme through interfering its active site or modifying its affinity to the substrate (Roger et al., 1992). Such negative effects of glycerine on rumen microbes and their fermentation activity are more pronounced particularly at high administration levels. Glycerine apparently does not affect much protein degradation and fermentation in the rumen as indicated by the NH_3 concentration and CP digestibility parameters.

Lower triglyceride and NEFA concentrations in the blood due to glycerine supplementation are apparently related to the enhanced energy status of the animals. Most of the supplemented glycerine is absorbed in the rumen and then transferred to the liver. According to Krehbiel (2008), the arterial concentration of glycerine increased, whereas glucose concentration was not affected due to the fate of absorbed glycerol across the ruminal epithelium would most likely towards gluconeogenesis and be converted to glucose in the liver. Since the availability of glucose is elevated, animals are minimally mobilized their body fat for generating energy thus lowering blood triglyceride and NEFA concentrations. Due to this fact, sometimes glycerine is used to treat or to prevent ketosis in dairy cows particularly during early lactation or the negative energy balance period (Kholif, 2019). The insignificant effect of glycerine on BUN is in agreement with the ruminal NH_3 concentration and CP digestibility, and all these parameters confirm the minimum influence of glycerine on nitrogen metabolism in ruminants.

Glycerine supplementation generally shows no changes on beef product characteristics with the exception of carcass percentage that tends to increase. It seems to be related to the propiogenic property of glycerine that produces more propionate as glucogenic precursor and lower acetate as lipid precursor, thus reflects greater tissue and lower fat deposit respectively. Glycerine effects on milk composition of dairy cattle are apparently related to its propiogenic property as well. Lower acetate proportion in the rumen that serves as lipid precursor explains the lowering effect of glycerine on milk fat proportion (DeFrain et al., 2004) and then oppositely affects milk protein proportion. Further, the propiogenic property of glycerine stimulates glucose synthesis in the liver that in turn elevates lactose production in mammary gland (Kass et al., 2012). Fatty acid profiles on beef and dairy products are of interesting concerns due to their effects on human health. The current meta-analysis reveals the alteration of fatty acid profiles towards a positive direction, that is, elevating MUFA and lowering SFA so that UFA/SFA ratio in the products is enhanced. Glycerine supplementation in ruminant diets may potentially inhibit lipid degradation in the rumen, a precondition of ruminal biohydrogenation, enhance ruminal passage of total lipid and thereby provide higher proportion of beneficial UFA for incorporation in animal products. It had been also demonstrated that DNA concentrations of *Butyrivibrio fibrisolvens* and *Clostridium proteoclasticum* declined when crude glycerine was used to replace corn in a continuous culture rumen fermentation system (Abo El-Nor et al., 2010). These bacteria species play important and central roles in ruminal biohydrogenation process of UFA (Maia et al., 2007).

5 | CONCLUSION

Glycerine supplementation on beef and dairy cattle reveals different response; it decreases beef cattle performance but has no negative effect on dairy cattle performance. The substance negatively affects fibre digestibility but shows the limited effect on protein digestion and metabolism of ruminants. Glycerine has a propiogenic property in the rumen by increasing propionate proportion at the expense of acetate, and this alteration is a major factor affecting the change of product characteristics in beef and dairy cattle. Supplementation of glycerine is favourable with regard to fatty acid profiles in animal products in which it enhances the UFA/SFA ratio.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a meta-analysis article with no original research data.

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