

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CAMPUS DE JABOTICABAL**

**USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR
A GLICERINA E MELAÇO NA DIETA DE BOVINOS**

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TÍTULO DA TESE: USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR A GLICERINA E MELAÇO
NA DIETA DE BOVINOS

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“Ninguém vai bater mais forte do que a vida.
Não importa como você bate e sim o quanto aguenta apanhar e continua lutando;
O quanto pode suportar e seguir em frente.
É assim que se ganha.”

Sylvester Stallone

“A vida não é necessariamente generosa com aqueles que buscam honestamente o
conhecimento; por isso é preciso paixão.”

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SUMÁRIO

	Página
RESUMO.....	vi
ABSTRACT.....	viii
LISTA DE TABELAS.....	x
LISTA DE FIGURAS.....	xii
CAPÍTULO 1 – Considerações gerais.....	1
Introdução.....	1
Revisão de literatura.....	3
Utilização de glicerina na alimentação animal	4
Utilização de melaço na alimentação animal.....	5
Uso da abordagem meta-analítica na nutrição de ruminantes.....	6
Referências.....	11
CAPÍTULO 2 – The effect of dietary inclusion of crude glycerin on performance, ruminal fermentation, meat quality and fatty acid profile of beef cattle: Meta-analysis.....	18
Abstract.....	19
1. Introduction.....	20
2. Material and Methods.....	21
2.1. Data set.....	21
2.2. Data extraction.....	21
2.3. Statistical analysis.....	21
3. Results.....	23
3.1. DM and nutrient intake and digestibility.....	23
3.2. Performance, carcass traits and physical and chemical characteristics and fatty acid profile of meat.....	23
3.3. Ruminal, blood parameter and nitrogen metabolism.....	24
3.4. Analysis of Meta-Regression and Asymmetry of the funnel plot.....	24
3.5. Subgroup analysis.....	24
4. Discussion	26
5. Conclusion.....	30
Acknowledgment.....	30

References.....	30
CAPÍTULO 3 – Meta-analysis of the effect of glycerin inclusion in dairy cattle diet on milk fatty acid profile.....	50
Abstract.....	51
1. Introduction.....	51
2. Material and Methods.....	52
2.1. Data set.....	52
2.2. Data extraction	53
2.3 Statistical analysis.....	53
2.3.1. Weighted mean difference and publication bias.....	53
2.3.2. Meta-Regression and Subgroup analysis.....	54
3. Results.....	55
3.1. Intake and digestibility of DM and nutrients.....	55
3.2. Performance, composition, and fatty acid profile of milk.....	55
3.3. Rumen and blood parameters.....	56
3.4. Meta-regression analysis and funnel plot asymmetry.....	56
3.5. Subgroup analysis.....	56
4. Discussion	58
5. Conclusion.....	62
Supplementary Data.....	62
Acknowledgement.....	62
References.....	62
CAPÍTULO 4 – Effects of molasses supplementation on animal performance and carcass parameters of beef cattle: a meta-analysis.....	78
Abstract.....	79
1. Introduction.....	80
2. Material and Methods.....	81
2.1. Data set.....	81
2.2. Data extraction.....	81
2.3. Statistical analysis.....	81
3. Results.....	83
3.1. Intake and digestibility of nutrients and ruminal parameters	83

3.2. Performance, carcass traits and blood parameters.....	84
3.3. Analysis of Meta-Regression and Asymmetry of the funnel plot.....	84
3.4. Subgroup analysis.....	84
4. Discussion	85
5. Conclusion.....	87
Acknowledgment.....	87
References.....	87
CAPÍTULO 5 – Meta-analysis to evaluate the effect of including molasses in the diet for dairy cows on performance, milk fat synthesis and milk fatty acid.....	
Abstract.....	99
1. Introduction.....	99
2. Material and Methods.....	101
2.1. Data set.....	101
2.2. Data extraction.....	101
2.3. Statistical analysis.....	101
3. Results.....	103
3.1. Intake and digestibility of DM and nutrients.....	103
3.2. Performance, composition and milk fatty acids.....	103
3.3. Ruminal and blood parameters and nitrogen metabolism.....	104
3.4. Meta-Regression analysis and asymmetry of the funnel plot.....	104
3.5. Subgroup analysis.....	104
4. Discussion	106
5. Conclusion.....	109
6. References.....	110

USO DA ABORDAGEM META-ANALÍTICA PARA AVALIAR A GLICERINA E MELAÇO NA DIETA DE BOVINOS

RESUMO – O aumento no uso de biocombustíveis (bioetanol e biodiesel) em substituição aos combustíveis de origem fóssil, foi acompanhado pela preocupação da adequada destinação dos seus resíduos (ex. melaço, grãos de destilaria, glicerina). A inclusão desses resíduos na dieta animal, além de incentivo à cadeia de produção de alimento, possibilita a adequada destinação desses resíduos. No entanto, são contraditórias as informações sobre o impacto da inclusão de melaço e glicerina na dieta de bovinos de corte e leite sobre o desempenho, parâmetros físico-químicos da carne e leite. Nesse contexto, objetivou-se a partir do uso da abordagem meta-analítica, o desenvolvimento de quatro estudos, avaliando o impacto da utilização do melaço e da glicerina na dieta de bovinos sobre o desempenho, características de carcaça, parâmetros físico-químicos e perfil de ácidos graxos da carne e do leite. No estudo 1, avaliou-se o efeito da glicerina bruta em dietas para bovinos de corte sobre o desempenho, parâmetros físico-químicos e o perfil de ácidos graxos da carne. A inclusão de glicerina bruta não afetou o ganho médio diário dos animais, aumentou a eficiência alimentar em 3,15%, reduziu o teor de colesterol (9,13%) e a concentração de ácidos graxos saturados totais em 1,05%, e aumentou os ácidos graxos insaturados totais (2,02%) e monoinsaturados (3,17%) na carne. No entanto, não afetou as concentrações de ácido linoleico conjugado e ômega-3. A concentração de ácido graxo na carne foi modificada com inclusão de glicerina bruta a partir de 70 dias de fornecimento. Inclusões de glicerina bruta em até 200 g/kg na dieta, não afetaram negativamente o desempenho, as características da carcaça e os parâmetros físico-químicos da carne, e proporcionaram aumento no total de monoinsaturado (17,33 mg), ácido oleico (12,29 mg) e ácido palmitoléico (1,24 mg), enquanto reduziram o ácido mirístico (3,08 mg) e o ácido esteárico (12,00 mg) na carne. No estudo 2, avaliou-se o efeito da inclusão de glicerina na dieta de vacas leiteiras sobre o perfil de ácidos graxos do leite. O efeito da inclusão de glicerina na dieta sobre a produção de gordura do leite foi dependente do grupo genético, em que vacas Holandesas e suas mestiças, produziram menos gordura no leite em comparação às vacas Jersey. Inclusões de glicerina acima de 150 g/kg na dieta reduziram a concentração de gordura, ácidos graxos insaturados, monoinsaturados, poli-insaturados e ácido linoleico conjugado (CLA C18: 2 cis-9 e trans-11) no leite. No estudo 3, avaliou-se o efeito do uso de melaço em dietas para bovinos de corte em sistema de confinamento sobre o desempenho e parâmetros de carcaça. O grupo genético e o período de suprimento do melaço foram as principais covariáveis que afetaram as respostas do metabolismo, desempenho e parâmetros de carcaça. A inclusão de melaço na dieta sobre o ganho médio diário (GMD) sofreu efeito do tipo de melaço e teor de concentrado da dieta. Quando utilizado o melaço de beterraba, houve redução no GMD em 0,24 kg/d. Quando incluído em dietas com 800-900 g/kg concentrado na matéria seca da dieta, reduziu o GMD em 0.18 kg/d. Além disso, a inclusão de melaço reduziu a gordura mesentérica em 1.61 kg e a gordura que envolve os rins, a cavidade pélvica e o coração em 1.59 kg. Independente do tipo de melaço, inclusões acima de 200 g/kg reduziram o GMD e peso de carcaça. Entretanto, inclusões entre 100-150 g/kg não apresentaram efeito sobre o desempenho e parâmetros de carcaça. No estudo 4, avaliou-se o efeito da inclusão do melaço em dietas para vacas leiteiras sobre a produção e concentração de gordura no leite. O efeito da inclusão de melaço na dieta

sobre a composição do leite foi dependente do grupo genético e dia de lactação. Quando vacas Jersey foram alimentadas com melaço a concentração de proteína no leite aumentou em 0,81 g/kg, sem alterar o consumo e a produção de leite. A inclusão de melaço acima de 60 g/kg de MS, independente do grupo genético ou período de lactação, reduziu a produção de leite e as concentrações de proteína e lactose. A inclusão de melaço até 120 g/kg MS aumentou em 11,52% a concentração de ômega-3 no leite, sem afetar a concentração de ácidos graxos poli-insaturados e CLA C18: 2 cis-9, trans-12 e produção e concentração de gordura no leite.

Palavras-chave: ácidos graxos, coprodutos, carcaça, qualidade da carne, gordura do leite

USE OF THE META-ANALYTICAL APPROACH TO ASSESS GLYCERIN AND MOLASSES IN THE BOVINE DIET

ABSTRACT – The increase in the use of biofuels (bioethanol and biodiesel) to replace fossil fuels was accompanied by the concern for the proper destination of their residues (e.g., molasses, distillery grains, glycerin). The inclusion of these residues in the animal diet, in addition to encouraging the food production chain, enables the proper disposal of these residues. However, information on the impact of including molasses and glycerin in the diet of beef and dairy cattle on performance, physicochemical parameters of meat and milk is contradictory. In this context, the objective was, from the use of the meta-analytical approach, the development of four studies, evaluating the impact of the use of molasses and glycerin in the diet of cattle on performance, carcass characteristics, physicochemical parameters and profile of fatty acids from meat and milk. In study 1, the effect of crude glycerin in beef cattle diets on performance, physicochemical parameters and fatty acid profile of meat was evaluated. The inclusion of crude glycerin did not affect the animals' average daily gain, increased feed efficiency by 3.15%, and reduced the cholesterol content (9.13%) and the concentration of total saturated fatty acids by 1.05%, and increased total unsaturated fatty acids (2.02%) and monounsaturated (3.17%) in meat. However, it did not affect conjugated linoleic acid and omega-3 concentrations. The fatty acid concentration in meat was modified with the inclusion of crude glycerin after 70 days of supply. Inclusions of crude glycerin up to 200 g/kg in the diet did not negatively affect meat performance, carcass characteristics and physicochemical parameters, and showed an increase in the total monounsaturated (17.33 mg), oleic acid (12.29 mg) and palmitoleic acid (1.24 mg), while it reduced myristic acid (3.08 mg) and stearic acid (12.00 mg) in meat. In study 2, the effect of including glycerin in the diet of dairy cows on the fatty acid profile of milk was evaluated. The effect of including glycerin in the diet on milk fat production was dependent on the genetic group, in which Holstein cows and their crossbreds produced less milk fat compared to Jersey cows. Inclusions of glycerin above 150 g/kg in the diet reduced the concentration of fat, unsaturated fatty acids, monounsaturated, polyunsaturated and conjugated linoleic acid (CLA C18: 2 cis-9 and trans-11) in milk. In study 3, the effect of using molasses in diets for beef cattle in a feedlot system on performance and carcass parameters was evaluated. The genetic group and the period of molasses supply were the main covariates that affected the responses of metabolism, performance and carcass parameters. The inclusion of molasses in the diet on the average daily gain (ADG) was affected by the type of molasses and the concentrate content of the diet. When beet molasses was used, there was a reduction in ADG by 0.24 kg/d. When included in diets with 800-900 g/kg concentrate in the total diet, it reduced ADG by 0.18 kg/d. In addition, the inclusion of molasses reduced mesenteric fat by 1.61 kg and fat kidneys, pelvic cavity and heart by 1.59 kg. Regardless of the type of molasses, inclusions above 200 g/kg reduced ADG and carcass weight. However, inclusions between 100-150 g/kg had no effect on performance and carcass parameters. In study 4, the effect of including molasses in diets for dairy cows on milk fat production and concentration was evaluated. The effect of including molasses in the diet on milk composition was dependent on genetic group and day of lactation. When Jersey cows were fed molasses, the protein concentration in milk increased by 0.81 g/kg, without altering milk consumption and production. The inclusion of molasses above 60 g/kg DM, regardless of genetic group or lactation

period, reduced milk production and protein and lactose concentrations. The inclusion of molasses up to 120 g/kg DM increased the concentration of omega-3 in milk by 11.52%, without affecting the concentration of polyunsaturated fatty acids and CLA C18: 2 cis-9, trans-12 and production and concentration of fat in milk.

Keywords: fatty acids, byproduct, carcass, meat quality, fat in milk

LISTA DE TABELAS

Página

CAPÍTULO 2

Table 1. Chemical composition of crude glycerin used in the studies.....	34
Table 2. Effect of inclusion of crude glycerin in beef cattle diet on intake and digestibility.....	35
Table 3. Effect of inclusion of crude glycerin in beef cattle diet on performance, carcass and blood parameters.....	36
Table 4. Effect of inclusion of crude glycerin in the diet of beef cattle on the physicochemical characteristics of meat.....	37
Table 5. Effect of inclusion of crude glycerin in beef cattle diet fatty acid (mg/100g of meat) profile of meat	38
Table 6. Effect of inclusion of crude glycerin in beef cattle diet on ruminal parameters and nitrogen balance.....	39
Table 7. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.....	40
Table 8. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.....	41
Table 9. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.....	42

CAPÍTULO 3

Table 1: Effect of the glycerin inclusion in dairy cow diets on the intake, digestibility and body weight change.....	66
Table 2: Effect of the glycerin inclusion in dairy cow diets on the ruminal and blood parameters.....	67
Table 3: Effect of the glycerin inclusion in dairy cow diets on the milk yield, composition and fatty acid profile in milk.....	68
Table 4. Meta-regression of the effect glycerin inclusion in dairy cow diets on weighted mean differences (WMD) for digestibility, ruminal, blood, and milk production variable.....	69

Table 5. Meta-regression of the effect glycerin inclusion in dairy cow diets on weighted mean differences (WMD) for milk fatty acid variables.....	70
--	----

CAPÍTULO 4

Table 1: Effect of inclusion of molasses in beef cattle diet on intake, digestibility and ruminal parameters.....	90
---	----

Table 2: Effect of inclusion of molasses in beef cattle diet on performance, carcass and blood parameters.....	91
--	----

Table 4: Meta-regression of the effect of inclusion the molasses in diets for beef cattle on weighted mean differences (WMD), between treatment and control.....	92
--	----

CAPÍTULO 5

Table 1: Effect of the molasses in the diet of dairy cow on the intake, digestibility performance and body weight.....	114
--	-----

Table 2: Effect of the molasses in the diet of dairy cow on milk yield and milk composition.....	115
--	-----

Table 3: Effect of the molasses in the diet of dairy cow on the ruminal parameters, nitrogen metabolism.....	116
--	-----

Table 4. Meta-regression of the effect molasses in the diet of dairy cow on weighted mean differences (WMD) between treatment and control.....	117
--	-----

LISTA DE FIGURAS

Página

CAPÍTULO 2

Figure 1. Forest plot (with subgroup analysis “Covariate=Glycerin in diet (g/kg)), showing the effect of the use of crude glycerin on the diet the beef cattle on monounsaturated fatty acid total (mg/100g of meat).....	43
Figure 2. Subgroup analysis (subgroup=Genetic Type) of the effect of the use of glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	44
Figure 3. Subgroup analysis (subgroup=Treatment period (day)) of the effect of the use of glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	45
Figure 4. Subgroup analysis (subgroup=Concentrate in diet (g/kg of DM)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	46
Figure 5. (A) Subgroup analysis (subgroup=Glycerin in diet (g/kg)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	47
Figure 5. (B cont) Subgroup analysis (subgroup=Glycerin in diet (g/kg)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	48
Figure 6. Subgroup analysis (subgroup=Feeding system) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.....	49

CAPÍTULO 3

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.....	71
Figure 2. Forest plot showing the effect of the use of glycerin on the diet of dairy cows on milk fatty acid profile.....	72
Figure 3. Subgroup analysis (Subgroup=Genetic type) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	73

Figure 4. Subgroup analysis (Subgroup=Days in Milk) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	73
Figure 5. Subgroup analysis (Subgroup=Experiment period) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	75
Figure 6. Subgroup analysis (Subgroup=Glycerin in diet (g/kg)) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	75
Figure 7. Subgroup analysis (Subgroup=glycerin type) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	77
Figure 7. Subgroup analysis (Subgroup=Concentrate in diet (g/kg)) of the effect of the use of glycerin on the diet of dairy cows, WMD=weighted mean differences between crude glycerin and control treatments.....	77

CAPÍTULO 4

Figure 1 Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of molasses in beef cattle diet.....	93
Figure 2. Forest plot (with subgroup analysis (covariate=Amount molasses in diet (g/kg of DM) showing the effect of the use of molasses on the diet the beef cattle on carcass weight (kg).....	94
Figure 3. Subgroup analysis (subgroup=Genetic Type) of the effect of the use of molasses in diet of the beef cattle, WMD=weighted mean differences between treatment and control treatments.....	95
Figure 4. Forest plot (with subgroup analysis (Covariate=Experimental period)) showing the effect of the use of molasses on the diet the beef cattle, WMD=weighted mean differences between treatment and control treatments.....	95
Figure 5 Subgroup analysis (subgroup=Amount of molasses in diet (g/kg of DM)) of the effect of the use of molasses in diet of the beef cattle, WMD=weighted mean differences between treatment and control treatments.....	96

Figure 6 Subgroup analysis (subgroup=Molasses type) of the effect of the use of molasses in diet of the beef cattle, WMD=weighted mean differences between treatment and control treatments.....97

Figure 7 Subgroup analysis (subgroup=Amount of concentrate in diet (g/kg of DM)) of the effect of the use of molasses in diet of the beef cattle, WMD=weighted mean differences between treatment and control treatments.....97

CAPÍTULO 5

Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of molasses in dairy cow diet.....118

Figure 2. Forest plot showing the effect of the use of molasses on the diet of dairy cows on milk fatty acid profile (mg/g FA milk).....119

Figure 3. Forest plot (with Subgroup analysis (Covariate=Molasses in diet (g/kg)) showing the effect of the use of molasses on the diet of dairy cows on milk fat (g/kg).....120

Figure 4. Subgroup analysis (Subgroup=Group Genetic) of the effect of the use of molasses on the diet of dairy cows, WMD=weighted mean differences between molasses and control treatments.....121

Figure 5. Subgroup analysis (Subgroup=Days in Milk) of the effect of the use of molasses on the diet of dairy cows, WMD=weighted mean differences between molasses and control treatments.....122

Figure 6. Subgroup analysis (Subgroup=Concentrate in diet (g/kg)) of the effect of the use of molasses on the diet of dairy cows, WMD=weighted mean differences between molasses and control treatments.....123

Figure 7 Subgroup analysis (Subgroup=Molasses of type) of the effect of the use of molasses on the diet of dairy cows, WMD=weighted mean differences between molasses and control treatments.....123

Figure 8. Subgroup analysis (Subgroup=Molasses in diet (g/kg)) of the effect of the use of molasses on the diet of dairy cows, WMD=weighted mean differences between molasses and control treatments.....124

CAPÍTULO 1 – Considerações gerais

INTRODUÇÃO

A preocupação pela redução do impacto ambiental a partir da extração e o uso de combustíveis fósseis associados à crise energética mundial, tem estimulado o mercado mundial de biocombustíveis à buscar por fontes renováveis, que apresentem menor impacto ao ambiente (Rodrigues e Rondina, 2013; Mahbub et al., 2019). Como parâmetro de desenvolvimento no atual cenário político, a redução no uso de combustíveis fósseis criou favoráveis condições para a produção e comercialização de biocombustíveis, tais como o biodiesel e o etanol. Tendo em vista este cenário, em 2005, o governo federal brasileiro sancionou a lei federal nº 11.097 em 13 de janeiro de 2005, instituindo a obrigatoriedade do acréscimo de 2% de biodiesel no diesel comum a partir de 2008, o chamado B2 (ANP, 2019). A partir de 2019 a proporção de biodiesel adicionada ao óleo diesel passou a ser de 11%, conforme a Lei nº13.263/2016 (ANP, 2020). Já o etanol, pode ser utilizado de duas formas: como combustível veicular, sendo como etanol hidratado, utilizado diretamente no tanque do veículo em substituição à gasolina, e também como etanol anidro, que pode ser usado como aditivo na gasolina, substituindo o chumbo tetraetila por razões ambientais e melhorando o desempenho dos motores (Zanardi e Junior, 2015), sendo adicionado na proporção obrigatória de 25% (EPE, 2005).

A produção de biocombustíveis está associada à geração de coprodutos como a glicerina e o melaço. Segundo a ANP (2019), no ano de 2019 foram produzidos no Brasil 440,6 mil m³ de glicerina bruta, resíduo da produção de biodiesel. Na produção de etanol, quando o substrato é a cana-de-açúcar, o resíduo é o bagaço de cana, ou quando é utilizado o milho como substrato, temos os grãos de destilarias como resíduos. Contudo, pode haver a comercialização de produtos intermediários da produção do etanol, como o melaço, sendo utilizado para a produção de açúcar ou para a alimentação animal. Coprodutos (glicerina) ou intermediários (melaço) da produção de biocombustíveis podem ser utilizados na alimentação de animais ruminantes (Andriguetto et al., 2002).

A utilização de coprodutos na alimentação animal, além de reduzir o problema do descarte inadequado, também possibilita sua conversão em alimentos de origem animal, tais como carne ou leite, e com menor custo, devido à redução na utilização de grãos como o milho e a soja na dieta dos animais. Outro ponto a ser considerado quanto ao uso de coprodutos na dieta animal é a menor competição de grãos de interesse na nutrição humana com a nutrição animal.

A glicerina e o melaço têm sido utilizados na alimentação animal com sucesso e apresentando bons resultados. Contudo, existem questionamentos sobre o nível ideal de inclusão e a possibilidade de alteração das características físico-químicas e do perfil de ácidos graxos da carne e do leite e a relação com o desempenho produtivo, os quais apresentam resultados controversos. Em relação a glicerina, a inclusão tem mostrado aumentos na proporção de ácidos graxos insaturados (Carvalho et al., 2014; Buttrey et al., 2015), ou mesmo redução nos parâmetros da cor da carne (Benedeti et al., 2016). Variações nas respostas com a inclusão de glicerina na dieta também foram observadas para a composição do leite, como o aumento no conteúdo proteico (Bajramaj et al., 2016), e redução na concentração de ácidos graxos insaturados no leite (Ariko et al., 2015; Gaillard et al., 2018). Contudo, resultados controversos também foram relatados (Ezequiel et al., 2015; Bajramaj et al., 2016)

Em relação ao melaço, a inclusão em dietas para bovinos de corte também está associada a uma grande variação nas respostas de parâmetros de carcaça, como o peso e rendimento de carcaça (Tomkins et al., 2014; Asimwe et al., 2015). Quando avaliado o efeito da inclusão em dietas para vacas leiteiras, foram observados desde redução na concentração de ácidos graxos de configurações trans (Razzaghi et al., 2020), como aumentos na concentração de ácidos graxos da família ômega-3 (Brito et al., 2017). Todas essas variações observadas com a inclusão de glicerina ou melaço, podem ser devidos não somente ao nível de inclusão, também associados a fatores como: raça, idade do animal, sistema de alimentação e ao período de oferta da dieta experimental. Todas estas fontes de variação são difíceis de avaliar em um único experimento, sendo, portanto, necessário o uso da abordagem meta-analítica para se alcançar avaliações mais robustas.

De acordo com Volpato (2019), a ciência é um fenômeno natural dinâmico de caráter acumulativo de suas informações. Neste contexto, a utilização de ferramentas

estatísticas como a meta-análise pode ajudar a agregar as informações formando conclusões mais robustas, possibilitando o agrupamento de informações e avaliação de fenômenos, e detecção de respostas mesmo em conjunto de dados com elevada variação. Como foi abordado por Sauvant et al. (2008), a utilização da meta-análise apresenta maior poder de detecção do que um único experimento para respostas com elevada variação. Esta abordagem ainda possibilita maior capacidade de explorar fontes de variação (covariáveis) que influenciam sobre uma resposta (Glass, 1976; Higgins e Green, 2011). Contudo, estudos meta-analíticos que avaliem as respostas do uso de glicerina e de melaço na dieta de bovinos de corte e leite sobre os parâmetros de desempenho e físico-químicos da carcaça bem como o perfil de ácidos graxos do leite e da carne ainda são escassos, e respostas mais concretas precisam ser dadas para ratificar a utilização desses ingredientes na alimentação de ruminantes.

Portanto, objetivou-se, a partir do uso da abordagem meta-analítica, avaliar os fatores que afetam as respostas, bem como o impacto da utilização do melaço e glicerina na dieta de bovinos de corte e leite, sobre os parâmetros de desempenho e de carcaça, físico-químicos da carne bem como o perfil de ácidos graxos do leite e da carne.

REVISÃO DE LITERATURA

Diante da necessidade de melhorar a eficiência alimentar dos animais através do uso de ingredientes com altos teores energéticos, práticas alimentares devem ser adotadas para evitar que os animais corram risco de queda na produtividade devido aos distúrbios metabólicos em consequência do novo padrão de fermentação ruminal. Muitos resíduos agroindustriais podem ser incluídos nas dietas dos animais, proporcionando o mesmo desempenho dos ingredientes comumente utilizados. A glicerina bruta, por exemplo, além de ser fonte de energia prontamente disponível no rúmen, também possui baixo poder de acidificação ruminal (Omazic et al., 2015), além de reduzir o custo final da dieta devido ao baixo custo de mercado. Outro importante coproduto para utilização na alimentação animal é o melaço, que além de fornecer energia prontamente disponível, é uma ótima fonte de sais minerais e também pode melhorar a aceitabilidade de alimentos grosseiros (Andriguetto et al., 2002; McDonald

et al., 2007). Entretanto, existem questionamentos sobre o nível ideal de inclusão e como a inclusão destes coprodutos alteram os parâmetros de desempenho e as características físico-químicas da carne e do leite e do perfil de ácidos graxos destes produtos.

Neste sentido, para o presente estudo foi realizado o uso da abordagem meta-analítica, para investigar o impacto da inclusão da glicerina e do melaço na dieta de bovinos de corte e leite, e as respostas sobre os parâmetros físico-químicos e de desempenho, além do perfil de ácidos graxos da carne e do leite. Também foi avaliado o nível de inclusão adequado destes coprodutos nas dietas através da abordagem meta-analítica.

Utilização de glicerina na alimentação animal

A produção de biodiesel é acompanhada pela geração de resíduos que necessitam de adequado descarte, para que não se tornem fontes de contaminação ao ambiente. A glicerina bruta, coproduto da produção do biodiesel já foi estudada como ingrediente energético na alimentação de animais desde os anos 1950 (Johns, 1953). Portanto, somando-se o fato do seu custo relativamente baixo, a glicerina bruta tornou-se novamente o foco dos estudos como fonte energética alternativa em dietas para ruminantes (Donkin et al., 2008; Ezequiel et al., 2015; Almeida et al., 2017).

No Brasil, a utilização de glicerina bruta na nutrição animal foi aprovada pela Instrução Normativa N° 42 do Ministério da Agricultura, Pecuária e Abastecimento (MAPA), de 16 de dezembro de 2010. A glicerina pode ser classificada em três diferentes graus de pureza: 1) Baixa pureza – obtida após a separação do biodiesel, contém baixos teores de glicerol (400 a 700 g/kg de glicerina) e elevados níveis de catalisadores e álcool; 2) Média pureza – é a glicerina bruta após o tratamento ácido, seguido de remoção dos ácidos graxos e sabões, possui entre 750 a 900 g/kg de glicerina; 3) Alta pureza – após sofrer bi destilação a vácuo e tratamento com absorventes, contendo mais de 990 g/kg de glicerina.

A glicerina bruta apresenta características energéticas semelhantes ao grão de milho e a comumente utilizada na alimentação animal é a de média pureza, apresentando em média 810 g de glicerol, 45 g de cinzas, 0,3 g de metanol, 0,1 g de

proteína bruta, 3 g de extrato etéreo e 3.656 calorias de energia bruta por kg de produto (TECPAR, 2010; LANA, 2010).

Ao associarem as características energéticas e o seu elevado potencial de regeneração do glicogênio em ruminantes a glicerina teve a sua aplicação inicialmente na dieta de vacas de leite como alternativa para a prevenção de cetose (Fisher et al., 1973, Defrain et al., 2004). Ao longo dos estudos com a inclusão de glicerina na dieta de vacas de leite, também foram associados efeitos como a alteração na composição do leite, como o aumento na concentração de proteína (Bajramaj et al., 2016) e no perfil de ácidos graxos (Gaillard et al., 2018).

Quando avaliado a inclusão de glicerina na dieta de bovinos de corte, que é realizada como alternativa a grãos como o milho, é associado a possibilidade na redução na relação ômega-6/ômega-3 (Silva et al., 2017). Bem como o aumento nos valores de cor da carne, como o brilho (L^*) e vermelho (a^*), (Carvalho et al., 2014), sem afetar as respostas de desempenho e os parâmetros de carcaça (Benedeti et al., 2016; D'Aurea et al., 2017). Entretanto, ainda existem divergências nas respostas dos estudos quanto ao nível de inclusão, bem como o efeito de variáveis como o grupo genético, sistema de alimentação e período de consumo da glicerina pelos animais e mesmo o efeito do tipo de glicerina (bruta e refinada) sobre as repostas de desempenho, parâmetros de carcaça, carne e leite.

Utilização de melaço na alimentação animal

O melaço é o produto intermediário da fabricação e refinamento do açúcar e do bioetanol da cana, soja e da beterraba, sendo a última, utilizada nos Estados Unidos da América. Quando o melaço não é destinado à alimentação animal, pode ser utilizado para a produção de etanol.

Devido ao elevado conteúdo de carboidratos solúveis em água, uma das suas primeiras aplicações na ciência animal foi como aditivo em silagens, atuando como estimulador de fermentação (Kung jr et al., 2003). Também, utilizada de forma direta, pela inclusão na dieta e com a finalidade de melhorar a aceitabilidade de alimentos grosseiros e de baixa aceitabilidade (Andriguetto et al., 2002).

Contudo, a inclusão de melaço em alta concentração na dieta de animais ruminantes foi associada a ocorrência de poliencefalomalacia, conhecida como “Molasses Toxicity” (Rowe et al., 1979).

Entretanto, uma das principais respostas com a inclusão de melaço na dieta de animais ruminantes é o aumento na retenção de nitrogênio, que foi associado ao aumento na eficiência de síntese de proteína microbiana (Hatch e Beeson, 1972; Osuji e Khalili, 1994; Araba e Byers, 2002). Também associaram a inclusão de melaço na dieta, como alternativa à redução do amido na dieta de vacas de leite, devido a sua fermentação ruminal não aumentar o risco de acidose e ou induzir a depressão na gordura do leite (Martel et al., 2011), em resposta a melhor estabilidade do pH ruminal (Razzaghi et al., 2020).

Enquanto, a inclusão de melaço na dieta de vacas de leite está associada à estabilidade do pH ruminal e na concentração de gordura no leite, a sua inclusão na dieta de bovinos de corte apresenta grande variação nas respostas de desempenho e parâmetros de carcaça, como exemplo no peso de carcaça como observado por Araba e Byers (2002) e Asimwe et al. (2015). Assim como observado para a inclusão de glicerina na dieta, a inclusão de melaço na dieta de bovinos ainda apresenta dúvidas quanto ao nível de inclusão, bem como o efeito de variáveis como o grupo genético, sistema de alimentação e período de consumo do melaço pelos animais e mesmo o efeito do tipo de melaço sobre as repostas de desempenho, parâmetros de carcaça, carne e leite.

Uso da abordagem meta-analítica na nutrição de ruminantes

A meta-análise pode ser realizada para o desenvolvimento de modelos matemáticos, que segundo Tedeschi, (2006) são representações matemáticas de mecanismos que governam os fenômenos naturais, que não são totalmente reconhecidos, controlados e compreendidos. Sendo representações matemáticas que buscam a simulação de um fenômeno, com a finalidade de compreensão e/ou estudo dos fatores que os influenciam (ex. fatores químicos, fisiológicos e metabólicos), neste contexto são atribuídos como modelos mecanicistas (buscam a máxima interação matemática-biológica de um fenômeno). Modelos matemáticos também são utilizados

para a predição de um fenômeno, como ocorre nos modelos empíricos, rotineiramente utilizados nos sistemas de exigências nutricionais.

A abordagem meta-analítica, também pode ser utilizada para a avaliação do efeito de grupo ou do tratamento baseada na média, que teve seus primeiros relatos por Glass (1976), quando inicialmente foi utilizado nas ciências médicas e sociais, com o objetivo de reduzir o número de unidades experimentais e aumentar a precisão de posteriores experimentos, bem como avaliar o efeito de fatores (covariáveis) que poderiam interferir nas respostas. Todos estes métodos possibilitam maior poder de inferência de uma resposta, quando comparada a um único experimento, além de possibilitarem a avaliação de fatores que influenciam as respostas “covariáveis” (Sauvant et al., 2008).

A abordagem meta-analítica traz maior precisão nas tomadas de decisão na aplicação e condução de tratamentos, inclusão de ingredientes nas dietas, ou ferramenta de tomada de decisão para posteriores experimentos. Podendo ser utilizada como ferramenta na busca por aumento na precisão dos estudos científicos.

O uso da abordagem meta-analítica pelo método de avaliação do efeito de tratamento baseado na média, já é bem conhecido na medicina humana (Vesterinen et al., 2014), porém, o seu uso na ciência animal é recente. Esse método é baseado, na quantificação do efeito da intervenção “Efeito de Tratamento”, pela diferença entre as médias do grupo controle e o tratamento em questão (Glass, 1976; Borenstein et al., 2009). Para que as diferenças existentes entre a qualidade de condução dos estudos (precisão) presentes no banco de dados não gerem respostas com viés, são atribuídos diferentes pesos para cada estudo, assim refletindo a relativa contribuição individual do estudo no efeito total da estimativa (DerSimonian e Laird, 1986; Vesterinen et al., 2014), sendo referenciado como a precisão do estudo. A quantificação da precisão ou peso do estudo, pode ser realizado pelo método do inverso da variância (DerSimonian e Laird, 1986), sendo onde o erro do estudo (medida de dispersão da média) apresenta relação inversa à precisão ou peso do estudo.

Além da avaliação da precisão dos estudos, é necessário entender as características dos estudos presentes no banco de dados e assim escolher o melhor modelo de avaliação da origem da variação do efeito de tratamento. Quando se

assume que os diferentes estudos fornecem uma estimativa do mesmo efeito, que é assumido como sendo fixo entre todas as comparações, usamos os modelos de efeito fixo (Hedges e Vevea, 1998; Vesterinen et al., 2014). Dessa forma o efeito de tratamento observado varia devido apenas ao erro aleatório dentro dos estudos (Borenstein et al., 2009).

Quando é assumido que o efeito de tratamento varia devido a variação existente dentro de cada estudo, bem como a variação entre estudos, são utilizados os modelos de efeito aleatório (Hedges e Vevea, 1998; Borenstein et al., 2009; Vesterinen et al., 2014). Os modelos de efeito aleatório são os mais indicados para os dados de experimentos com animais, devido à variação no grupo genético, sistemas de alimentação, características dos alimentos, período e delineamento experimental. Essa mesma linha de raciocínio é utilizada nos métodos meta-analíticos que usam modelos matemáticos mistos, sendo observado melhor estratificação das origens de variação das respostas, como foi sugerido por St-Pierre, (2001), que o uso de modelos mistos possibilita a melhor extração quantitativa da relação entre os dados, quando comparado a simples métodos de regressão linear.

Além destes conceitos, uma essencial avaliação neste método de abordagem meta-analítica é a avaliação e quantificação da heterogeneidade. Devido a inclusão de diferentes estudos com diferentes respostas, que podem influenciar nas conclusões. Na tentativa de estabelecer se os estudos são consistentes, os testes de heterogeneidade procuram determinar se as diferenças existentes dos resultados dos estudos (heterogeneidade) são devido a variação dentro ou entre os estudos, devendo-se avaliar os efeitos das covariáveis ou se a variação dos resultados é apenas ao acaso (Higgins et al., 2003; Higgins e Thompson, 2009).

Os métodos de quantificação da heterogeneidade utilizados são os Cochran's Q (Cochran, 1954). Entretanto, esse teste é susceptível ao número de estudos incluídos no banco de dados (Higgins et al., 2003). Na busca por resolver esse problema foi desenvolvido um novo método de quantificação, denominado de estatística I^2 , que pode variar de 0 a 100%, que representa o percentual da variação total entre estudos, devido à heterogeneidade ou diferença entre as condições experimentais (Higgins et al., 2003; Borenstein et al., 2009), como vantagem pode ser utilizada em bancos de dados com elevada variação nas condições experimentais

(Higgins et al., 2003). Para norteio da avaliação do valor de I^2 , Higgins et al. (2003) sugeriram que valores de $I^2 \leq 25\%$, 25 a 50% e $\geq 50\%$, representam baixa, moderada e alta heterogeneidade, respectivamente. Em um contexto mais prático, valores de I^2 próximo de 0%, representa que toda variação é devido ao acaso. Enquanto, valores próximos a 100% toda variação reflete a real diferença entre o verdadeiro efeito de tratamento entre estudos (Vesterinen et al., 2014). Neste contexto, a variação do efeito de tratamento ou diferença média, está sendo influenciada pelas características dos estudos inseridos no banco de dados, como exemplo a diferença no grupo genético, sistema de alimentação, idade dos animais, período e delineamento experimental.

Contudo, a elevada heterogeneidade não deve ser apenas associada às diferenças entre características experimentais entre os estudos, outro fator importante é a presença de outliers. Publicações viesadas, podem ser originadas, segundo Borenstein et al. (2009), por erro de digitação dos dados, inadequada escolha do delineamento e métodos estatísticos para avaliação dos dados experimentais, bem como fraudes dos dados na busca por encontrar o valor de “p” significativo. Todos esses erros acabam por reduzir a precisão dos estudos, bem como perturbar as respostas da meta-análise, por proporcionar aumento desproporcional da heterogeneidade. Entretanto, existe formas de detecção de estudos viesado, para a sua retirada do banco de dados.

De acordo com Borenstein et al. (2009), os dois principais métodos de detecção são: o método gráfico do funnel plot (Light e Pillemer, 1984), que plota a relação entre efeito estimado (diferencia média bruta e ou ponderada) contra o efeito do tratamento no *funnel plot*. Havendo a detecção subjetiva da assimetria do *funnel plot*, ocorre a retirada do estudo que está ocasionando a deformação do gráfico. O segundo método é o teste de assimetria da regressão de Egger (Egger et al., 1997) que por meio de regressão linear mensura a assimetria do *funnel plot*, pela regressão entre a estimativa do tamanho de efeito do tratamento contra suas respectivas variâncias. Os dois métodos apresentam sucesso na detecção de possíveis outliers (Egger et al., 1997). Quando avaliou em uma meta-análise o uso de leveduras na dieta de cordeiros em crescimento, Sales (2011) utilizou os dois métodos citados acima, juntamente com um terceiro teste estatístico, o teste “*Begg’s rank correlation*”, para a detecção de viés

de publicação, concluiu que os três métodos foram aptos em detectar possíveis vies de publicação em bancos de dados com elevada variação.

Mesmo havendo a retirada de vies de publicação, pode ocorrer a permanência de alta heterogeneidade, demonstrando forte impacto das diferentes características entre os estudos inseridos no banco de dados. Esse comportamento pode ser comum na ciência animal, devido as diferenças no material genético animal dentro de uma mesma espécie, condições edafoclimáticas que influenciam o metabolismo animal, as características nutricionais dos alimentos utilizados em suas dietas, bem como o sistema de alimentação que podem ser a pasto, ração completa misturada e fornecida no cocho, e entre outras características.

Neste contexto, é necessário eleger as características que podem interferir nas respostas e utilizá-las como covariáveis. Para a avaliação da heterogeneidade das respostas são utilizados a análise de meta-regressão, que conforme descrito por Harbord e Higgins, (2008), utiliza modelos mistos para ajustar os dados da meta-análise, usando os resultados de efeito de tratamento como variável dependente. Ao gerar o cálculo do R^2 ajustado, que representa a proporção da variância entre estudo (heterogeneidade) explicada pelas covariáveis (Harbord e Higgins, 2008; Vietchbauer, 2010).

Quando se conhece qual covariável está influenciando sobre a heterogeneidade da estimativa do efeito de tratamento, pode ser realizado a análise de subgrupo. Essa análise compara a média absoluta entre o grupo controle e tratamento, dentro do conjunto de dados pertencente à covariável (Borenstein e Higgins, 2013). Quando observado que a resposta ao determinado tratamento sofre efeito do grupo genético, por exemplo, é realizada a análise de subgrupo, sendo avaliado o efeito do tratamento dentro de cada raça ou espécie separadamente. Estas duas abordagens possibilitam melhor avaliação das características entre os estudos adicionados no banco de dados, possibilitando conhecer o impacto das covariáveis sobre as estimativas de efeito de tratamento, bem como avaliá-las de forma isolada, tornando a tomada de decisão com maior precisão.

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CAPÍTULO 2 - The effect of dietary inclusion of crude glycerin on performance, ruminal fermentation, meat quality and fatty acid profile of beef cattle: Meta-analysis

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista **“Research in Veterinary Science”** exceto a posição das tabelas. Em tramitação.

The effect of dietary inclusion of crude glycerin on performance, ruminal fermentation, meat quality and fatty acid profile of beef cattle: Meta-analysis

Abstract

This meta-analysis was carried to evaluate the effect of the use of crude glycerin in diets for beef cattle on the physical and chemical characteristics, and fatty acid profile of meat through meta-analysis. Data from forty-four peer-reviewed publications with 161 treatments means were included in the data set. The effect glycerin in diet was evaluated using random-effect models to examine the weighted mean differences (WMD) between glycerin treatment (diets with crude glycerin) and control diet (without crude glycerin). Heterogeneity was explored by meta-regression and subgroup analysis using genetic type, treatment period, crude glycerin in the diet (g/kg DM), feed systems (pasture or total mixed ration), and concentrate in the diet (g/kg DM). The inclusion of crude glycerin inclusion had no effect on the average daily gain, but increased feed efficiency by 3.15% while reducing subcutaneous fat thickness by 3.13%. Inclusion of crude glycerin reduced meat cholesterol by 9.13%, and total saturated fatty acids by 1.05%, and increased total unsaturated (2.02%), monounsaturated (3.17%) fatty acids. However, it did not affect the concentrations of conjugated linoleic acid and omega-3. Crude glycerin inclusions of up to 200 g/kg in the diet, did not have a negative effect on performance, carcass and physical-chemical characteristics of meat, and supported an increase in total monounsaturated (1.73%), oleic acid (12.29 mg) and palmitoleic acid (1.24 mg), while reducing myristic acid (3.08 mg), stearic acid (12.00 mg) in meat.

Keywords: biofuels; MUFA; glycerol; PUFA; ruminants.

1 Introduction

The reduction in dependence on the use of fossil fuels has become a parameter of sustainable development in the current political scenario, a fact that has stimulated the use of alternative sources such as biodiesel. Biodiesel is considered one of the best alternatives to fossil fuels because it allows total substitution of oil without the need for major changes in machinery, added to the low impact on their performance (Johnson and Taconi, 2007). Further, biodiesel production results in the production of by-products such as crude glycerin, which due to the large surplus is being directed to animal nutrition.

The inclusion of crude glycerin has led to a reduction in the concentration of saturated fatty acids, as well as a reduction in the omega-6/omega-3 fatty acid ratio in meat (Silva et al., 2017). However, in other studies its use had no effect on the unsaturated fatty acid profile or carcass parameters (Carvalho et al., 2014; Buttrey et al., 2015; Benediti et al., 2016; D'Aurea et al., 2017). The variation in responses with inclusion of the crude glycerin in diet on lipid concentration, as well as the fatty acid profile, may be associated with factors such as genetic group, animal age or feeding period. According to Krueger et al. (2010), the feeding of glycerin over time may increase the population of glycerol-fermenting microorganisms, thus changing the rumen fatty acid accumulation rate, as well as their saturation activity (biohydrogenation). Another factor affecting these responses is the level of crude glycerin inclusion in the diet. Carvalho et al. (2014), by including 18% DM crude glycerin in lamb diets, observed a reduction in meat moisture as well as an increase in meat fat values and values of lightness (L^*), redness (a^*) and yellowness (b^*). In other studies, inclusion of up to 10% DM crude glycerin in cattle diet linearly reduced subcutaneous fat thickness (Buttrey et al., 2015).

However, the variation in responses with the inclusion of glycerin in the diet on the characteristics of carcass and meat composition may be affected by factors such as breed, inclusion level, treatment period and feeding system (pasture or TMR). For these reasons the use of meta-analytical approach becomes necessary to better evaluate the responses with high variation (Sauvant et al., 2008), besides allowing greater exploration of the "covariates" that are sources of variation in the responses (Glass, 1976; Higgins and Green, 2011).

In this context, our hypothesis is that factors such as breed, inclusion level, treatment period present effects on the physical and chemical characteristics and fatty acid profile of beef cattle meat. In this sense, the objective was to evaluate from a meta-analytical approach the effect of the diet inclusion of crude glycerin on the physical and chemical composition of meat, animal performance, and characteristics of ruminal fermentation and metabolism in beef cattle.

2. Material and Methods

2.1 Data set

A comprehensive literature search was conducted using three search engines: Web of Science (<https://login.webofknowledge.com>), PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>), and Science Direct (<http://www.sciencedirect.com/>). Around 1276 publications were retrieved using the following search terms: “glycerin and beef cattle” and “glycerol and ruminant”. Of the papers that were retrieved, only those that met the predetermined inclusion criteria were included in the meta-analysis. For inclusion into the meta-analysis, studies had to have the following (standardized criteria): 1) The control treatment without crude glycerin inclusion in diet; 2) Data were only from animals intended for meat production.

2.2 Data Extraction

Based on inclusion criteria, 44 studies peer-reviewed publications, with 49 studies with 161 means of treatment were rated by the first author, publication reference, country, genetic type, experimental period, experimental design, age (month), additive type, glycerin type, glycerin in diet (g/kg DM), feed systems (pasture; TMR= total mixed ration), and concentrate in the diet (g/kg DM). The number of replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables for control and crude glycerin treatments. The complete data set is available in an Excel file in Supplemental File S1 (<https://doi.org/.....>).

2.3 Statistical analysis

A meta-analysis was conducted using R Statistical Software (Metafor package, version 3.4.2; Viechtbauer, 2010). The Forest Graph (forest plot) was created using STATA software (Version 16.0; StataCorp LP, College Station, TX). The effects of crude glycerin inclusion in beef cattle diets were evaluated using random-effect models to examine the weighted mean difference (WMD) between control diet (without crude glycerin) and diet with crude glycerin inclusion (treatment). Treatments means were weighted by the inverse of the variance, according to the method proposed by Der-Simonian and Laird (1986) for random effects model.

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% indicate low heterogeneity, whereas values between 25 and 50% denote moderate heterogeneity and those above 50% denote 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 analysis was conducted to identify categorical covariate effects (described previously). Mixed model was applied to adjust the data to the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by.

$$\Theta_i = \beta + \beta_j x_{ij} + \dots \beta_p x_{ip} + \mu_i$$

Where Θ_i = the true effect treatment in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_j = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, t^2)$. Here t^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

Tests of null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010), was calculated by comparing the estimated between-study variance when covariates were included in the model (σ^2) with corresponding values when they were excluded (σ_o^2); adjusted R^2 (%) = $(\sigma_o^2 - \sigma^2) / \sigma_o^2 \sigma_o$.

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 residuals out of the range -2.5 to 2.5 (outliers). WMD was evaluated by subgroup analysis when the categorical covariates were significant at P -value ≤ 0.10 .

The covariates were divided as following: Genetic type (*Bos taurus*, *Bos indicus*, Crossbreed, and Holstein steers); treatment period (≤ 70 , 70-100, 100-130, 130-170, 170-200 and 200-280 days); feeding system (Total mixed ration (TMR) and Pasture); amount of

concentrate in diet (<400, 400-600, 600-800 and ≥ 900 g/kg of DM); amount of glycerin in the diet (≤ 100 , 100-200, 200-300 and 300-400 g/kg of DM).

3. Results

The data used to evaluate the effect of inclusion of crude glycerin in the diet for beef cattle on the physical and chemical characteristics of meat, consisted of 49 studies on 161 means of treatment. The predominant genetic group in the studies was Crossbreed (46.53% studies), following *Bos indicus* (Nelore=40.88% studies), *Bos taurus* (North Red+Simmental+Limousin=6.90%), Holstein steers (3.77%) and not informed, representing 1.92% of the studies. The feeding systems used in the studies were total mixed ration (TMR; 83.85%) and pasture plus supplementation (16.15%). Regarding the level of concentrate in the diet dry matter (only for TMR diets), ratios of ≤ 400 (5.19%), 400-600 (14.81%), 600-800 (38.52%) and >900 g/kg DM (17.77%) and not informed representing 23.70% of the studies. All studies used crude glycerin, and the inclusion levels used were ≤ 100 , 100-200, 200-300, 300-400 g/kg of DM and not informed, representing 57.76, 27.33, 11.80, 1.86, 1.24% of the studies, respectively.

Table 1 shows the chemical composition of glycerin used in the studies included in the database. Tables (2, 3, 4, 5 and 6) are the first approach to determine whether inclusion of glycerin in the diet globally affects each response parameter.

3.1 DM and nutrient intake and digestibility

Inclusion of crude glycerin in the diets led to increases in the intake of ether extract ($P = 0.004$), and increases in digestibility of dry matter ($P = 0.008$), ether extract ($P = 0.001$) and neutral detergent fiber (NDF; $P = 0.003$, Table 2). However, the inclusion of crude glycerin reduced dry matter intake (DMI; $P = 0.001$), neutral detergent fiber intake ($P = 0.003$).

3.2 Performance, carcass traits and physical and chemical characteristics and fatty acid profile of meat

The inclusion of crude glycerin in the diets increased the feed efficiency ($P = 0.001$), and reduced the subcutaneous fat thickness (SFT; $P = 0.004$; Table 3). Inclusion of crude glycerin in the diets increased the contents of moisture ($P = 0.007$), palmitoleic acid ($P = 0.002$), oleic acid ($P = 0.018$), total unsaturated fatty acid (UFA; $P = 0.003$), total monounsaturated fatty acid (MUFA; $P = 0.001$; Figure 1), and total omega-6 ($P = 0.001$) of meat. However, glycerin inclusion reduced meat colour parameters (Lightness (L^* , $P = 0.001$), Redness (a^* , $P = 0.002$),

Yellowness (b^* , $P = 0.001$), and ash concentrations ($P = 0.001$), cholesterol ($P = 0.007$), myristic acid ($P = 0.042$), stearic acid ($P = 0.001$), linoleic acid ($P = 0.044$), arachidonic acid ($P = 0.046$), and total saturated fatty acid (SFA; $P = 0.049$; Table 5).

3.3 Ruminal, blood parameters and nitrogen metabolism

Inclusion of crude glycerin increased the concentration of propionate ($P = 0.001$; Figure 2), butyrate ($P = 0.001$), and rumen microbial nitrogen (RNM; $P = 0.002$). However, it reduced pH values ($P = 0.004$), and concentrations of N-NH₃ ($P = 0.001$) and acetate ($P = 0.001$). It also reduced the populations of Entodinium ($P = 0.006$), Isotrich ($P = 0.019$). The N-Intake ($P = 0.001$), blood urea nitrogen (BUN; $P = 0.006$), and β -OH-butyrate ($P = 0.001$; Table 3) were also reduced with the inclusion of crude glycerin in the diets.

3.4 Analysis of Meta-Regression and Asymmetry of the funnel plot

As observed in Tables 2, 3, 4, 5 and 6, the presence of publication bias from the funnel plot asymmetry test was not evident ($P > 0.05$). Based on meta-regression analysis in Tables 7, 8 and 9 (Presented in supplementary data), among all covariates analyzed (genetic type, treatment period, feeding system, concentrate in the diet and crude glycerin in the diet), those that represented the factors that most influenced variability of performance responses and physical and chemical characteristics of meat were the genetic group and treatment period.

3.5 Subgroup Analysis

To evaluate the effect of covariates with $P \leq 0.10$ in the meta-regression analysis, subgroup analysis was performed. When the effect of the covariate genetic group was evaluated (Figure 2), the inclusion of crude glycerin in the diet for crossbreed animals reduced the DMI (WMD = -0.22 kg/d; $P = 0.003$), myristic acid (WMD = -3.98 mg/100g of meat; $P = 0.001$), and palmitic acid concentration in meat (WMD = -11.7 mg/100g of meat; $P = 0.004$).

Inclusion of crude glycerin in diet for *Bos indicus* animals increased palmitoleic acid (WMD = 1.94 mg/100 g of meat; $P = 0.009$), and total MUFA in meat (WMD = 1.49%; $P = 0.001$). Inclusion of crude glycerin in diet for the *Bos taurus* animals, increased the ADG of animals (WMD = 0.05 kg/d; $P = 0.051$).

For the covariate “treatment period” (Figure 3), the supply of crude glycerin of periods between 130-170 days, there was a reduction in the SFT (WMD = -0.66 mm; $P = 0.001$). Glycerin period supply of 100-130 days increased the blood glucose concentrations (WMD =

8.97 mg/dL; $P = 0.001$). For the period supply between 130-170 days and 200-280 days, glycerin promoted a reduction in myristic acid concentration (WMD = -5.20 mg/100 g of meat; $P = 0.001$ and WMD = -5.53 mg/100 g of meat; $P = 0.001$), respectively. The supply of crude glycerin for 200-280 days reduced the palmitic acid concentration in meat (WMD = -28.8 mg/100 g of meat; $P = 0.001$). Glycerin period supply of 70-100 days increased palmitoleic acid concentrations (WMD = 1.92 mg/100 g of meat; $P = 0.006$).

Periods supply of glycerin in diet of 70-100 and 200-280 days increased Total MUFA (WMD = 1.59%; $P = 0.002$ and WMD = 3.21%; $P = 0.001$, respectively). While period supply between 70-100 days reduced total PUFA in meat (WMD = -0.98%; $P = 0.001$). However, periods supply between 130-170 and 200-280 days increased total PUFA in meat (WMD = 0.82%; $P = 0.021$ and WMD = 2.10%; $P = 0.001$, respectively).

For the covariate “amount concentrate in the diet” (Figure 4), the inclusion of crude glycerin in diets with 600-800 g/kg concentrate increased ADG (WMD = 0.07 kg/d; $P = 0.003$), and when includes in diets with ≥ 900 g/kg concentrate reduced ADG of animals (WMD = -0.05 kg/d; $P = 0.001$) and SFT (WMD = -0.54 mm; $P = 0.001$). The inclusion of glycerin in diets with 400-600g/kg concentrate promoted a reduction in myristic acid (WMD = -6.20 mg/100 g of meat; $P = 0.001$), and palmitic acid in meat (WMD = -37.4 mg/100 g of meat; $P = 0.001$). However, diets containing between 600-800 g/kg concentrate increased palmitoleic acid concentration (WMD = 1.79 mg/100g of meat; $P = 0.023$). The inclusion of glycerin in diets with 400-600 and 600-800g/kg concentrate resulted in increased total MUFA in meat (WMD = 3.29%; $P = 0.001$ and WMD = 1.09%; $P = 0.041$, respectively).

For the covariate “amount glycerin in diet” (Figure 5), inclusion ≤ 100 g glycerin in diet/kg of DM, increased DM digestibility (WMD = 18.4 g/kg; $P = 0.008$), NDF digestibility (WMD = 17.4 g/kg of DM; $P = 0.001$), palmitoleic acid (WMD = 1.58 mg/100g of Meat; $P = 0.042$), total MUFA (WMD = 1.26%; $P = 0.051$). However, inclusion ≤ 100 g glycerin in diet/kg of DM, decreased steric acid (WMD = -18.9 mg/100g of meat; $P = 0.001$), linoleic acid in meat (WMD = -4.64 mg/100g of Meat; $P = 0.047$). While inclusion of glycerin between 100-200 g/kg of DM, increased palmitoleic acid (WMD = 1.24 mg/100g of meat; $P = 0.011$), oleic acid (WMD = 12.3 mg/100g of meat; $P = 0.051$), total MUFA in meat (WMD = 1.73%; $P = 0.036$). However, inclusion 100-200g glycerin in diet/kg of DM reduced DMI (WMD = -0.43 kg/d; $P = 0.001$), myristic acid (WMD = -3.08 mg/100g of meat; $P = 0.042$), stearic acid (WMD = -12.0 mg/100g of meat; $P = 0.001$), arachidonic acid (WMD = -0.80 mg/100g of meat; $P = 0.041$). The inclusion of glycerin between 200-300 g/kg of DM, reduced the DMI

(WMD = -0.80 kg/d; $P = 0.004$), SFT (WMD = -1.01 mm; $P = 0.001$), while increased NDF digestibility (WMD = 11.5 g/kg; $P = 0.031$). With respect to the feeding system (Figure 6), the inclusion of glycerin in TMR diets reduced the DMI (WMD = -0.20 kg/d; $P = 0.001$), while for pasture, there was no effect ($P = 0.160$).

4. Discussion

Control of food intake by animals is a multifactorial response mechanism that includes physical factors of the food as well as chemostatic factors of the animal metabolism. Following this premise, the 2.3% reduction in DMI (Table 2) with the inclusion of crude glycerin may be attributed to the increased digestibility of dietary fractions, which may have resulted in higher energy availability for the animal, in which satiety was due to chemostatic regulation. This fact can be confirmed by the 14.27% reduction in β -OH-Butyrate (Table 3) in the blood concentration of animals supplemented with crude glycerin. According to Corrêa et al. (2010), the increase of this metabolite in plasma is related to energy deficiency for the animal. The search to meet the energy demand via chemostatic control, when there is no rumen filling effect, is the main mechanism of intake control in ruminant animals (Forbes, 2007).

This fact is confirmed when the covariates (subgroup analysis) were analyzed, in which there was a reduction in DMI only for the TMR feeding system (Figure 6) in relation to pasture, since animals in feedlot receive diets with higher concentrate content and consequently higher total digestibility. In this sense, it also justified the reduction of NDF intake (2.77%), which is associated with the reduction in DMI, since there was an increase in NDF digestibility (1.43%) in this system. The increase in the intake of ether extract observed when glycerin was included in the diets can be associated with an effect of overestimation of the results of the chemical analysis of the material not consumed by animal.

The positive effect of the inclusion of crude glycerin on the higher digestibility of DM and nutrients (OM, EE, and NDF; Table 2). Thus, glycerin can be readily used, as according to Omazic et al. (2015), approximately 75% of the glycerol escaped rumen fermentation can be absorbed by the rumen wall and approximately 25% can be used by rumen microorganisms. In addition to the fact that glycerin does not contain NDF, resulting in a dilution effect on NDF intake and digestibility of diet. Added to the fact that the inclusion of glycerin did not promote changes in the ruminal pH of the animals, suggesting that ruminal functions occurred without major variations in the conditions of the ruminal environment (stability), associated with the maintenance of ruminal pH above 6.2 (Table 6). In a study by Krueger et al. (2010), stability

was verified in the growth of the population of *Megasphaera elsdenii* when crude glycerin was included in the diet, precisely because of ruminal pH stability, because they consume lactate, which is therefore favored by maintaining the population of fibrolytic microorganisms such as *Ruminococcus albus*, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* (El-Nor et al., 2010; Castagnino et al., 2015).

The increase in the digestibility of nutrients in diet with glycerin, can also explain the high heterogeneity observed for ADG (71.20%; Table 3), requiring the of meta-regression to identify potential sources of heterogeneity followed by subgroup analysis to examine their influences on the response (Higgins et al., 2003). However, the effect of the inclusion of glycerin in ADG was affected by the covariates genetic group and amount of concentrate in the diet. At concentrate inclusion greater than 900 g/kg DM, inclusion of glycerin showed a reduction in the ADG of the animals, which can be attributed to the occurrence of metabolic disorders, such as ruminal acidosis.

The negative effect with the inclusion of crude glycerol on subcutaneous fat in the present meta-analysis may be associated with a ruminal acetate concentration reduced by 5.34% (Table 6). As observed by Carvalho et al. (2014), in subcutaneous adipose tissue glucose contributed to less than 5% total acetyl, with ruminal acetate being the main precursor of fat. Supporting this explanation, the period of dietary supply of crude glycerin between 100 - 130 days increased the blood glucose concentration by 8.97 mg/dL, while the period of 130 - 170 days of consumption showed a reduction in subcutaneous fat thickness (-0.66 mm; Figure 3). Even with a reduction in subcutaneous fat deposition, no negative effect of the inclusion of crude glycerin on water holding capacity, cooking loss, thawing or shear force on meat was observed. The absence of effect may be associated with the mean value above 5.0 mm subcutaneous fat (7.98 mm; Table 3), which according to Baldassini et al. (2017) in Nellore cattle prevent fiber shortening by cold and excessive loss of water by dripping and other phases of processing.

However, crude glycerin reduced meat colour parameters, with reductions of 3.84, 5.15 and 11.56%, respectively for L*, a* and b* (Table 4). The reduction in the b* value in meat is associated with reduced carotenoid intake due to the replacement of corn with crude glycerin in diet formulation (Favaro et al., 2016). Moreover, the increased concentration of unsaturated fatty acids is also related to meat colour change due to susceptibility to lipid oxidation (Faustman and Suman, 2017). However, the inclusion of crude glycerin had no effect on malonaldehyde concentration, which is used as an indicator of lipid oxidation (Wood et al.,

2008; Faustman and Suman, 2017). The absence of the effect of the increase in the concentration of unsaturated fatty acids in the meat in response to the inclusion of glycerin on the oxidative stability of the meat, may be associated with the increase of monounsaturated fatty acids (i.e., oleic acid), then in polyunsaturated fatty acids as was observed in our work. As noted by (Wood et al., 2008) monounsaturated fatty acids are less prone to lipid oxidation than polyunsaturated. It should also be noted that changes in meat colour parameters were of low magnitude, and the highest accuracy of inference the meta-analytical approach should be considered in consideration of a single experiment (Sauvant et al., 2008).

However, it was not possible to detect the effect of covariates (i.e., breed, treatment period) on meat colour variation. Our hypothesis being partially confirmed that factors such as breed (Figure 2), inclusion level (Figure 5), treatment period (Figure 3) present effects on the physical and chemical characteristics and fatty acid profile of beef cattle meat. These results demonstrate that the change in fatty acid profile in meat is therefore a multifactorial response. However, the inclusion of crude glycerin demonstrated great potential for fatty acid profile alteration, with beneficial implications for human health. The reduction in myristic and palmitic acid was 4.29 and 3.62% (Table 5) respectively and according to Wood et al. (2003), the intake of those fatty acids is associated with the increase of low-density lipoproteins in the blood that is considered harmful to human health.

The reduction in saturated fatty acids, and the increase in monounsaturated and unsaturated fatty acids in meat may be associated with the reduction of the rumen biohydrogenation process, because according to Krueger et al. (2010), glycerol is associated with in vitro lipolysis reduction by 48 to 77%, thus being the first step for biohydrogenation. Besides that, the reduction in *Butyrivibrio fibrisolvens* population reported by some authors (El-Nor et al., 2010; Edwards et al., 2012) may justify such effect, as this is a key species for the biohydrogenation process (Stewart et al., 1997; Harfoot and Hazlewood, 1997). In this context, it is expected a greater escape of unsaturated fatty acids for post-ruminal absorption and incorporation into meat.

The effect of the genetic group on the fatty acid profile of meat may be associated with intrinsic factors of the breed as it occurs in zebu animals and their crossbred offspring with higher levels of unsaturated fatty acids in the muscle (Ducatti et al., 2009; Rotta et al., 2009). Thus, we observed for *Bos indicus* animals that received crude glycerin in the diet, presented higher concentration of monounsaturated fatty acids in meat in relation to the other genetic groups (Figure 2). Our results demonstrate that *Bos taurus* animals are less sensitive to the

effect of diet on the fatty acid profile of meat (Figure 2). When evaluating the genetic analysis, Teixeira et al. (2017) concluded that the expression of genes involved in lipid metabolism is less affected by diet in Angus animals when compared to Nellore animals.

The effect of dietary concentrate on the fatty acid profile can be attributed to its increase in diet with consequent alteration in the rumen and microbial profile, which resulted in reduced efficiency of the biohydrogenation process, resulting in saturation of the rumen with unsaturated fatty acids, providing greater passage for their absorption in the intestine and incorporation in meat.

Another possibility is that due to the glycogenic potency of glycerin, it caused greater stimulation of the expression of the SCD-1 gene that encodes the $\Delta 9$ enzyme desaturase (stearoyl-CoA desaturase) which is responsible for synthesizing monounsaturated fatty acids from saturated fatty acids such as stearic acid (Smith et al., 2009), as observed herein. The level of SCD-1 activity is increased by dietary glucose, fructose and insulin (Flowers and Ntambi, 2009).

The effect of the crude glycerin supply period on the fatty acid profile incorporated in the meat is affected by the type of fatty acid. When crude glycerin was supplied from 70 days, the total monounsaturated and palmitoleic acids increased, in the same period there was a reduction in total polyunsaturated fatty acids. However, supply periods over 130 days increased the total polyunsaturated fatty acids. The mechanism by which the supply period acts on the incorporation of different fatty acids in meat is not yet known. The hypothesis is that long-term supply of crude glycerin results in changes of the population and metabolism of the rumen microbiota, as well as expression of animal enzymes, which favors the incorporation of unsaturated fatty acids in meat. The observed that long-term supply of crude glycerin resulted in an increase in the population of *Megasphaera elsdenii* and *Selenomona ruminatum* (Krueger et al., 2010), well as increased activity of enzymes such as glycerol kinase in the liver (Ladeira et al., 2016) and SCD-1 in adipose tissue (Smith et al., 2009), supporting the above hypothesis.

Inclusions of glycerin in 100-200 g/kg in the diet, did not have a negative effect on performance and showed an increase in total MUFA (1.73%), oleic acid (12.29 mg/100g of meat) and palmitoleic acid (1.24 mg/100g of meat), however reduced myristic acid (3.08 mg/100g of meat), and stearic acid (12.00 mg/100g of meat; Figure 5) in meat. In addition to changes in fatty acid profile with the inclusion of crude glycerin, which may be beneficial to human health, there was also a reduction in meat cholesterol. Thus, its reduction in the human diet helps to reduce the risk of cardiovascular diseases (Wood et al., 2003; Wood et al., 2008).

Reduction in cholesterol level may be associated with reduced rumen acetate, once it is the main precursor for cholesterol biosynthesis in ruminants (Palmquist and Mattos, 2011).

5. Conclusion

The inclusion of crude glycerin in diet for beef cattle reduced meat cholesterol by 9.13%, and total saturated fatty acids by 1.05%, and increased total unsaturated (2.02%), monounsaturated (3.17%) fatty acids. The main covariates, period of supply, greater than 70 days of supply the glycerin in diet, reducing saturated fatty acids and increasing unsaturated, monounsaturated and polyunsaturated fatty acids in meat. The main covariates influencing the responses to the inclusion of crude glycerin were the genetic group and treatment period.

Crude glycerin inclusions of 100-200 g/kg in the diet, did not have a negative effect on performance, carcass characteristics and physical chemical parameters of meat, and showed an increase in total MUFA (1.73%), oleic acid (12.29 mg) and palmitoleic acid (1.24 mg), while reducing myristic acid (3.08 mg), stearic acid (12.00 mg/100g of meat) in meat.

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Table 1. Chemical composition of crude glycerin used in the studies.

Item ²	Mean	Median	Maximum	Minimum	SD	<i>n</i> ¹
DM, g/kg	882.7	885.0	950.0	768.0	56.5	22
CP, g/kg DM	6.86	8.00	17.0	0.00	5.09	13
Ash, g/kg DM	56.9	50.7	86.8	47.6	11.3	21
Salts, g/kg DM	68.8	60.0	84.7	55.0	14.6	5
Phosphorus, g/kg DM	1.06	0.50	2.39	0.30	1.15	3
Potassium, g/kg DM	3.20	1.00	4.07	7.90	0.70	3
Sodium, g/kg DM	19.5	21.2	31.0	5.00	9.24	8
Methanol, g/kg DM	1.92	0.40	15.2	0.03	3.55	19
Glycerol, g/kg DM	828.4	812.0	900.0	735.0	39.2	23
GE, MJ/ kg DM	14.5	14.2	15.3	14.2	0.62	3
EE, g/kg DM	10.3	10.8	18.1	0.20	6.42	15
Fatty acid, g/kg DM						
Palmitic, C16:0	199.5	122.4	369.0	107.1	146.9	3
Stearic, C18:0	134.7	81.0	257.7	65.3	106.8	3
Oleic, C18:1	407.7	357.9	516.2	349.0	94.1	3

¹Treatments mean from 23 studies (Table S1 Online Data Supplement).

²DM= dry matter in diet; CP = crude protein in diet; EE= ether extract in diet; GE= gross energy SD=standard deviation.

Table 2. Effect of inclusion of crude glycerin in beef cattle diet on intake and digestibility

Item ¹	Control ²	N ³	Crude glycerin		Heterogeneity ⁵		Funnel test ⁶
	mean (SD)		⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
Intake, kg/d							
Dry Matter	9.13 (1.63)	129	-0.21 (-0.32, -0.11)	0.001	0.001	90.55	0.290
DM-supplement	2.24 (1.02)	11	-0.02 (-0.09, 0.07)	0.728	1.000	0.00	0.964
DM-pasture	8.76 (1.86)	13	0.09 (-0.63, 0.80)	0.813	0.029	47.47	0.143
Crude protein	1.53 (0.48)	28	-0.01 (-0.03, 0.02)	0.422	0.160	21.07	0.166
Extract ether	0.41 (0.18)	10	0.02 (0.01, 0.28)	0.004	0.761	0.00	0.071
NDF	3.82 (1.41)	43	-0.11 (-0.18, -0.04)	0.003	0.006	38.88	0.919
Digestibility, g/kg of DM							
Dry matter	624.2 (74.5)	41	13.2 (3.48, 22.9)	0.008	0.001	84.29	0.151
Organic matter	659.7 (80.9)	39	10.8 (1.15, 20.5)	0.028	0.001	86.90	0.154
Crude protein	669.5 (74.9)	32	-3.79 (-11.9, 4.27)	0.357	0.001	50.68	0.145
Extract ether	777.9 (65.3)	20	38.5 (24.7, 52.3)	0.001	0.001	74.14	0.084
NDF	539.3 (132.1)	34	7.72 (2.58, 12.9)	0.003	0.744	0.00	0.089

¹DMsupplement= dry matter supplement; DMpasture= dry matter pasture; NDF= neutral detergent fiber.

²Control treatment (without glycerin in diet).

³N = number of comparisons of control and with treatments (with glycerin in diet) (complete data set is available in Supplementary File S1).

⁴WMD = weighted mean differences between control and with treatments.

⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test.

Table 3. Effect of inclusion of crude glycerin in beef cattle diet on performance, carcass and blood parameters

Item ¹	Control ² mean (SD)	N ³	Crude glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
Body Weight Final, kg	470.5 (90.5)	65	-2.47 (-5.25, 0.31)	0.081	0.832	0.00	0.188
ADG, kg/d	1.38 (0.29)	86	0.02 (-0.01, 0.04)	0.173	0.001	71.20	0.065
Feed Efficiency, kg/kg	0.16 (0.04)	75	0.01 (0.00, 0.00)	0.001	0.829	0.00	0.126
Carcass weight, kg	308.8 (44.7)	43	1.39 (-0.22, 4.08)	0.078	0.239	0.00	0.648
Dressing, %	58.1 (3.39)	41	0.07 (-0.12, 0.26)	0.465	0.496	0.00	0.165
SFT, mm	7.98 (4.28)	55	-0.25 (-0.42, -0.08)	0.004	0.002	39.35	0.868
Ribeye area, cm ²	74.1 (20.5)	52	-0.32 (-0.83, 0.20)	0.232	0.717	0.00	0.066
Blood Parameters							
BUN, mg/dL	15.7 (5.29)	8	-0.91 (-1.57, -0.25)	0.006	0.709	0.00	0.232
Glucose, mg/dL	69.6 (11.8)	23	0.27 (-1.15, 1.69)	0.708	0.009	45.69	0.183
β –OH-Butyrate, mg/dL	4.42 (1.2)	7	-0.63 (-1.02, -0.24)	0.001	0.052	51.78	0.264

¹ADG= average daily gain; BUN= blood urea nitrogen; SFT= subcutaneous fat thickness;

²Control treatment (without glycerin in diet).

³N=number of comparisons of control and with treatments (with glycerin in diet) (complete data set is available in Supplementary FileS1).

⁴WMD = weighted mean differences between control and with treatments.

⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test.

Table 4. Effect of inclusion of crude glycerin in the diet of beef cattle on the physicochemical characteristics of meat

Item ¹	Control ² mean (SD)	N ³	Crude glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
pH Final	5.65 (0.25)	21	0.001 (-0.01, 0.02)	0.917	0.955	0.00	0.136
Meat colour							
Lightness, L*	35.4 (3.01)	28	-1.36 (-1.89, -0.82)	0.001	0.984	0.00	0.837
Redness, a*	16.5 (4.04)	30	-0.85 (-1.39, -0.31)	0.002	0.004	45.23	0.943
Yellowness, b*	6.92 (3.63)	30	-0.79 (-1.28, -0.32)	0.001	0.004	46.00	0.736
Shear force, kgf/cm ²	4.19 (0.96)	27	0.05 (-0.13, 0.23)	0.604	0.802	0.00	0.319
WHC, g/kg	734.6 (30.1)	13	3.13 (-4.07, 10.32)	0.395	0.999	0.00	0.447
Thawing loss, g/kg	79.4 (47.1)	10	-6.75 (-16.1, 2.62)	0.158	0.319	13.42	0.633
Cooking loss, g/kg	281.4 (80.6)	29	-1.19 (-9.30, 6.90)	0.772	0.848	0.00	0.293
Malonaldehyde, mg/kg Meat	0.41 (0.09)	10	0.02 (-0.02, 0.06)	0.300	0.979	0.00	0.861
Chemical Composition, g/kg of DM							
Moisture	745.4 (25.4)	18	3.86 (1.01, 6.71)	0.007	0.001	96.25	0.184
Protein	216.5 (19.5)	19	-1.98 (-5.16, 1.20)	0.222	0.001	98.19	0.365
Fat	26.5 (11.5)	17	-0.85 (-4.46, 2.75)	0.642	0.001	94.25	0.066
Ash	10.3 (1.55)	16	-0.24 (-0.33, -0.13)	0.001	0.001	86.99	0.254
Cholesterol, mg/100g Meat	35.3 (3.8)	10	-3.22 (-5.42, -0.83)	0.007	0.001	75.39	0.287

¹WHC= water holding capacity²Control treatment (without glycerin in diet).³N = number of comparisons of control and with treatments (with glycerin in diet) (complete data set is available in Supplementary File S1). ⁴RMD = raw mean differences between control and with treatments. ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test.

Table 5. Effect of inclusion of crude glycerin in the diet of beef cattle on the fatty acid (mg/100g of meat) profile of meat

Item ¹	Control ² mean (SD)	N ³	Crude glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
Lauric C12:0	1.14 (0.57)	21	0.01 (-0.04, 0.06)	0.720	0.717	0.00	0.092
Myristic C14:0	43.8 (23.4)	31	-1.88 (-3.64, -0.07)	0.042	0.001	79.31	0.062
Palmitic C16:0	345.2 (174.9)	27	-12.5 (-26.4,1.42)	0.071	0.001	96.05	0.076
Palmitoleic C16:1 c-9	41.3 (22.0)	30	1.46 (0.50, 2.41)	0.002	0.006	43.55	0.102
Stearic C18:0	232.3 (108.6)	17	-14.4 (-19.4, -9.29)	0.011	0.029	43.50	0.136
Oleic C18:1 c-9	473.6 (184.3)	27	9.32 (1.54, 17.09)	0.018	0.001	74.36	0.723
Linoleic C18:2 c-9 c-12	63.9 (45.1)	28	-4.07 (-8.05, -0.09)	0.044	0.001	77.65	0.197
CLA C18:2 c-9 t-11	4.67 (3.31)	29	-0.002 (-0.01, 0.01)	0.827	0.466	0.00	0.171
α – Linolenic C18:3 ω 3	5.59 (4.07)	25	-0.06 (-0.24, 0.11)	0.483	0.001	72.47	0.066
Arachidonic C20:4	19.58 (10.3)	22	-0.44 (-0.87, -0.01)	0.046	0.544	0.00	0.507
UFA/SFA	1.09 (0.16)	25	0.02 (-0.001, 0.005)	0.216	0.028	38.26	0.474
Total SFA, %	47.3 (2.91)	21	-0.52 (-1.04, -0.002)	0.049	0.044	37.30	0.505
Total UFA, %	49.5 (6.22)	13	0.99 (0.32, 1.71)	0.003	0.505	0.00	0.188
Total MUFA, %	45.2 (3.59)	20	1.44 (0.57, 2.29)	0.001	0.001	75.50	0.457
Total PUFA, %	6.56 (2.45)	24	0.13 (-0.45, 0.72)	0.653	0.001	88.61	0.139
Total Omega-3, %	0.91 (0.19)	7	-0.001 (-0.05, 0.04)	0.977	0.671	0.00	0.327
Total Omega-6, %	3.99 (1.76)	9	0.77 (0.31, 1.23)	0.001	0.001	90.71	0.171

¹CLA= conjugated linoleic acid; SFA= saturated fatty acid; UFA= unsaturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid; ²Control treatment (without glycerin in diet). ³N = number of comparisons of control and with treatments (with glycerin in diet) (complete data set is available in Supplementary File S1). ⁴WMD = weighted mean differences between control and with treatments. ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test.

Table 6. Effect of inclusion of crude glycerin in beef cattle diet on ruminal parameters and nitrogen balance

Item ¹	Control ² mean (SD)	N ³	Crude glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
pH	6.45 (0.25)	41	-0.06 (-0.09, -0.02)	0.004	0.001	60.48	0.450
N-NH ₃ , mg/dL	17.6 (6.19)	34	-1.42 (-1.98, -0.86)	0.001	0.001	84.23	0.138
Acetate, mol/100mol	68.4 (9.17)	30	-3.66 (-4.45, -2.86)	0.001	0.002	47.52	0.761
Propionate, mol/100mol	19.4 (6.28)	32	0.98 (0.59, 1.36)	0.001	0.055	30.41	0.307
Butyrate, mol/100mol	11.8 (3.42)	33	2.08 (1.68, 2.46)	0.001	0.001	58.00	0.351
Total Protozoa, log 10 ⁴ /mL	20.9 (7.37)	6	0.25 (-1.18, 1.67)	0.734	0.966	0.00	0.822
Entodinium, log 10 ⁴ /mL	6.13 (1.26)	6	-0.10 (-0.21, 0.00)	0.006	0.331	13.05	0.853
Isotrich, log 10 ⁴ /mL	4.28 (1.82)	6	-0.22 (-0.36, -0.09)	0.002	0.586	0.00	0.754
RNM, g/d	104.6 (9.73)	6	11.1 (1.79, 20.4)	0.021	0.944	0.00	0.453
EPMS	116.8 (11.6)	10	6.19 (-1.67, 14.1)	0.122	0.912	0.00	0.510
Nitrogen Metabolism, g/d							
N-Intake	200.6 (42.3)	9	-24.1 (-38.7, -9.48)	0.001	0.056	47.24	0.295
N-Urine	73.7 (1.96)	5	2.20 (-4.33, 8.74)	0.508	0.988	0.00	0.582
N-Fecal	57.1 (21.5)	11	-1.09 (-5.62, 3.43)	0.635	0.999	0.00	0.421
N-Balance	45.8 (12.3)	5	-2.93 (-9.06, 3.19)	0.348	0.139	42.40	0.065

¹ RNM= rumen nitrogen microbial; EPMS= Rumen microbial protein synthesis efficiency. ² Control treatment (without glycerin in diet). ³ N = number of comparisons of control and with treatments (with glycerin in diet) (complete data set is available in Supplementary File S1). ⁴WMD = weighted mean differences between control and with treatments. ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test.

Table 7. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ³
	Intercept	Genetic type	PT (day)	Age (animal)	SF	CON	Glycerin in diet		
DMI, kg/d	-2.03 (0.248)	0.03 (0.051)	-0.03 (0.975)	-1.01 (0.018)	1.02 (0.057)	-0.46 (0.259)	0.95 (0.463)	54.71	129
DMI pasture, kg/d	-0.11 (0.884)	1.88 (0.124)	-2.32 (0.022)	--	--	--	0.02 (0.981)	5.79	23
NDFI, kg/d	0.06 (0.853)	0.79 (0.166)	-0.83 (0.191)	0.04 (0.881)	--	-0.43 (0.288)	0.12 (0.771)	43.52	43
DMd	-182 (0.401)	69.7 (0.391)	73.2 (0.332)	0.05 (0.993)	--	57.7 (0.572)	0.36 (0.991)	0.00	41
OMd	-80.4 (0.291)	136 (0.050)	1.16 (0.985)	-50.3 (0.233)	--	36.7 (0.242)	3.24 (0.881)	29.85	39
CPd	-263 (0.101)	54.4 (0.331)	138 (0.091)	-47.6 (0.439)	--	-53.2 (0.380)	-18.4 (0.011)	100	32
EEd	-7.22 (0.901)	15.7 (0.954)	42.2 (0.143)	-76.2 (0.672)	--	98.0 (0.011)	-1.50 (0.992)	38.47	20
ADG, kg/d	-0.71 (0.023)	0.74 (0.061)	0.30 (0.012)	-0.47 (0.013)	0.12 (0.265)	0.20 (0.071)	0.08 (0.263)	54.81	86
SFT, mm	-0.79 (0.808)	-0.19 (0.951)	2.03 (0.011)	0.05 (0.988)	-1.21 (0.605)	-2.74 (0.021)	0.02 (0.981)	100	55

¹PT= treatment period (day); SF= system feeding (pasture; total mixed ration (TMR); partial-TMR; CON= concentrate in diet.

² DMI= dry matter intake; NDFI= neutral fiber detergent intake; DMd= dry matter digestibility; CPd= crude protein digestibility; EEd= ethereal extract digestibility; Omd= organic matter digestibility; ADG= average daily gain; SFT= subcutaneous fat thickness.

Adjusted R²= proportion of the between-study variance (heterogeneity) explained by the covariate.

³N = number of comparisons between treatment (glycerin in diet) and control (complete data set is available in Supplementary file S1).

Table 8. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ³
	Intercept	Genetic type	PT (day)	Age (animal)	SF	CON	Glycerin in diet		
Cholesterol, mg/100 g meat	-6.10 (0.031)	--	--	--	--	--	3.13 (0.381)	0.00	10
Lightness, a*	-3.92 (0.281)	4.62 (0.271)	-0.84 (0.653)	--	--	0.42 (0.784)	-0.09 (0.941)	0.00	30
Yellowness, b*	-4.36 (0.58)	4.25 (0.578)	0.13 (0.975)	--	--	2.00 (0.709)	0.10 (0.989)	0.00	30
Moisture, g/kg	34.8 (0.092)	-43.8 (0.012)	14.4 (0.391)	--	--	12.9 (0.432)	-5.40 (0.779)	0.00	18
Protein, g/kg	-4.28 (0.791)	10.8 (0.122)	7.00 (0.073)	--	--	--	-2.55 (0.832)	90.78	19
Fat, g/kg	-47.7 (0.031)	58.6 (0.012)	--	--	--	-19.5 (0.152)	8.61 (0.472)	0.00	17
Ash, g/kg	0.83 (0.455)	0.18 (0.478)	0.01 (0.998)	--	--	--	-1.11 (0.280)	0.00	16
Myristic, mg/100g of meat	9.54 (0.671)	-27.4 (0.083)	-34.2 (0.013)	--	--	-34.5 (0.013)	0.80 (0.939)	91.01	31
Palmitic, mg/100g of meat	-11.9 (0.931)	-149 (0.011)	-140 (0.012)	--	--	-184 (0.013)	0.24 (0.991)	45.83	27
Palmitoleic, mg/100g of meat	12.4 (0.535)	28.8 (0.022)	-30.0 (0.012)	--	--	-31.0 (0.013)	-0.77 (0.902)	51.91	30
Stearic, mg/100g of meat	-128 (0.123)	39.3 (0.651)	--	--	--	--	-4.00 (0.395)	90.69	17

¹; PE= experimental period (day); SF= system feeding (pasture; total mixed ration (TMR); partial-TMR; CON= concentrate in diet.

Adjusted R³ = proportion of the between-study variance (heterogeneity) explained by the covariate.

³N = number of comparisons between treatment (glycerin in diet) and control (complete data set is available in Supplementary file S1).

Table 9. Meta-regression of the effect of inclusion the crude glycerin in diets for beef cattle on weighted mean differences (WMD), between treatment and control.

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ³
	Intercept	Genetic type	PT (day)	Age (ani- mal)	SF	CON	Glycerin in diet		
Linoleic, mg/100g of meat	-39.2 (0.501)	0.70 (0.981)	-13.1 (0.602)	--	--	22.2 (0.193)	1.93 (0.961)	67.86	28
Total SFA, %	18.1 (0.832)	-22.7 (0.761)	-10.5 (0.781)	--	--	28.4 (0.101)	-2.79 (0.962)	0.00	21
Total MUFA,%	27.1 (0.593)	-25.1 (0.094)	-22.2 (0.032)	--	--	-33.7 (0.022)	2.84 (0.955)	84.48	20
Total PUFA,%	34.6 (0.690)	-13.4 (0.730)	-13.4 (0.012)	--	--	-5.96 (0.462)	-1.73 (0.978)	95.77	24
pH rumen	0.06 (0.901)	0.05 (0.792)	-0.03 (0.823)	--	--	-0.47 (0.124)	-0.01 (0.986)	86.43	41
N-NH ₃ , mg/dL	3.23 (0.712)	-0.99 (0.893)	1.87 (0.054)	-4.96 (0.492)	--	-1.43 (0.851)	1.05 (0.641)	100	34
Acetate, mol/100mol	-6.10 (0.126)	-5.91 (0.015)	8.70 (0.014)	-7.24 (0.013)	--	-3.08 (0.022)	1.75 (0.041)	100	30
Propionate, mol/100mol	1.18 (0.841)	6.12 (0.032)	-8.34 (0.253)	5.02 (0.124)	--	0.11 (0.524)	-1.09 (0.068)	100	32
Butyrate, mol/100mol	0.48 (0.933)	8.27 (0.012)	1.64 (0.882)	1.39 (0.469)	--	0.39 (0.961)	-1.38 (0.017)	100	33
N-intake, g/d	-51.1 (0.012)	37.1 (0.050)	--	--	--	--	25.4 (0.182)	38.02	9
Glucose, mg/dL	10.3 (0.091)	6.56 (0.176)	-9.95 (0.126)	--	--	--	3.80 (0.386)	100	23

¹PT= treatment period (day); SF= system feeding (pasture; total mixed ration (TMR); partial-TMR; CON= concentrate in diet. ² SFA= saturated fatty acid; UFA= unsaturated fatty acid; MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid. Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariate. ³N = number of comparisons between treatment (glycerin in diet) and control (complete data set is available in Supplementary file S1).

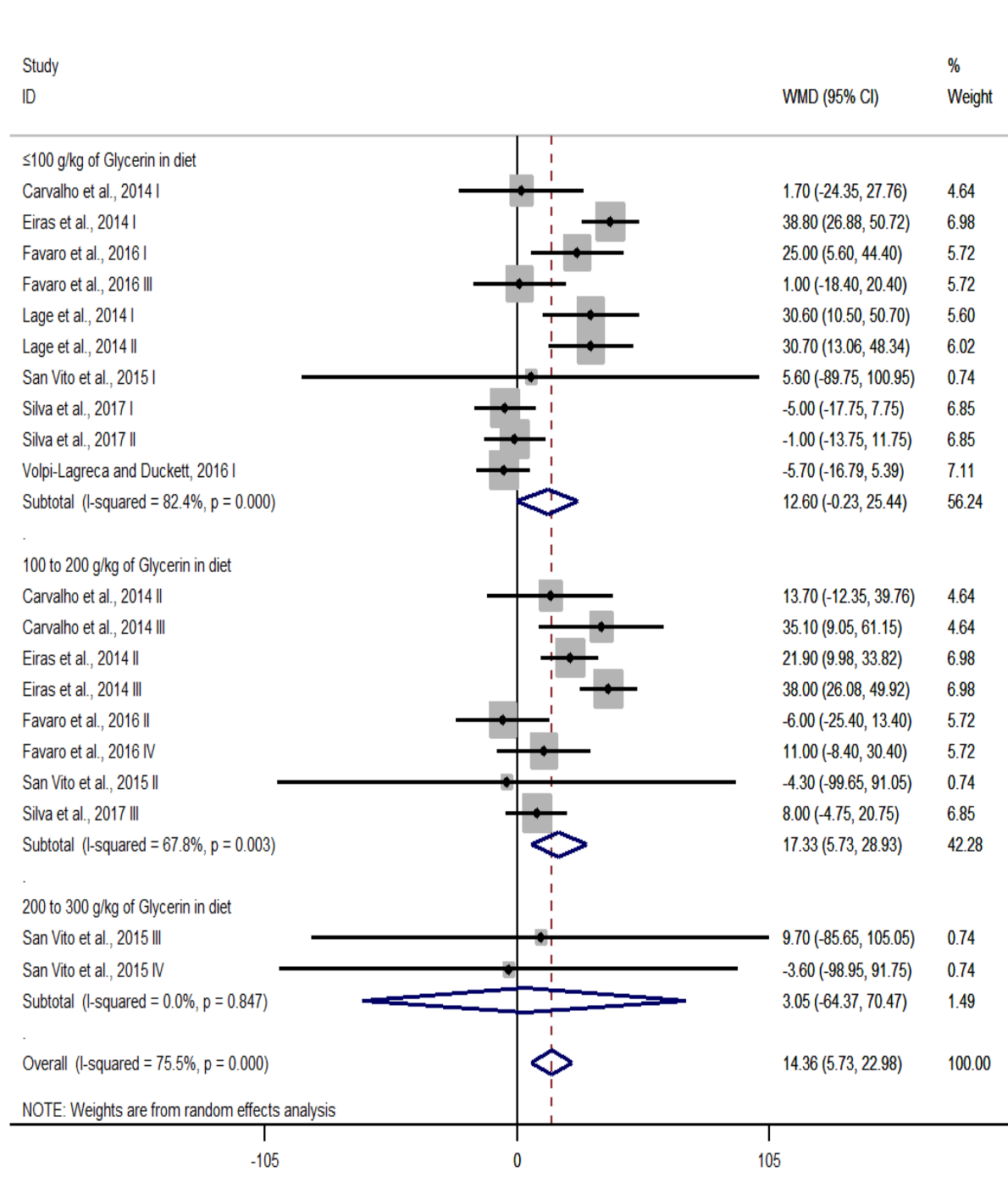


Figure 1. Forest plot (with subgroup analysis “Covariate= Glycerin in diet (g/kg)”), showing the effect of the use of crude glycerin on the diet the beef cattle on monounsaturated fatty acid total (g/kg). The x-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent 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 treatment 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.

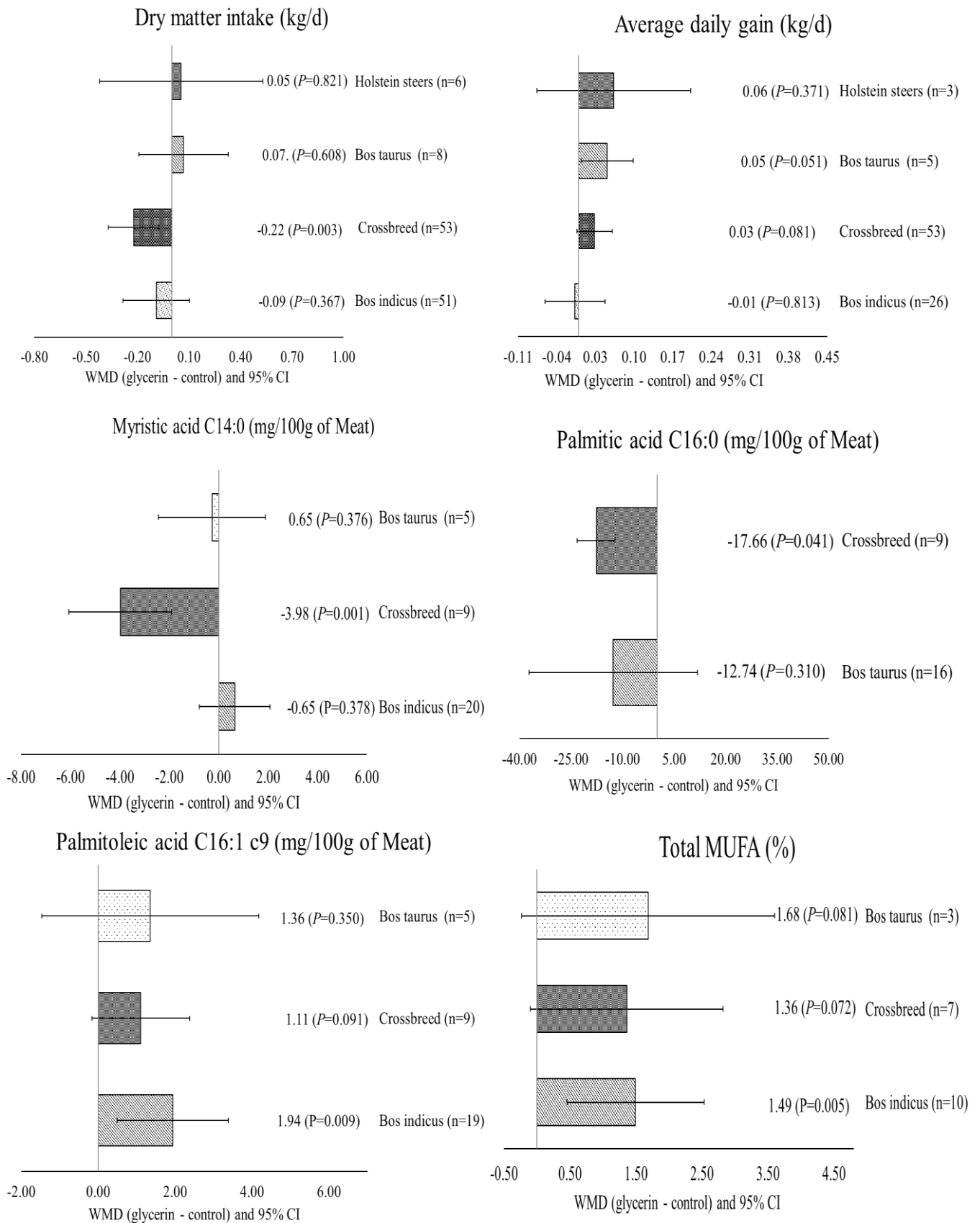
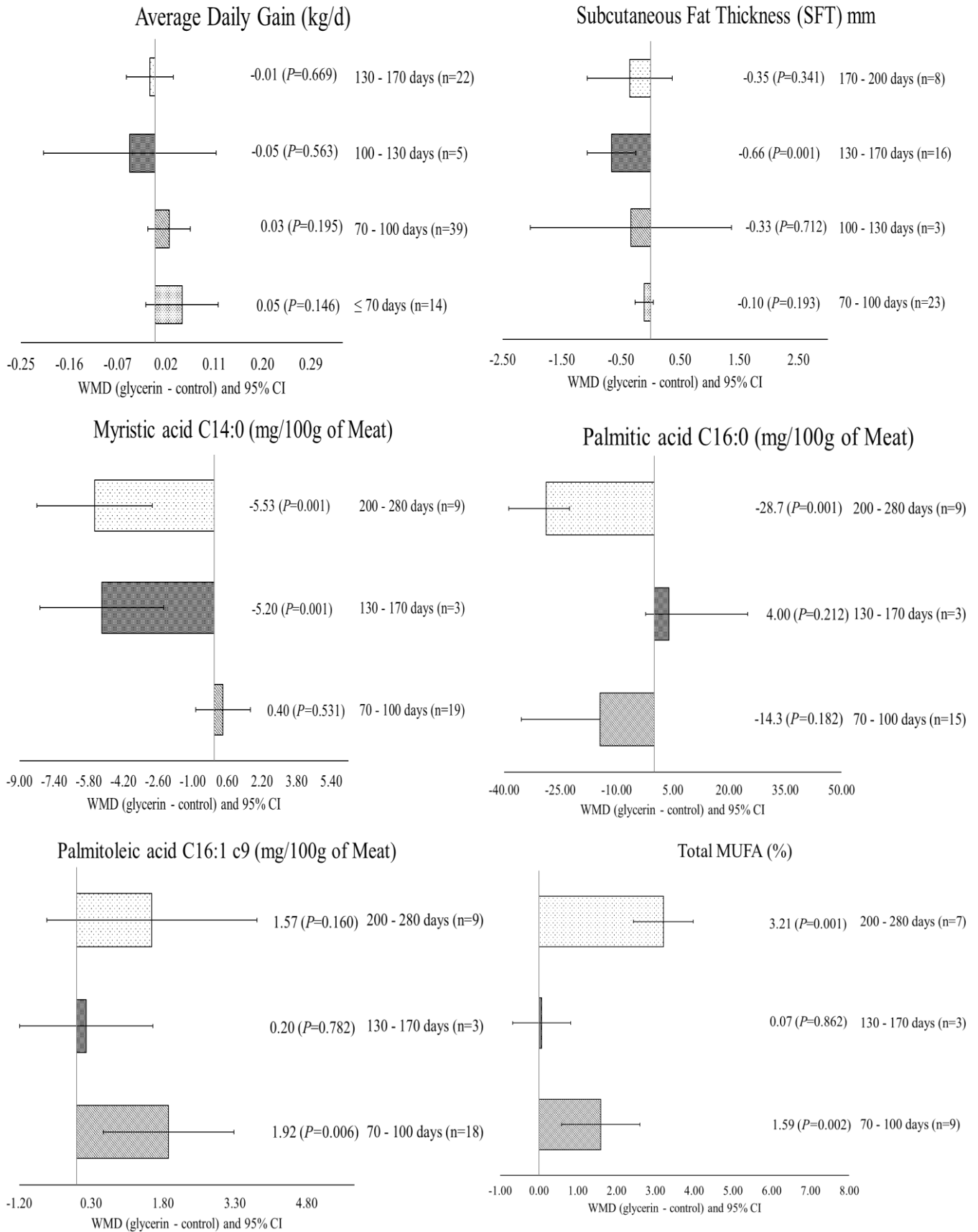


Figure 2. Subgroup analysis (subgroup=Genetic Type) of the effect of the use of glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.



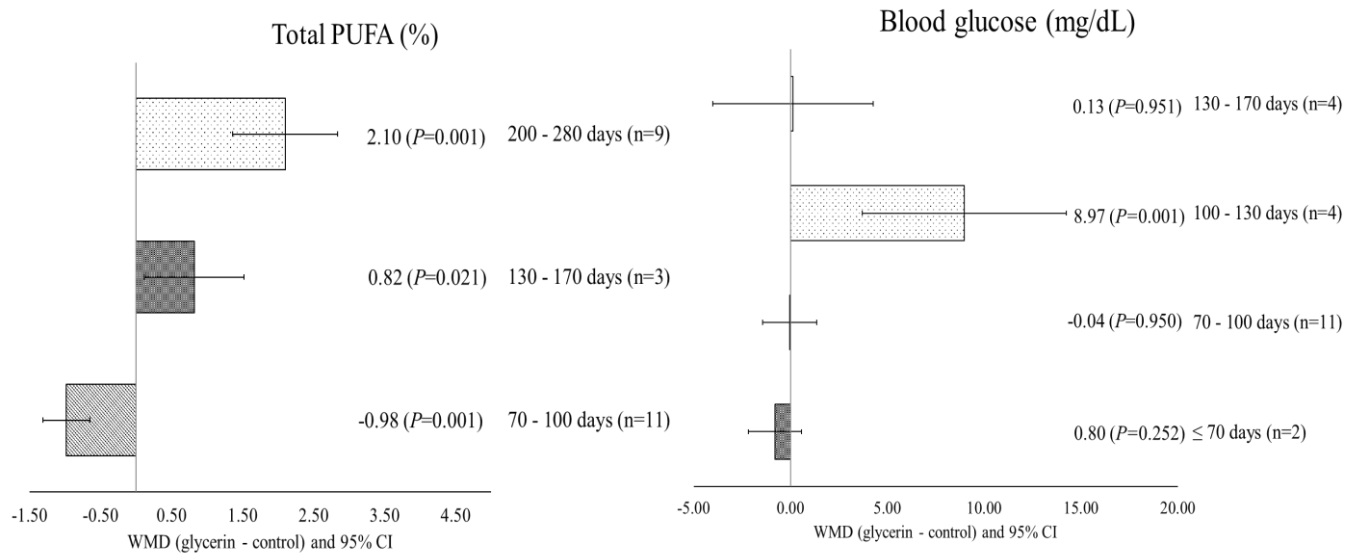
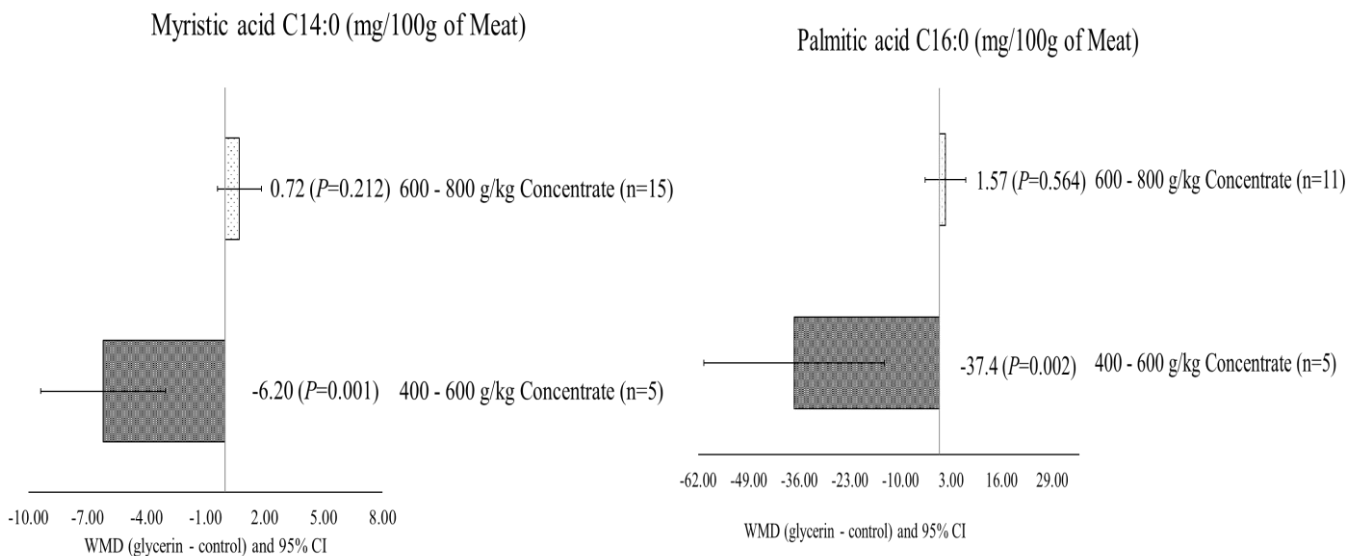
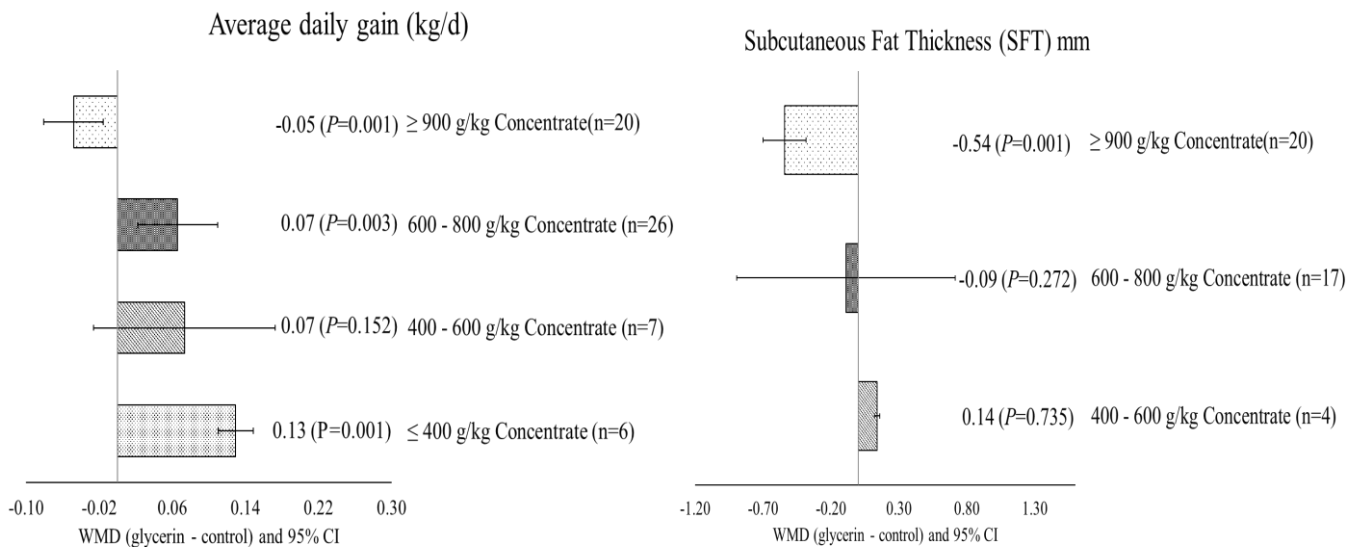


Figure 3. Subgroup analysis (subgroup= Treatment period (day)) of the effect of the use of glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.



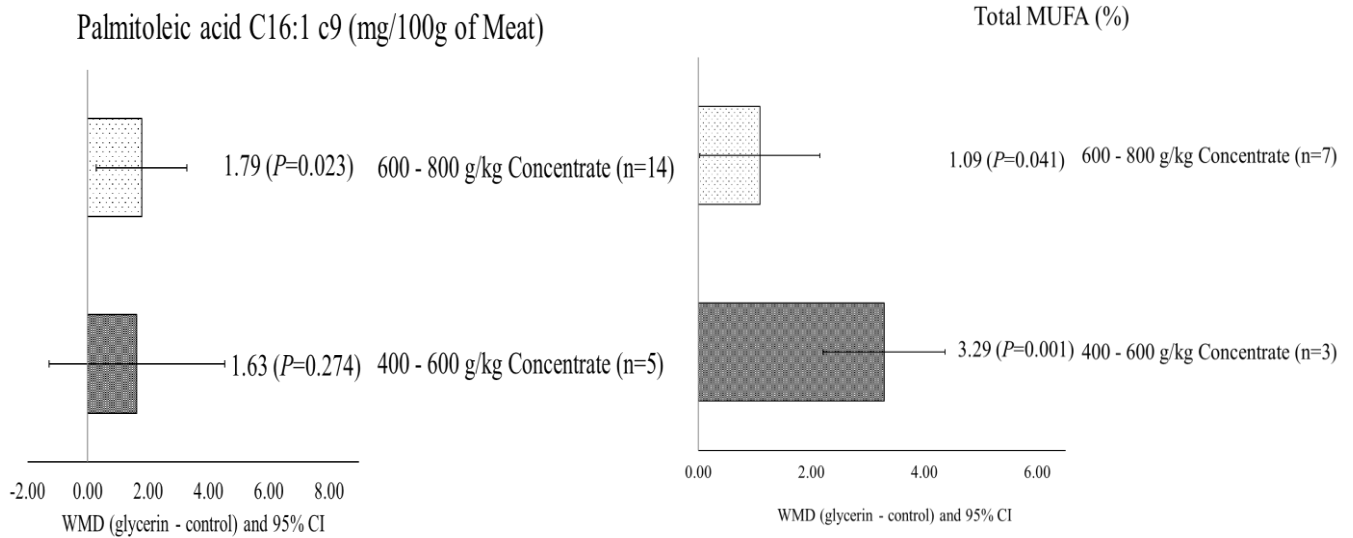
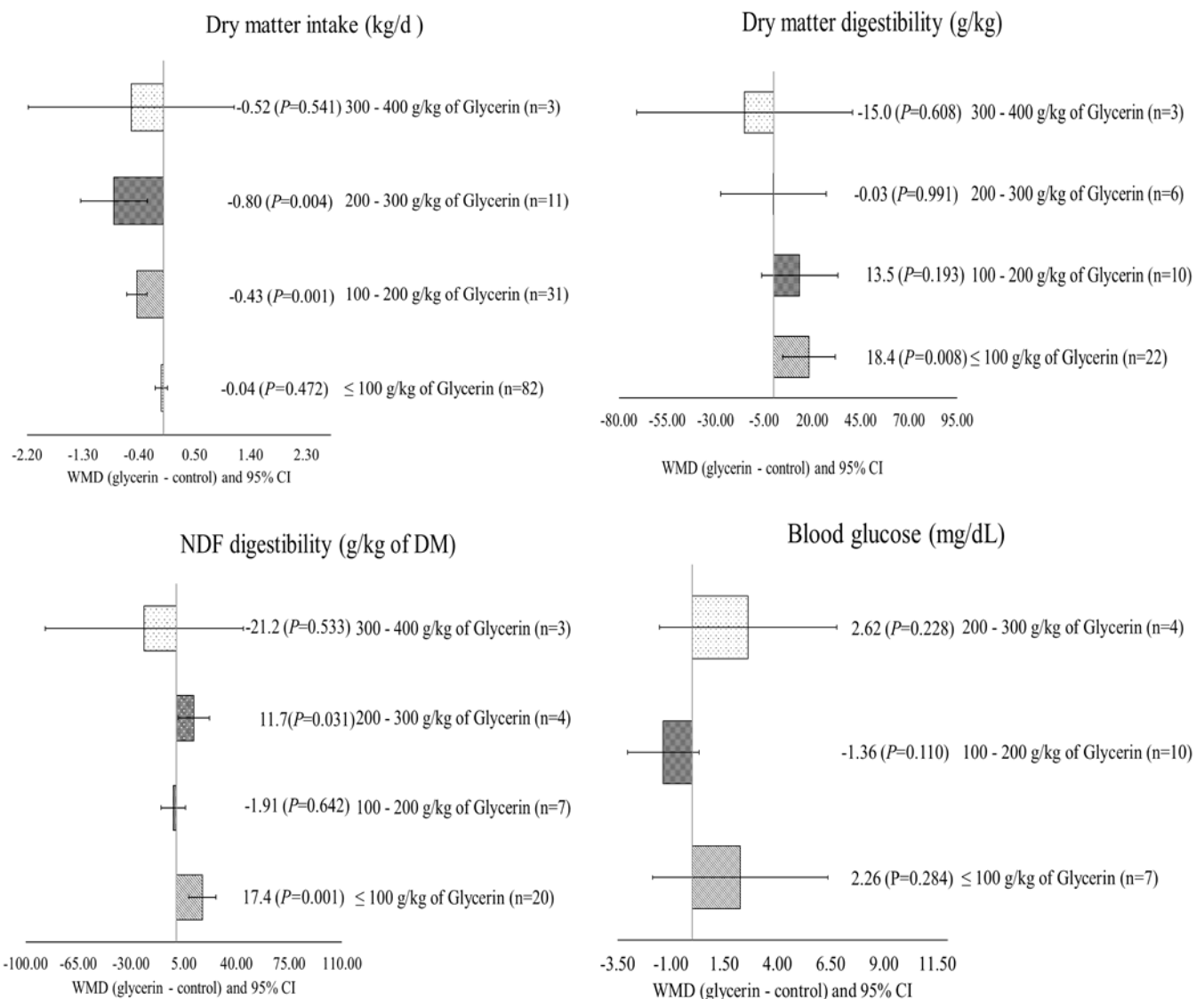


Figure 4. Subgroup analysis (subgroup=Concentrate in diet (g/kg of DM)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.



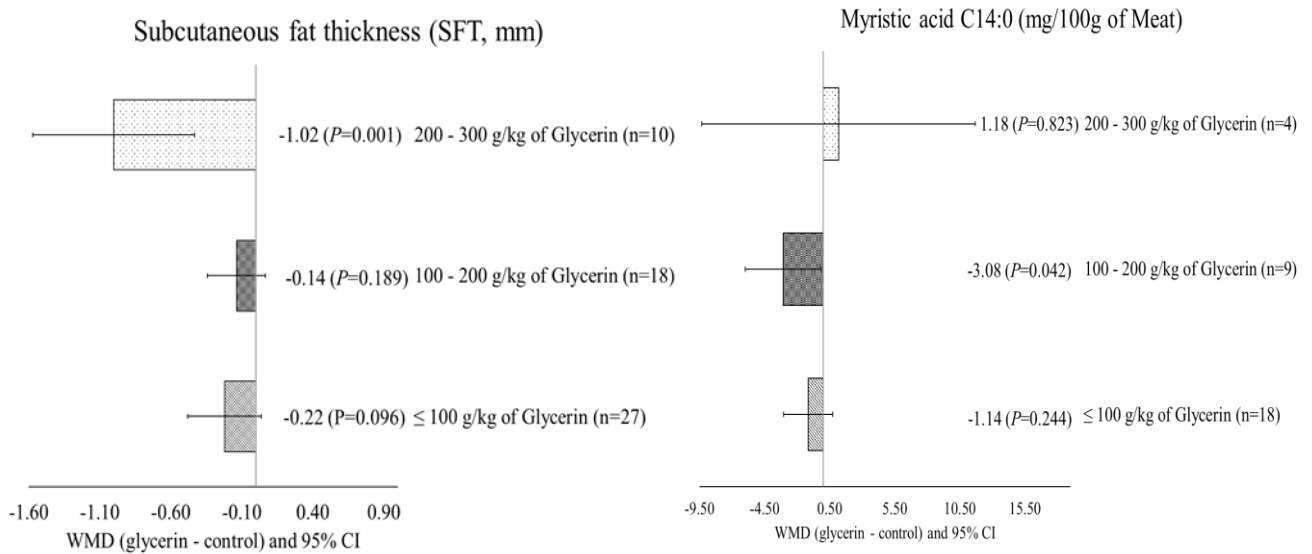
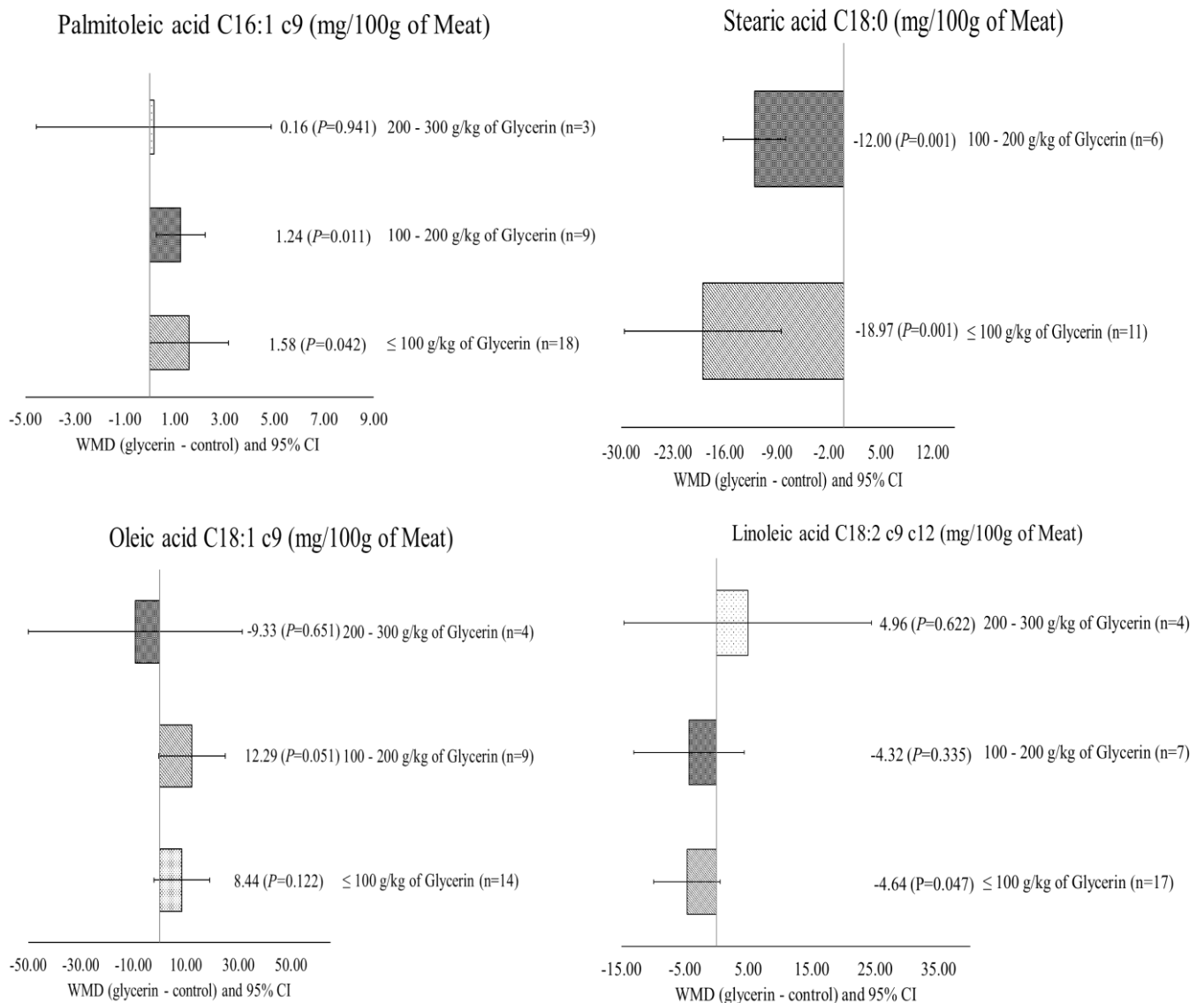


Figure 5. (A) Subgroup analysis (subgroup=Glycerin in diet(g/kg)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.



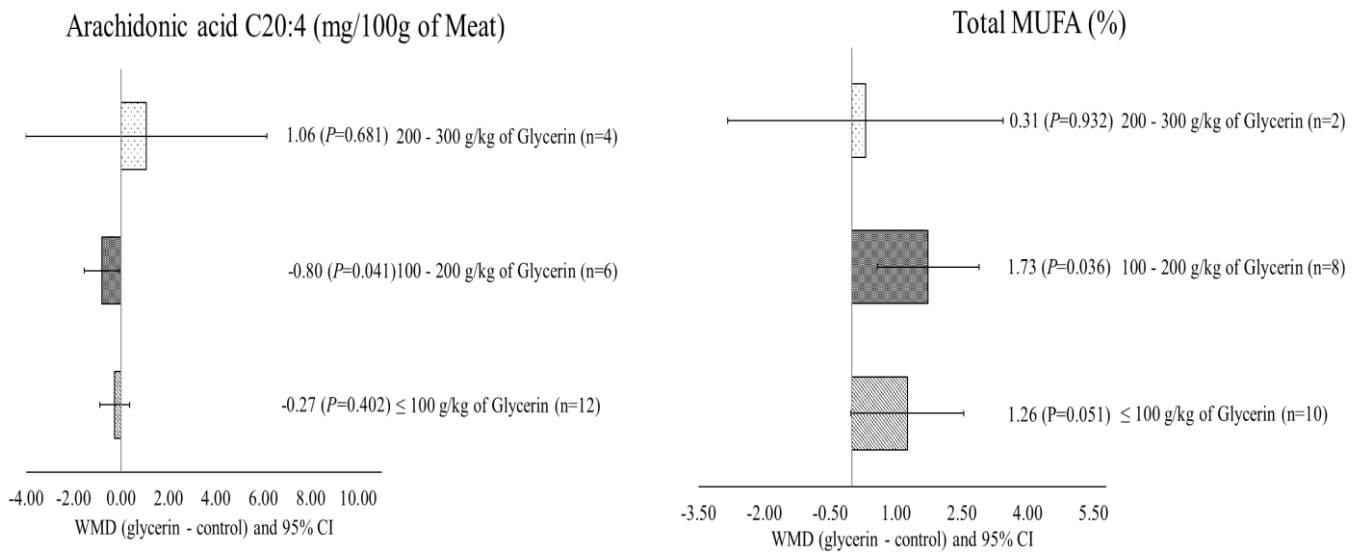


Figure 5. (B. Cont.) Subgroup analysis (subgroup=Glycerin in diet(g/kg)) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

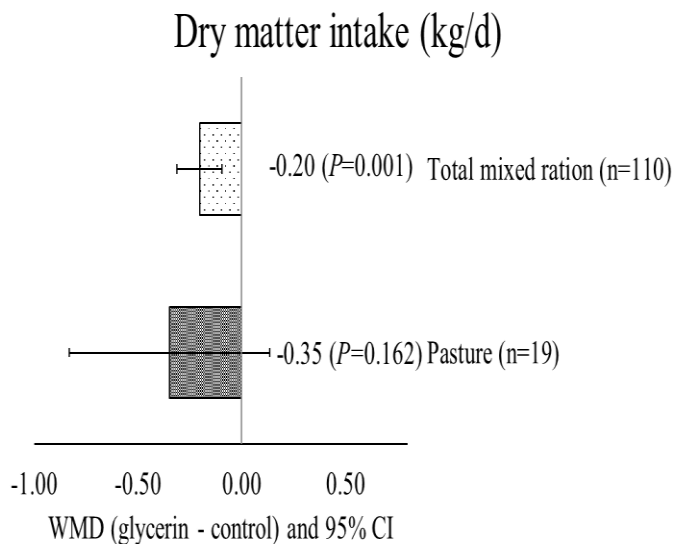


Figure 6. Subgroup analysis (subgroup= Feeding system) of the effect of the use of crude glycerin in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

CAPÍTULO 3 - Meta-analysis of the effect of glycerin inclusion in dairy cattle diet on milk fatty acid profile

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista **“Translational Animal Science”** exceto a posição das tabelas. Publicado em janeiro de 2021 sob identificação DOI: doi.org/10.1093/tas/txab012

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

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; $P<.0001$) cows produced less fat in milk compared to Jersey cows, when glycerin was included in the diets. Glycerine 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

1. Introduction

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

2. Materials and Methods

2.1 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” and “dairy cows”, and “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.

2.2 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.

2.3 Statistical analysis

2.3.1. 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 Der-Simonian 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).

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 to 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 $P \leq 0.05$.

2.3.2. 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

$$\Theta_i = \beta + \beta_1 x_{ij} + \dots \beta_p x_{ip} + \mu_i$$

Where Θ_i = the true effect treatment in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_j = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, \tau^2)$. Here τ^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

To measure of between-study variance ($\text{Tau-squared} = T^2$). 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) $P\text{-value} \leq 0.05$, for the heterogeneity test; 2) $P\text{-value} \geq 0.05$, for the funnel plot; 3) no observations with values for studentized residual out of the variation -2.5 to 2.5 (outliers); 4) Performed on all variables "Fatty acid in milk" in Table 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 the tables 1, 2 and 3.

The covariates were divided as following: genetic type (Jersey, Swedish, Holstein x Gyr and Holstein); days in milk (≤ 60 , 60 to 90, 90 to 120, 120 to 150, 150 to 180 and 180 to

210 days); Experimental period (≤ 60 , 60 to 90, 90 to 120, 120 to 150, 150 to 180 and 180 to 210 days). The level of concentrate in diet (300 to 500 and 500 to 700 g/kg DM). The type of glycerin (crude glycerin and refined glycerin). The levels of glycerin in the diet (≤ 50 , 50 to 100, 100 to 150, 150 to 200 and 200 to 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 by Roma-Garcia et al. (2016).

3. Results

Based on the inclusion criteria, 22 peer-reviewed publication 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 x Gyr (6.08%), Jersey (4.54%) and Swedish Red (4.54%). The lactation period in the studies were ≤ 60 , 60 to 90, 90 to 120, 120 to 150, 150 to 180, 180 to 210 days in milk and not presented, representing 22.7, 15.2, 24.2, 13.6, 9.1, 4.5, 10.7 % studies, respectively. The experimental period in the studies were ≤ 60 (25.8%), 60 to 90 (9.1%), 90 to 120 (24.2%), 120 to 150 (13.6 %), 150 to 180 (9.1%), 180 to 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 to 500, 500 to 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 to 100, 100 to 150, 150 to 200 and 200 to 300 g/kg DM representing 26.7, 20.0, 24.4, 13.3 and 15.6 % of the studies, respectively.

3.1. 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 ($P < .0001$) and NDF digestibility ($P < .0001$). The glycerin inclusion increased the digestibility of dry matter ($P < .0001$), organic matter ($P = 0.03$), crude protein ($P = 0.01$), ether extract ($P < .0001$).

3.2. 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 ($P < .0001$; Table 1), protein concentration ($P = 0.04$) and total saturated fatty acids in milk ($P = 0.03$).

With the inclusion of glycerin in the diet, there was a reduction in fat production and concentration ($P < .0001$ 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; $P < .0001$), total unsaturated ($P < .0001$), monounsaturated ($P < .0001$) and polyunsaturated fatty acids ($P < .0001$), the omega-3 ($P < .0001$) and omega-6 families ($P < .0001$; Table 3).

3.3. Rumen and blood parameters

The glycerin inclusion in the diet reduced the concentrations of ammonia ($P = 0.03$), rumen acetate ($P < .0001$), 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 ($P < .0001$), glucose ($P = 0.01$) and β -OH-Butyrate (BHB; $P < .0001$) increased. Glycerin inclusion did not affect rumen pH ($P = 0.33$).

3.4. 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, 2 and 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 in the digestibility of dry matter, ammonia, acetate, ruminal propionate and butyrate, as well as BUN, milk fat yield and Total SFA values.

3.5. 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 x Gyr cows reduced the milk fat yield (WMD= -0.04 kg/d; $P=0.01$; and WMD= -0.10 kg/d; $P<.0001$, 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 to 120 (WMD= 11.0 g/kg; $P=0.04$), 150 to 180 (WMD=30.3 g/kg; $P<.0001$) and 180 to 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 to 210 days 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 to 150 and 180 to 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 days in milk.

The lactation period also affected BUN values, in which periods of 60 to 90, 90 to 120 and 180 to 210 days in milk reduced BUN concentrations, while 120 to 150 days in milk increased BUN values (WMD= 5.20 mg/dL; $P=0.03$; Figure 4E). For blood glucose, days in milk 90 to 120, 150 to 180 and 180 to 210 days, showed an increase in values (Figure 4F). Days in milk 150 to 180 increased the total SFA values in milk fat (WMD= 22.7 mg/g; $P<.0001$; Figure 4G). The same period reduced the concentration of CLA (WMD= -0.73 mg/g; $P<.0001$; Figure 4H). Days in milk between 120 to 150 and 150 to 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; $P<.0001$; Figure 4I, respectively).

The covariate “experimental period” (Figure 5A) in periods between 60 to 90 days accounted for the increase in blood glucose (WMD= 2.91 mg/dL; $P<.0001$) in the animals receiving glycerin in the diet. Levels of inclusion of glycerin in the diet (Covariate = glycerin in the diet) of 100 to 150 and 200 to 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; $P<.0001$; 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= -

1.43mg/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. On the other hand, 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; $P < .0001$; 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 to 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 to 500 and 500 to 700 g/kg DM, reduced the total MUFA (WMD= - 17.0 mg/g; $P < .0001$; and WMD= - 6.66 mg/g; $P = 0.04$, Figure 8B, respectively).

4. 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 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 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 to C10 and C5 to C17 fatty acids, while C14 to C16 reduced. In the present meta-analysis, it was not possible to individually evaluate fatty acids, however, the reduction of C14 to C16 with the inclusion of glycerin found by the authors mentioned above, may explain the reduction in the total MUFA found herein, since C14 to 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. Fuertes 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 (Mephram, 1988), reducing the glucose intake, thus affecting the synthesis of lactose in the mammary gland. Even though there 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 days 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 days 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.

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

Item ¹	Control ² mean (SD)	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	<i>P</i> -value	<i>P</i> -value	I ² (%)	<i>P</i> value
Intake, kg/d							
Dry matter	19.4 (3.55)	49	-0.11 (-0.34, 0.12)	0.35	0.33	7.06	0.91
Organic matter	17.6 (3.85)	10	-0.55 (-0.93, -0.17)	0.01	0.76	0.00	0.38
Crude protein	2.34 (0.65)	5	-0.09 (-0.18,-0.00)	0.04	0.23	28.4	0.06
NDF	7.66 (0.62)	10	-0.65 (-0.94,-0.35)	<.0001	0.14	33.3	0.54
Digestibility, g/kg of DM							
Dry matter	645 (73.5)	20	14.6 (7.63, 21.52)	<.0001	0.01	45.7	0.30
Organic matter	668 (60.8)	13	6.59 (0.70,12.48)	0.03	0.60	0.00	0.28
Crude protein	678 (53.3)	16	16.2 (5.97, 26.48)	0.01	<.0001	69.2	0.91
Ethereal extract	580 (115)	6	125 (111, 140)	<.0001	0.63	0.00	0.93
NDF	462 (120)	19	-19.2 (-28.5, -9.82)	<.0001	0.22	19.1	0.15
Feed Efficiency, kg/kg	1.32 (0.42)	20	0.01 (-0.01, 0.04)	0.35	0.81	0.00	0.69
Body Weight, kg/d							
Change	-0.12 (0.85)	27	0.06 (0.04, 0.08)	<.0001	0.82	0.00	0.57

¹NDF= neutral detergent fiber. Feed efficiency = kg milk yield/kg DMI.² Control treatment (without glycerin).³N = number of comparisons of control and treatments (glycerin) (complete data set is available in Supplementary File S1).⁴WMD = weighted mean differences between control and with treatments.⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.⁶Egger's regression asymmetry test

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

Item ¹	Control ²	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
	mean (SD)		⁴ WMD _{Random effect} (95% CI)	<i>P</i> -value	<i>P</i> -value	I ² (%)	<i>P</i> value
Ruminal Parameters							
pH	6.65 (0.45)	16	-0.01 (-0.04, 0.04)	0.93	0.33	10.7	0.42
NH ₃ -N, mg/dL	12.9 (7.89)	11	-1.85 (-3.53, -0.16)	0.03	<.0001	86.1	0.88
Short- chain fatty acid, mol/100mol							
Acetate	61.5 (2.77)	19	-7.30 (-9.57, -5.03)	<.0001	<.0001	94.7	0.20
Propionate	21.2 (1.88)	18	2.94 (1.97, 3.91)	<.0001	<.0001	74.7	0.13
Butyrate	14.1 (1.51)	19	3.27 (2.09, 4.44)	<.0001	<.0001	94.5	0.26
Blood Parameters, mg/dL							
Blood urea nitrogen	28.4 (12.6)	17	-1.68 (-2.59, -0.77)	0.01	0.01	63.9	0.11
Glucose	59.5 (11.7)	45	1.51 (0.40, 2.61)	0.01	<.0001	67.4	0.09
Non-esterified fatty	20.4 (11.8)	11	-1.14 (-1.94, -0.34)	0.01	0.06	42.9	0.61
β-OH-Butyrate	9.40 (5.13)	14	1.37 (0.84, 1.90)	<.0001	0.67	0.00	0.13

¹ N=nitrogen² Control treatment (without glycerin).³N = number of comparisons of control and treatments (glycerin) (complete data set is available in Supplementary File S1).⁴WMD = weighted mean differences between control and with treatments.⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.⁶Egger's regression asymmetry test

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

Item ¹	Control ² mean (SD)	N ³	Glycerin		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random Effect} (95% CI)	P-value	P-value	I ² (%)	P value
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)	<.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)	<.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)	<.0001	0.216	31.5	0.102
Total MUFA	220 (43.6)	7	-10.9 (-15.8, -6.09)	<.0001	0.415	1.50	0.153
Total PUFA	32.2 (9.40)	7	-2.73 (-3.73, -1.72)	<.0001	0.204	29.7	0.744
Omega-3	7.17 (1.06)	6	-0.53 (-0.69,-0.37)	<.0001	0.672	0.00	0.635
Omega-6	21.2 (2.19)	6	-2.52 (-3.40, -1.63)	<.0001	0.161	37.0	0.946

¹ SNF= solid not fat; MUN=milk urea nitrogen; CLA= conjugated linoleic acid; SFA= saturated fatty acids; UFA=unsaturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids. ² Control treatment (without glycerin); ³N = number of comparisons of control and with treatments (glycerin) (complete data set is available in Supplementary File S1). ⁴WMD = weighted mean differences between control and with treatments; ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test.

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.

Dependent variable (Y, RMD)	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ³
	Intercept	Genetic type	Days in milk (day)	ED (day)	Glycerin diet	Glycerin type	CON in diet		
DMd, g/kg	35.2 (0.05)	-7.62 (0.65)	-63.8 (0.04)	--	-18.3 (0.01)	32.4 (0.10)	--	100	20
CPd, g/kg	28.1 (0.40)	9.33 (0.64)	-23.6 (0.29)	-2.03 (0.92)	5.69 (0.81)	--	--	4.16	16
N-NH ₃ , mg/dL	-6.83 (0.01)	--	6.86 (0.01)	--	3.98 (0.01)	--	--	100	11
Acetate, mol/100mol	-16.2 (0.01)	--	-1.15 (0.73)	4.21 (0.47)	6.07 (0.02)	--	--	85.4	19
Propionate, mol/100mol	8.10 (0.01)	--	-2.99 (0.07)	-1.38 (0.80)	-7.40 (0.01)	--	--	40.2	18
Butyrate, mol/100mol	3.74 (0.06)	--	5.35 (0.01)	-3.67 (0.06)	1.57 (0.03)	--	--	99.2	19
BUN, mg/dL	-4.29 (0.34)	0.31 (0.80)	2.71 (0.01)	0.34 (0.92)	0.22 (0.89)	--	--	100	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)	100	45
NEFA, mg/dL	-5.96 (0.08)	1.12 (0.20)	2.28 (0.58)	--	0.73 (0.82)	--	--	0.00	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)	100	37
MUN, mg/dL	-1.19 (0.68)	0.45 (0.86)	0.87 (0.39)	--	-0.80 (0.52)	--	-0.40 (0.80)	100	19

¹ ED =experiment in day; CON= concentrate in diet (g/kg); DMd=dry matter digestibility, CPd= crude protein digestibility, BUN=blood urea nitrogen; NEFA=non esterified fatty acids; MUN= milk urea nitrogen. Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariate.

³N = 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.

Dependent variable (Y, RMD)	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ³
	Intercept	Genetic type	Days in milk (day)	ED (day)	Glycerin diet	Glycerin Type	CON in diet		
CLA C18:2 cis-9 trans-11	-0.82 (0.01)	--	0.57 (0.06)	--	0.32 (0.09)	--	--	100	6
Total SFA, mg/g	46.2 (0.19)	--	-28.5 (<.001)	--	-11.7 (0.74)	--	--	100	8
Total UFA, mg/g	-8.30 (0.78)	--	--	--	-5.70 (0.87)	--	--	0.00	5
Total MUFA, mg/g	-15.7 (0.47)	--	--	--	6.70 (0.76)	--	--	100	7
Total PUFA, mg/g	-3.54 (0.01)	--	6.26 (0.04)	--	1.74 (0.07)	--	--	100	7
Total Omega-3, mg/g	-0.39 (0.01)	--	0.20 (0.75)	--	-0.31 (0.10)	--	--	0.00	6
Total Omega-6, mg/g	-3.17 (0.01)	--	-0.65 (0.64)	--	1.87 (0.01)	--	--	100	6

¹ED =experiment in day; CON= concentrate in diet (g/kg); CLA= conjugated linoleic acid; SFA= saturated fatty acids; UFA=unsaturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariate.

³N = number of comparisons between treatment with (glycerin) and control (without crude glycerin) (complete data set is available in Supplementary file S1).

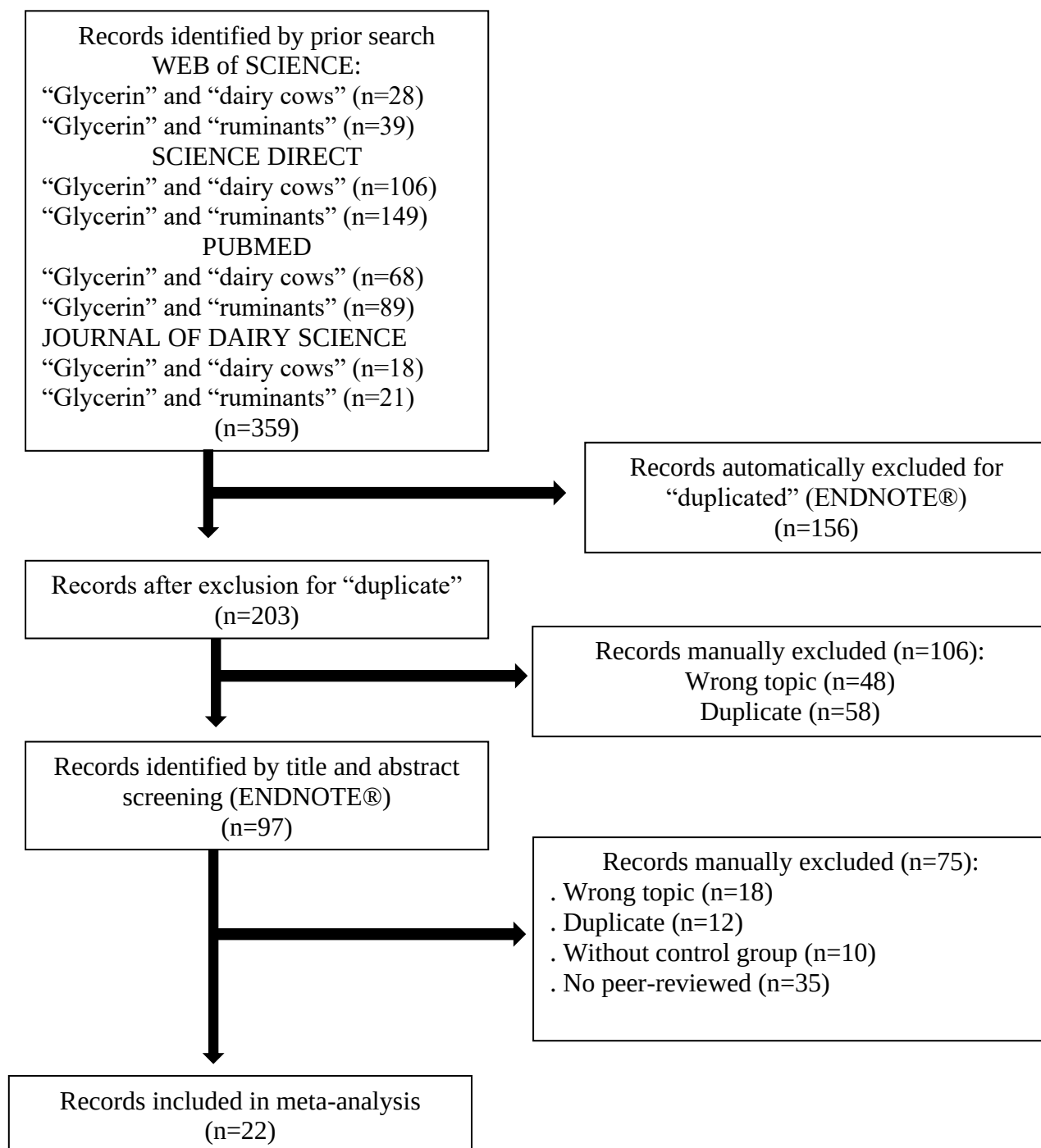


Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of glycerin in dairy cow diet.

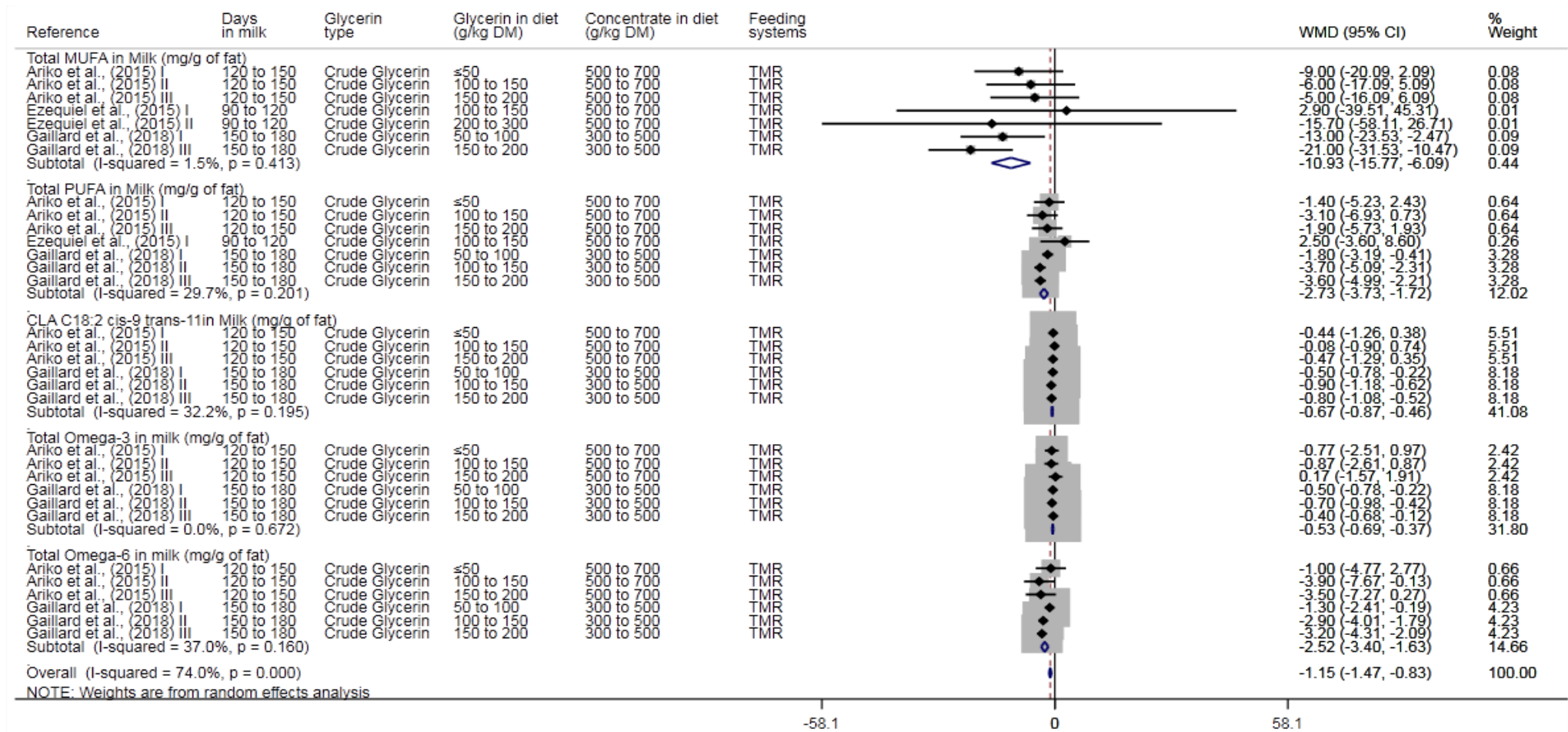


Figure 2. Forest plot showing the effect of the use of Glycerin on the diet of dairy cows on milk fatty acid profile. The x-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the line indicate an 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.

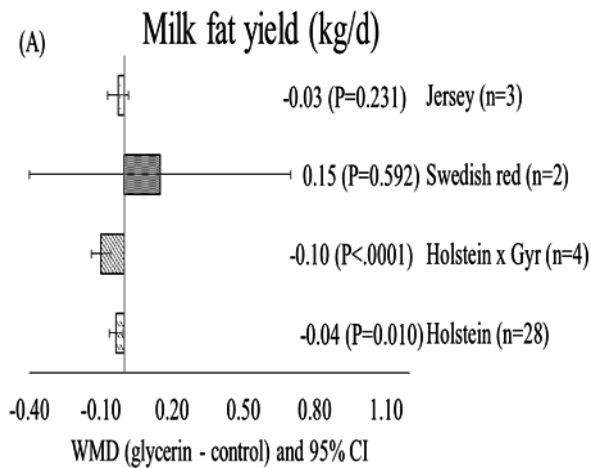
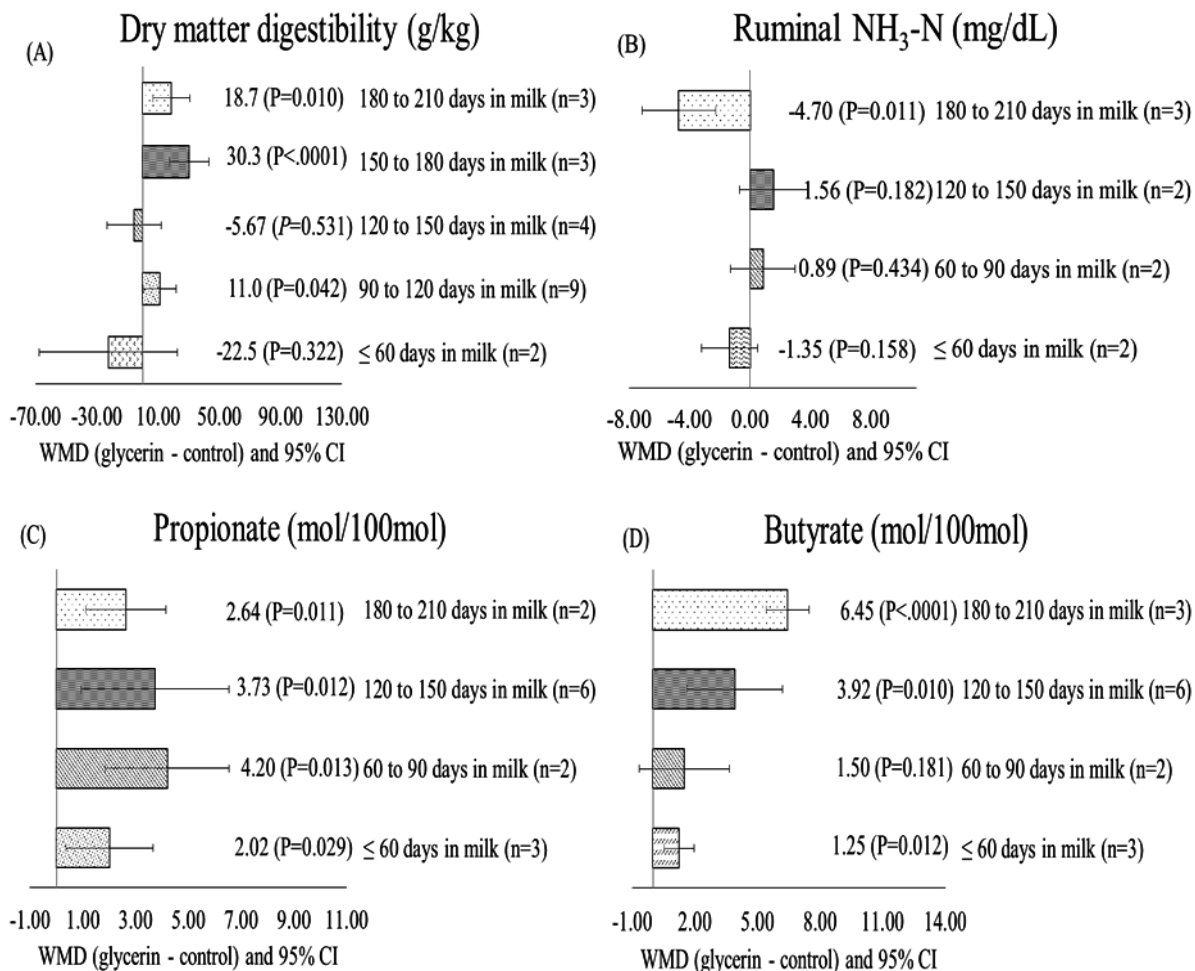


Figure 3. Subgroup analysis (Subgroup = Genetic type) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.



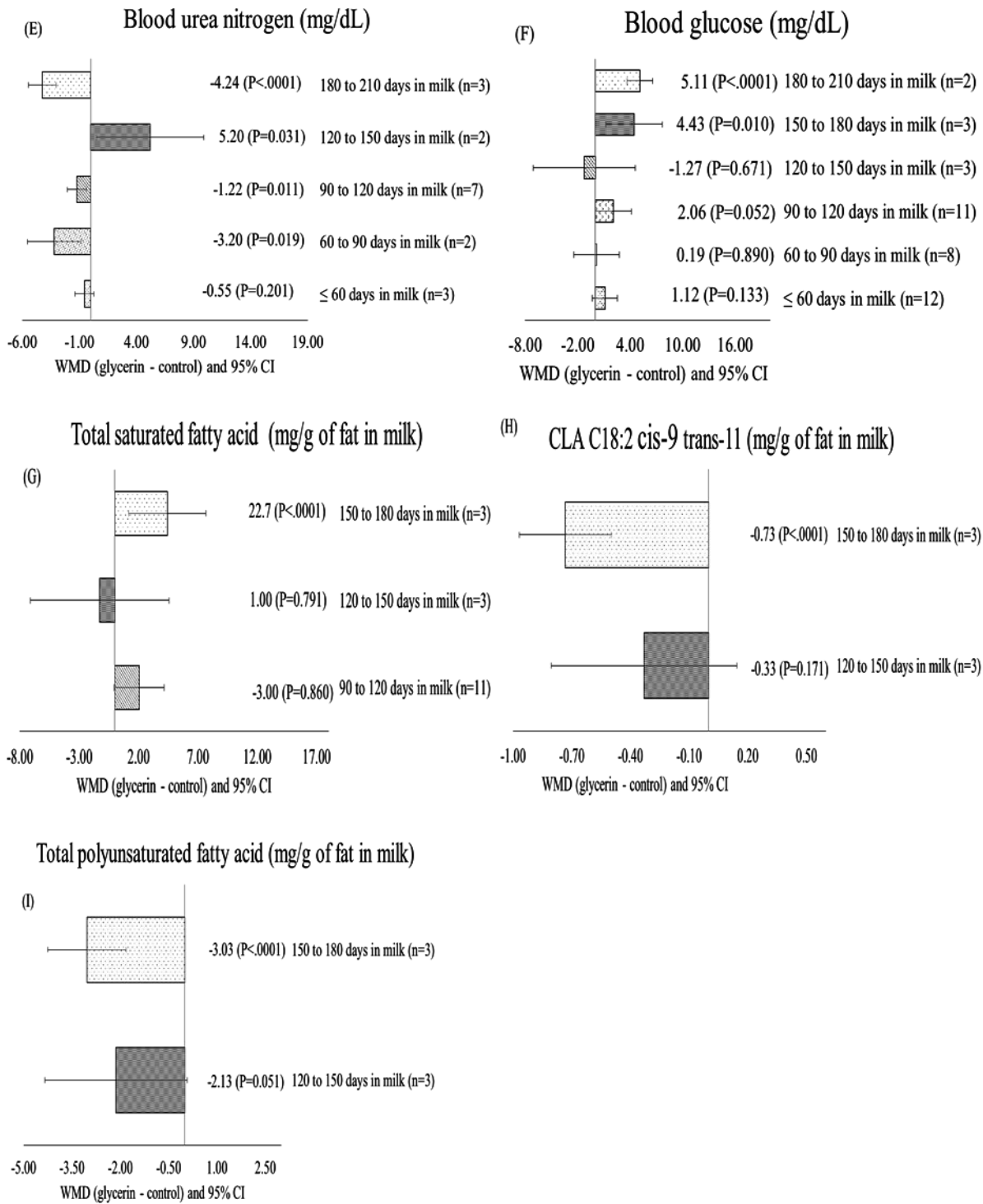


Figure 4. Subgroup analysis (Subgroup = Days in Milk) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.

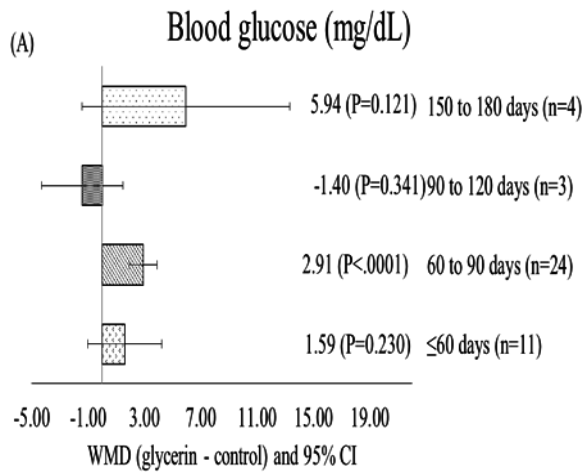
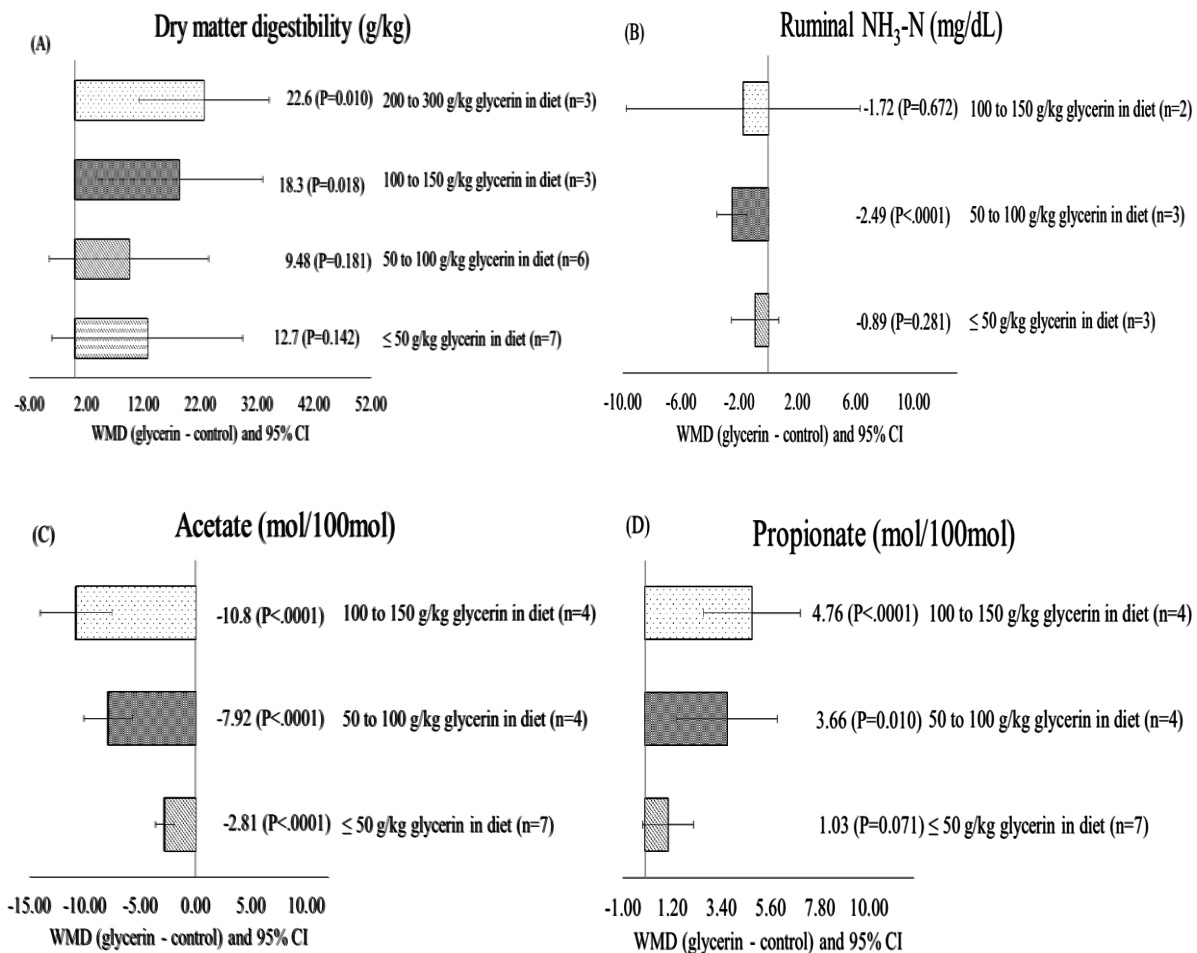
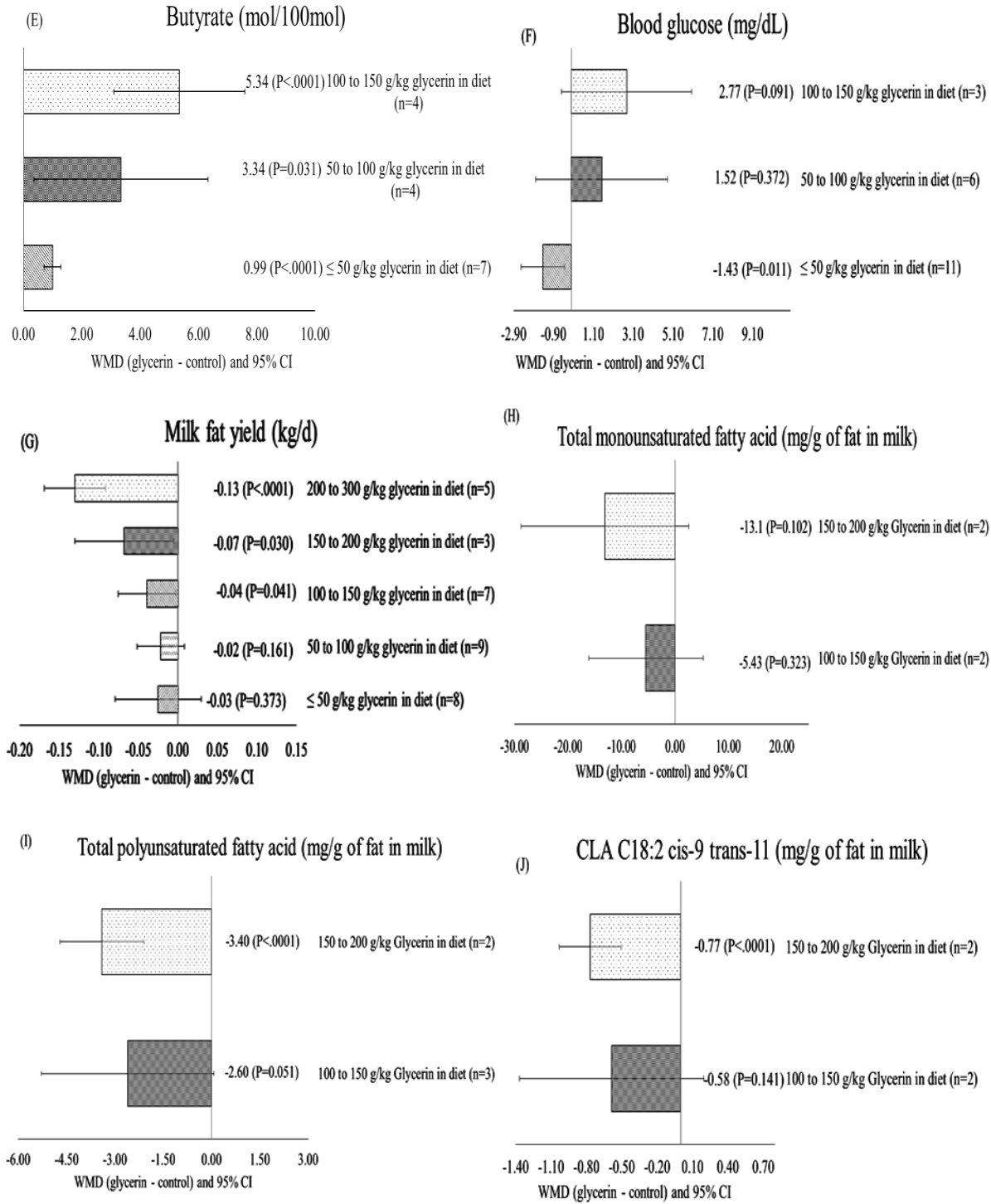


Figure 5. Subgroup analysis (Subgroup = Experiment period) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.





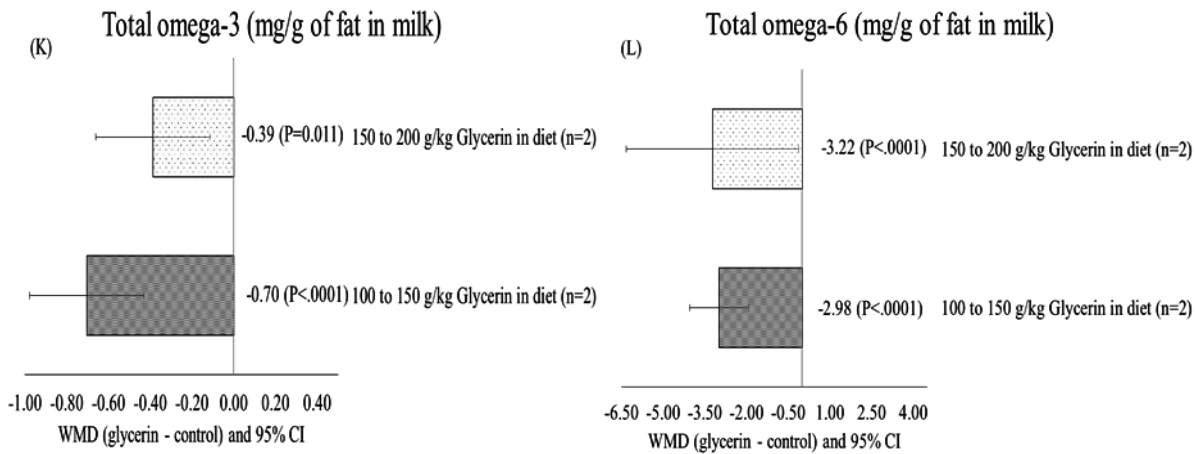


Figure 6. Subgroup analysis (Subgroup = Glycerin in diet (g/kg)) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.

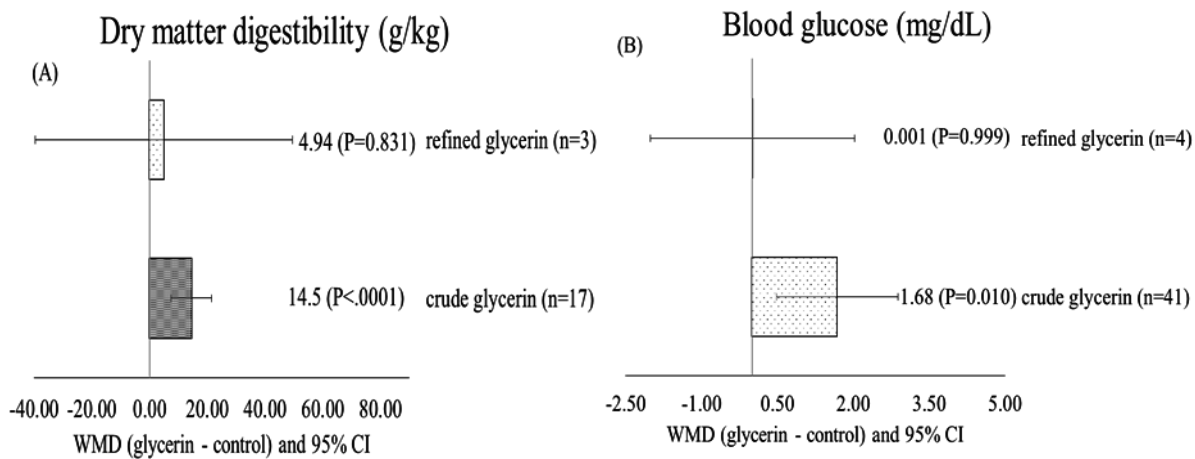


Figure 7. Subgroup analysis (Subgroup = glycerin type) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.

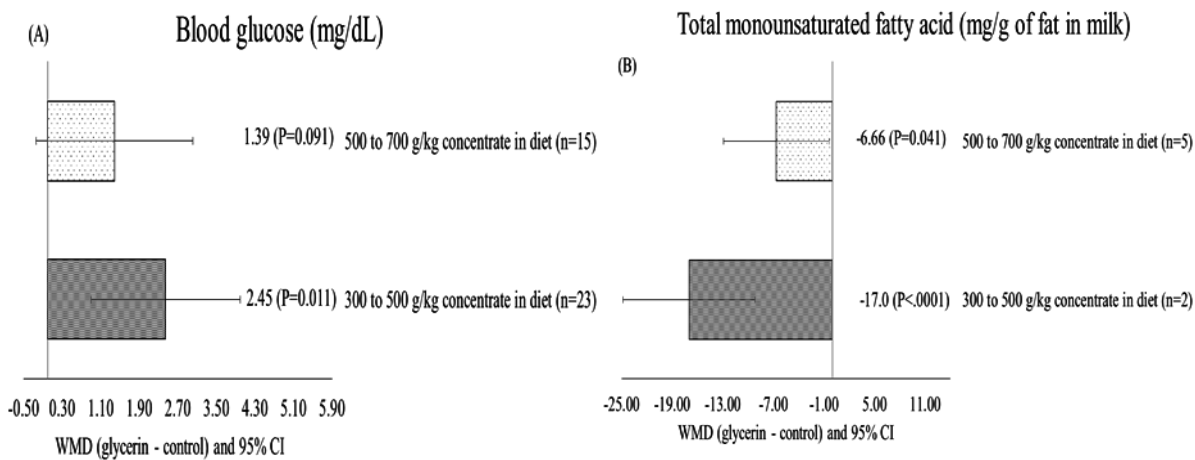


Figure 8. Subgroup analysis (Subgroup = Concentrate in diet(g/kg)) of the effect of the use of glycerin on the diet of dairy cows, WMD = weighted mean differences between glycerin and control treatments.

CAPÍTULO 4 - Effects of molasses supplementation on animal performance and carcass parameters of beef cattle: a meta-analysis

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista **“Animal Production Science”** exceto a posição das tabelas. Em tramitação.

Effects of molasses supplementation on animal performance and carcass parameters of beef cattle: a meta-analysis

Abstract

Positive effects of the supplementation of molasses in beef cattle diets are basically associated with the efficient use of dietary nitrogen. However, a large variation on performance and carcass parameters is also observed. In order to better understand these controversial results, this study aimed to investigate, from a meta-analytical approach, the effect of molasses in total mixed ration (TMR) on beef cattle performance and carcass parameters. Thirteen peer-reviewed publications with 45 treatment means were included in the data set. The effect of molasses in beef cattle diets were evaluated by examining the weighted mean differences (WMD) between molasses treatment (diet with molasses) and control diet (diet without molasses). Heterogeneity was explored by meta-regression and subgroup analysis using genetic type; experimental period, molasses in diet (g/kg DM), molasses type, concentrate in diet (g/kg DM) and forage type. When sugar beet molasses was included, there was a reduction in the average daily gain (ADG) by 0.24 kg. When included in high concentrate diets (800-900 g/kg concentrate), a reduction in ADG of 0.18 kg/d was observed. Regardless of concentrate type and level, the inclusion of molasses reduced carcass weight by 11.54 kg, mesenteric fat by 1.61 kg, and kidney, pelvic and heart fat by 1.59 kg. Given the results of this meta-analysis, it was possible to conclude that the inclusion of molasses greater than 200 g/kg DM negatively affects animal performance by reducing the average daily gain of animals, with a consequent reduction in carcass weight. However, inclusions between 100 to 150 g/kg DM of molasses in diets for beef cattle do not negatively affect animal performance and carcass parameters, being therefore indicated for use.

Keywords: by-product, bioethanol, carcass weight, molasses

1. Introduction

Molasses is the by-product of the manufacture or refinement of ethanol and sugar, from sugar cane, tubers or grains (Andrighetto et al., 2002; McDonald et al., 2007). It is a food rich in sugars, and is initially used in animal feed to improve the acceptability of coarse foods (Andrighetto et al., 2002). Since 1960, there has been intensive use in amounts above 60% in the cattle diet (Hunter, 2012). However, the provision of high levels of molasses in the diet for ruminant animals has been associated with a laxative effect (Andrighetto et al., 2002), and polioencephalomalacia, also known as “molasses toxicity” (Rowe et al., 1977).

However, one of the main responses to the use of molasses in cattle diet is the increase in rumen nitrogen retention, which is associated with the increased efficiency of conversion of urea into microbial protein, due to the higher energy and protein synchrony in the rumen (Hatch and Beeson, 1972; Osuji and Khalili, 1994; Araba and Byers, 2002). In this context, studies seeking the best nitrogen/energy synchrony for greater microbial efficiency and consequent greater animal performance with the use of molasses in the diets should be carried out, thus avoiding losses of dietary nitrogen, and consequently lower environmental pollution (Tamminga, 1992; NRC beef cattle 2016).

Another issue associated with the use of this by-product in diets for beef cattle is the effect on the carcass characteristics of the animals, but with results that are still very controversial in the literature, since there is large variation in the carcass weight of the animals (Garret et al., 1989; Araba and Byers, 2002; Asimwe et al., 2015). This variation can be a response depending on the level of inclusion of molasses in the diet, genetic group or even the feeding system (pasture or feedlot) used in the studies. These factors make it difficult to detect the difference between the treatments with molasses, especially when they are evaluated in individual experiments, thus making it necessary to use statistical tools such as the meta-analysis to assess the interference of these factors with the variable responses.

The meta-analysis has higher detection power than a single experiment for a highly variable response (Sauvant et al., 2008). This approach also allows greater capacity to explore sources of variation (covariates) on a response (Glass 1976; Higgins and Green, 2011). Thus, this study aimed to investigate, from a meta-analytical approach, the effect of molasses supplementation on animal performance and carcass parameters of beef cattle, with the hypothesis of molasses inclusions improves animal performance, with a positive effect on carcass parameters.

2. Materials and Methods

2.1 Data set

A comprehensive literature search was conducted using three search engines: Web of Science, PubMed, and Science Direct. Around 700 publications were retrieved using search terms including “molasses and beef cattle” and “molasses and ruminant”. Of the papers retrieved, only those that satisfied the predetermined inclusion criteria were included in the analysis. Of the papers that were retrieved, only those that met the predetermined inclusion criteria were included in the meta-analysis. For inclusion into the meta-analysis, studies had to have the following: 1) The control treatment without addition of molasses; 2) The data were only from animals intended for the production of meat and 3) Studies using only the total mixed ration as the feeding system. A flowchart detailing the process of study identification and selection for analyze is shown in Figure 1.

2.2 Data Extraction

Based on the inclusion criteria, 13 peer-reviewed papers were selected and classified by first author, publication reference, country, genetic type; experimental period; experimental design, age (month), additive type, molasses type; molasses in diet (g/ kg of dry matter (DM)); forage type and concentrate in diet (g/kg of dry matter). The number of replicates, means, and variances (i.e., SE, SD) were extracted for the following response variables for control and treatments. The complete data set is available in an Excel file in Supplemental File S1 (<https://doi.org/.....>).

2.3 Statistical analysis

The meta-analysis was performed using the R Software Metafor package (Viechtbauer, 2010) version 3.4.2. Forest plots were created using Stata software version 14.2 (StataCorp LP, College Station, TX). The effect of the molasses on the performance and carcass parameters of beef cattle were evaluated using random-effect models to examine the weighted means difference (WMD) between control diet data, and diet with the inclusion of molasses (treatment). Treatment means were weighted by inverse of the variance, according to the method proposed by Der-Simonian and Laird (1986) for a random effect model.

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% indicate low heterogeneity, whereas values between 25 and 50% denote moderate heterogeneity and those above 50% denote 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 $P \leq 0.05$. The presence of outliers in the dataset was tested using the studentized residual outside the -2.5 to 2.5 range.

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 to the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by

$$\emptyset = \beta + \beta_i x_{ij} + \dots \beta_p x_{ip} + \mu_i$$

Where $\emptyset$ = the true effect treatment in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_i = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, \tau^2)$. Here τ^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). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010), was calculated by comparing the estimated between-study variance when covariates were included in the model (σ^2) with corresponding values when they were excluded (σ_0^2); adjusted R^2 (%) = $100 \times ((\sigma_0^2 - \sigma^2) / \sigma_0^2 \sigma_0)$.

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 residuals out of the range -2.5 to 2.5 (outliers); 4) High heterogeneity (statistic $I^2 > 50\%$).

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). The covariates were divided as following: Genetic type (*Bos indicus*, *Bos taurus*, Crossbreed); Experimental period (30 to 70, 70 to 100 and 100 to 140 days); Experimental design (rotative= crossover, Latin square and continuos= randomized complete block design); Amount of molasses in diet (30 to 90, 100 to 150, 200 to 300 and 400 to 600 g/kg of DM); Molasses type (Sugar beet molasses, Cane molasses and Dried molasses product); Amount of concentrate in diet (100 to 400 and 800 to 900 g/kg of DM) and forage type (Straw and hay).

3. Results

The dataset was composed by 13 peer-reviewed publications with 49 treatment means selected to investigate the effect of molasses on beef cattle performance and carcass parameters. The predominant genetic group was the *Bos taurus* (Holstein steers + Hereford = 38.78% studies), followed by crossbreed (Crossbreed + Brahman-crossbreed + Angus-crossbreed = 28.89% studies), *Bos indicus* (Tamzania shorthorn zebu + Bunaji = 20.00 % of the studies) and not informed (13.33%). The experimental period was 30 to 70 (26.67%), 70 to 100 (40.00%), 100 to 140 days (26.67%) and not informed (6.67% the studies). The experimental design used in the studies were rotative (35.56%), continuos (57.78%) and not informed (6.67% studies).

The types of molasses used in the diets were cane-molasses, dried molasses product, sugar beet molasses, representing 70.73, 17.07 and 12.19 % of the studies, respectively. The level of inclusion of molasses in diet were 30.0 to 90.0 (26.67%), 100 to 150 (35.56%), 200 to 300 (8.89%), 400 to 600 (20.00%) and not informed 8.89% of the studies, respectively. The level of concentrate in diet were 100 to 400 (46.67%), 800 to 900 (28.89%) and not informed 24.45% of the studies. The forage type in diet were straw (15.56%), hay (hay + grass hay= 62.22%) and not informed represented 22.22% of the studies.

3.1. Intake and digestibility of nutrients and ruminal parameters

The inclusion of molasses in diet increased the digestibility dry matter ($P<.0001$), organic matter ($P<.0001$) and rumen concentration of butyrate ($P<.0001$) of the animals. However, the inclusion of molasses in the diets reduced the NDF intake and digestibility ($P<.0001$) and rumen acetate ($P=0.033$). The inclusion of molasses had no effect ($P>0.05$)

in dry matter intake (DMI), organic matter, ruminal pH, ammonia (N-NH₃), propionate and crude protein intake and digestibility (Table 1).

3.2. Performance, carcass traits and blood parameters

The inclusion of molasses in the diets reduced body weight final ($P=0.035$), carcass weight ($P=0.001$), subcutaneous fat thickness (SFT; $P=0.012$), kidney, pelvic and heart fat (KPH; $P=0.001$), and mesenteric fat ($P=0.007$). Inclusion of molasses had no effect ($P>0.05$) on the dressing, ribeye area, feed conversion ratio (FCR), blood ureic nitrogen (BUN) and blood glucose (Table 2).

3.3. Analysis of meta-regression and asymmetry of the funnel plot

High heterogeneity (I^2 statistic $> 50\%$) was shown by the organic matter and crude protein intake, NDF and crude protein digestibility, ruminal concentration of N-NH₃, body weight final, ADG, carcass weight and mesenteric fat (Table 1 and 2). As can be seen in Tables 1 and 2, the presence of publication bias ($P>0.05$) was not evident, from the funnel plot asymmetry test. Based on the meta-regression analysis presented in Tables 3, the covariates “Amount of molasses in diet” and “experimental period” were the factors that most influenced the variability of responses with the inclusion of molasses in the beef cattle diets.

3.5. Subgroup Analysis

To evaluate the effect of the covariate “Genetic type” (Figure 3), the inclusion of molasses in the diet of animal crossbreed reduced body weight final (WMD= 15.4 kg; $P=0.03$) and carcass weight (WMD= 17.49 kg; $P<.0001$).

To evaluate the effect of the covariate “experimental period” (Figure 4), the inclusion of molasses in the diet in periods of 100 to 140 days increased the dry matter intake (WMD = 0.45 kg/d; $P=0.01$). However, caused reductions in NDF digestibility (WMD= -54.02 g/kg of DM; $P<.0001$).

For the “amount of molasses in diet” covariate (Figure 5), inclusions between 100 to 150 g/kg increased the dry matter digestibility (WMD= 31.30 g/kg; $P<.0001$), whereas reduced NDF digestibility (WMD= -39.14g/kg of DM; $P=0.009$). However, amounts between 200 to 300 g/kg molasses reduced carcass weight (WMD= -12.76 kg; $P<.0001$) and ADG (WMD= -0.05 kg/d; $P<.0001$). While, amount between 400 to 600 g/kg molasses,

reduced carcass weight (WMD= -10.52 kg; $P=0.02$; Figure 2) and ADG (WMD= -0.10 kg/d; $P=0.008$).

To evaluate the effect “type of molasses” (Figure 6) covariate, in studies that use sugar beet molasses, reduced ADG (WMD= -0.24 kg/d; $P=0.002$). However, in studies that use cane molasses increased DMI (WMD= 0.21 kg/d; $P=0.03$). For the “concentrate in diet” covariate (Figure 7), inclusion the molasses in diet with 800 to 900 g/kg of concentrate reduced ADG (WMD= -0.18kg/d; $P=0.001$).

4. Discussion

The increase in DMI observed when molasses was included in the diets, for periods between 100 to 140 days, may be associated with an increase in requirements of nutritional maintenance in response to an increase in visceral organ weight in response to molasses consumption. Since the increase in DMI did not influence the carcass parameters (the inclusion reduced the SFT and carcass weight), and performance (there was no effect of the period of molasses consumption on the ADG), it is an adjustment response to changes in animal requirements when receiving molasses in the diet. Oxygen consumption by visceral organs may represent the use of 40-50% of all oxygen consumed by cattle and sheep, and may represent the use of up to 14% of the digestible energy consumed by cattle (Lindsay and Reynolds, 2005). The increase in DMI may also be associated with greater acceptability of the diet in response to the inclusion of molasses, as observed by Nombekela et al. (1994), in which ruminant animals had a preference for the consumption of foods rich in sugar (sucrose).

The increase in visceral organ mass in response to the inclusion of molasses in diets may also explain the lower deposition of mesenteric fat and KPH observed in this work, which may be associated with the stimulus for growth of visceral organs generated due to the energy input by the inclusion of molasses in the diet. In this context, lower deposition of visceral fat is expected in response to the greater energy demand of these organs in response to the increase in mass and metabolic activity, added to the lower availability of acetate, which is their main metabolic fuel (Lindsay and Reynolds, 2005), thus resulting in lower availability of nutrients for the synthesis and deposition of visceral fat. These facts corroborate the results of Garret et al (1989) and Araba and Byers (2002), which included molasses in the diet, and reported an increase in the liver and kidney weight of the animals. Increases in energy intake of the animals, with increases in the mass of visceral organs in

relation to the total body mass and the metabolic activity of these tissues were also observed by Burrin et al. (1990) and Reynold et al. (1995).

The reduction of 31.92 and 28.39% for KPH and mesenteric fat, respectively, observed with the inclusion of molasses in diet, may partially explain the 2.57% reduction in the final body weight and 5.55% in the carcass weight. As observed in Figure 2, regardless of the experimental period, genetic group and level of concentrate in the diet, the inclusion of molasses above 200 g/kg of DM in the diets reduced the carcass weight. High molasses concentrations in cattle diets were associated with a reduction in the animal's growth rate, even after correction of dietary protein and mineral deficiencies (Tomkins et al., 2004), which may be associated with the occurrence of diarrhea (Araba et al., 2002), as well as the greatest susceptibility to metabolic disorders. As noted by Araba and Byers (2002), who included 60% molasses in the diet for crossbred animals, showed increases of 44.4% in the confinement period, with reductions in ADG, carcass weight and dressing, compared to the control treatment (without molasses), in addition to the tendency for tympanism to occur.

The type of molasses used in the studies also influenced the animal performance responses. The reduction of 0.24 kg/d in ADG of animals when sugar beet molasses was included in the diets may indicate greater susceptibility of the animals to the occurrence of diarrhea (Araba et al., 2002) and metabolic disorders, due to their higher concentration of sucrose, which can represent almost 66% of the sugars (OECD, 2002), its ruminal fermentation results in greater production of ketogenic short-chain fatty acids that can make ruminants deficient in glucose when fed diets with a high concentration of molasses (Dryden, 2008).

When evaluating the effect of the "concentrate in diet" covariate, we observed that regardless of the level of molasses inclusion, diets containing between 800 to 900 g/kg of concentrate provided lower ADG for the animals, corroborating the hypothesis of the occurrence of diarrhea and/or nutritional metabolic disorders. In the present meta-analysis, the effect of the inclusion of molasses on the blood glucose concentration was not observed. It should be noted that the studies used in the database did not show the variation in glucose concentration at different times after feeding and this can impair the detection of the change in glucose concentration in response to the inclusion of molasses in the diet, not allowing an adequate assessment of the hypothesis of glucose deficiency when high amounts of molasses are included in diets. However, glucose availability is one of the main factors affecting lipogenesis and meat quality in ruminants (Ladeira et al., 2018).

In this context, the reduction in the availability of glucose added to the reduction in the ruminal concentration of acetate may explain the reduction in SFT in carcasses with the inclusion of molasses in the diets. Based on our results, our hypothesis that the inclusion of molasses in the diet would improve animal performance with a positive effect on carcass parameters was denied. The inclusion of molasses above 200 g/kg reduced ADG, carcass weight and SFT. However, inclusion between 100-150 g/kg had no effect on animal performance and carcass parameters.

5. Conclusion

The supplementation of molasses greater than 200 g/kg DM in TMR negatively affected animal performance by reducing the average daily gain and carcass weight. Inclusions between 100 to 150 g/kg DM molasses in the diet did not affect animal performance and carcass parameters of beef cattle.

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Table 1: Effect of inclusion of molasses in beef cattle diet on intake, digestibility and ruminal parameters

Item ¹	Control ² mean (SD)	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	<i>P</i> -value	<i>P</i> -value	I ² (%)	<i>P</i> -value
Intake, kg/d							
Dry matter	7.01 (1.74)	26	0.07 (-0.07, 0.20)	0.339	0.046	34.12	0.109
Organic matter	5.33 (1.46)	9	0.11 (-0.05, 0.28)	0.195	0.011	59.75	0.105
Crude protein	0.82 (0.36)	14	0.01 (-0.01, 0.03)	0.290	0.007	54.96	0.161
NDF	3.20 (0.32)	7	-0.07 (-0.16, 0.01)	0.089	0.815	0.00	0.792
Digestibility, g/kg of DM							
Dry matter	660.38 (70.45)	12	27.18 (17.94, 36.41)	<.0001	0.604	0.00	0.825
Organic matter	593.58 (58.36)	12	21.63 (12.70, 30.56)	<.0001	0.552	0.00	0.208
Crude protein	481.62 (75.56)	10	16.63 (-12.01, 45.27)	0.255	<.0001	80.82	0.433
NDF	626.16 (90.38)	13	-43.45 (-61.31, -25.58)	<.0001	0.002	61.80	0.269
Ruminal Parameters							
pH	6.77 (0.22)	5	-0.03 (-0.08, 0.02)	0.221	0.909	0.00	0.783
N-NH ₃ , mg/dL	21.76 (8.84)	4	0.97 (-4.27, 6.22)	0.716	0.035	64.94	0.174
SCFA, mol/100mol							
Acetate	66.05 (5.41)	5	-1.34 (-2.57, -0.10)	0.033	0.708	0.00	0.797
Propionate	20.82 (2.37)	5	0.11 (-1.31, 1.54)	0.871	0.987	0.00	0.964
Butyrate	10.02 (2.06)	5	1.03 (0.57, 1.47)	<.0001	0.300	17.95	0.312

¹SCFA= short- chain fatty acid; NDF= neutral detergent fiber. ²Control treatment (without molasses in diet). ³N = number of comparisons of control and with treatments (with molasses in diet) (complete data set is available in Supplementary File S1). ⁴WMD = weighted mean differences between control and with treatments. ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test.

Table 2: Effect of inclusion of molasses in beef cattle diet on performance, carcass parameters and blood parameters

Item ¹	Control ² mean (SD)	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
Body weight Final, kg	317.91 (102.67)	14	-8.16 (-15.73, -0.59)	0.035	<.0001	76.89	0.262
ADG, kg/d	0.68 (0.53)	29	0.01 (-0.06, 0.05)	0.929	<.0001	99.95	0.719
FCR, kg/kg	7.89 (1.67)	9	0.16 (-0.01, 0.33)	0.067	0.489	0.00	0.695
Carcass weight, kg	207.78 (61.28)	12	-11.54 (-18.11, -4.97)	0.001	<.0001	90.58	0.183
Dressing, %	52.54 (1.34)	9	0.25 (-0.14, 0.65)	0.215	0.293	16.70	0.128
SFT,mm	8.55 (5.74)	8	-0.86 (-1.53, -0.19)	0.012	0.718	0.00	0.108
Ribeye area, cm ²	80.31(18.64)	8	0.71 (-1.21, 2.62)	0.470	0.969	0.00	0.704
KPH, kg	4.98 (0.91)	5	-1.59 (-2.45, -0.74)	0.001	0.174	37.11	0.164
Mesenteric fat, kg	5.67 (0.56)	4	-1.61 (-2.78, -0.43)	0.007	0.028	66.95	0.071
Blood Parameters							
BUN, mg/dL	6.87 (3.10)	10	-0.12 (-0.81, 0.58)	0.739	0.154	31.82	0.937
Glucose, mg/dL	74.70 (3.49)	8	1.35 (-2.76, 5.47)	0.519	0.195	29.22	0.252

¹ADG= average daily gain; BUN= blood urea nitrogen; FCR= feed conversion ration in total mixed ration; SFT= subcutaneous fat thickness; KPH= kidney, pelvic and heart fat;

²Control treatment (without molasses in diet).

³N = number of comparisons of control and with treatments (with molasses in diet) (complete data set is available in Supplementary File S1).

⁴RMD = raw mean differences between control and with treatments.

⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test

Table 3: Meta-regression of the effect of inclusion the molasses in diets for beef cattle on weighted mean differences (WMD), between treatment and control.

Dependent variable (Y, WMD) ²	Meta-regression parameters (<i>P</i> -value) ¹								Adjusted R ² (%)	N ³
	Intercept	Genetic type	Experimental period (day)	Experimental design	Molasses type	Molasses in diet (g/kg)	CON (g/kg)	Forage type		
DMI, kg/d	-2.17 (0.14)	1.17 (0.05)	1.30 (0.09)	0.42 (0.35)	0.81 (0.08)	1.07 (0.04)	--	--	100	26
CPI, kg/d	0.07 (0.29)	-0.01 (0.63)	-0.06 (0.42)	--	--	-0.01 (0.66)	--	--	0.00	14
CPd, g/kg	73.00 (0.13)	-47.25 (0.39)	-40.25 (0.34)	--	--	-22.75 (0.63)	--	--	0.00	10
NDFd, g/kg	-50.76 (0.01)	-11.49 (0.41)	59.25 (0.01)	--	-20.04 (0.46)	53.25 (0.07)	--	--	100	13
ADG, kg/d	0.55 (0.17)	-0.21 (0.35)	-0.59 (0.01)	0.02 (0.89)	-0.68 (0.01)	0.21 (0.04)	0.52 (0.01)	--	75.84	29
BW final, kg	33.00 (0.01)	-34.50 (0.03)	-28.00 (0.03)	--	--	2.40 (0.89)	--	--	70.45	14
Carcass Weight, kg	-15.15 (0.01)	26.69 (0.01)	-10.58 (0.01)	--	--	11.45 (0.01)	--	--	88.9	12

¹Experimental design (rotative, continue); CON= concentrate in diet; forage type (straw and hay).

² BW= body weight; DMI= dry matter intake; CPI= crude protein intake; NDFd= neutral fiber detergent digestibility; CPd= crude protein digestibility; ADG= average daily gain.

Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariate.

³N = number of comparisons between treatment (molasses in diet) and control (complete data set is available in Supplementary file S1).

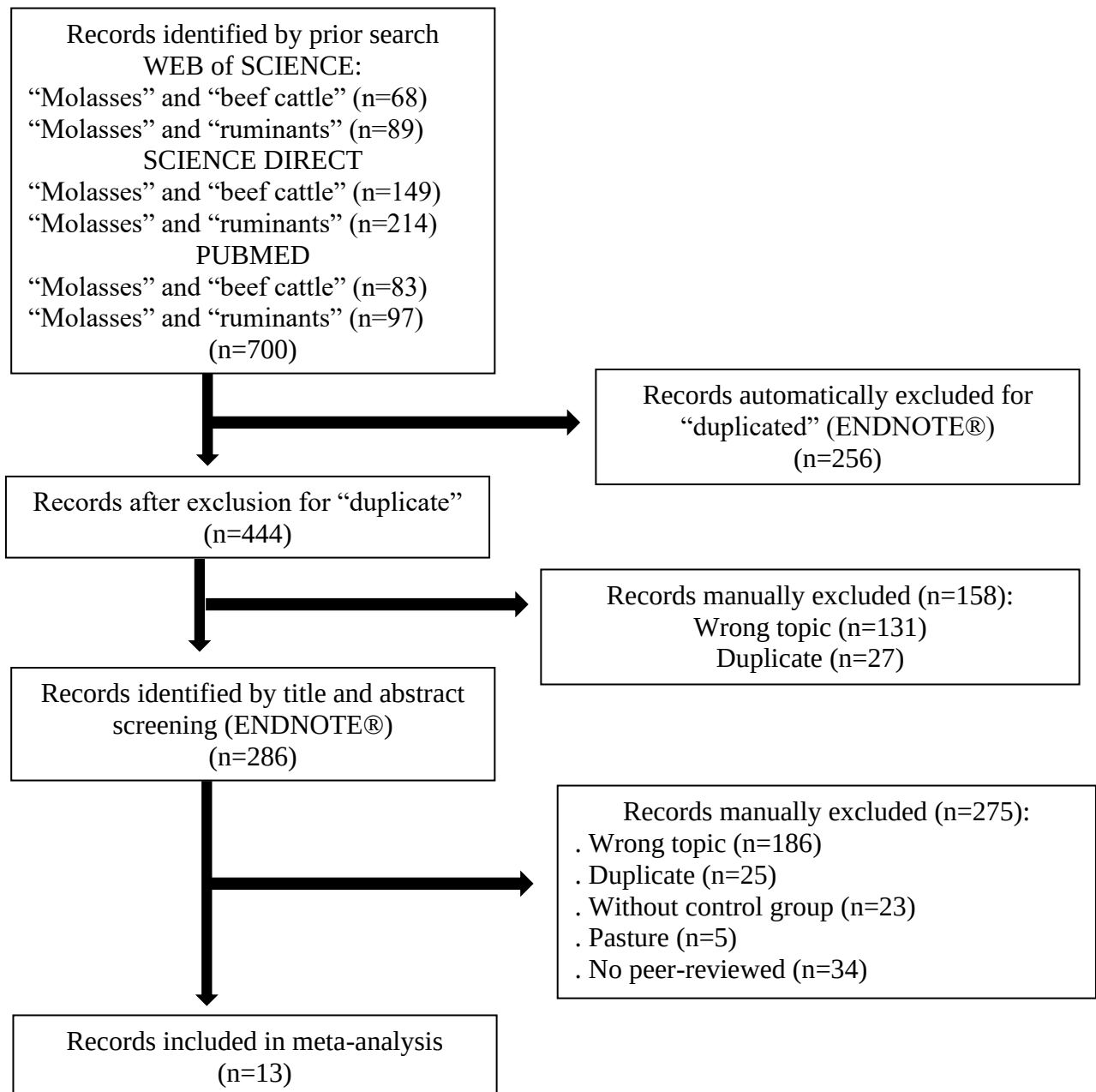


Figure 1 Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of molasses in beef cattle diet.

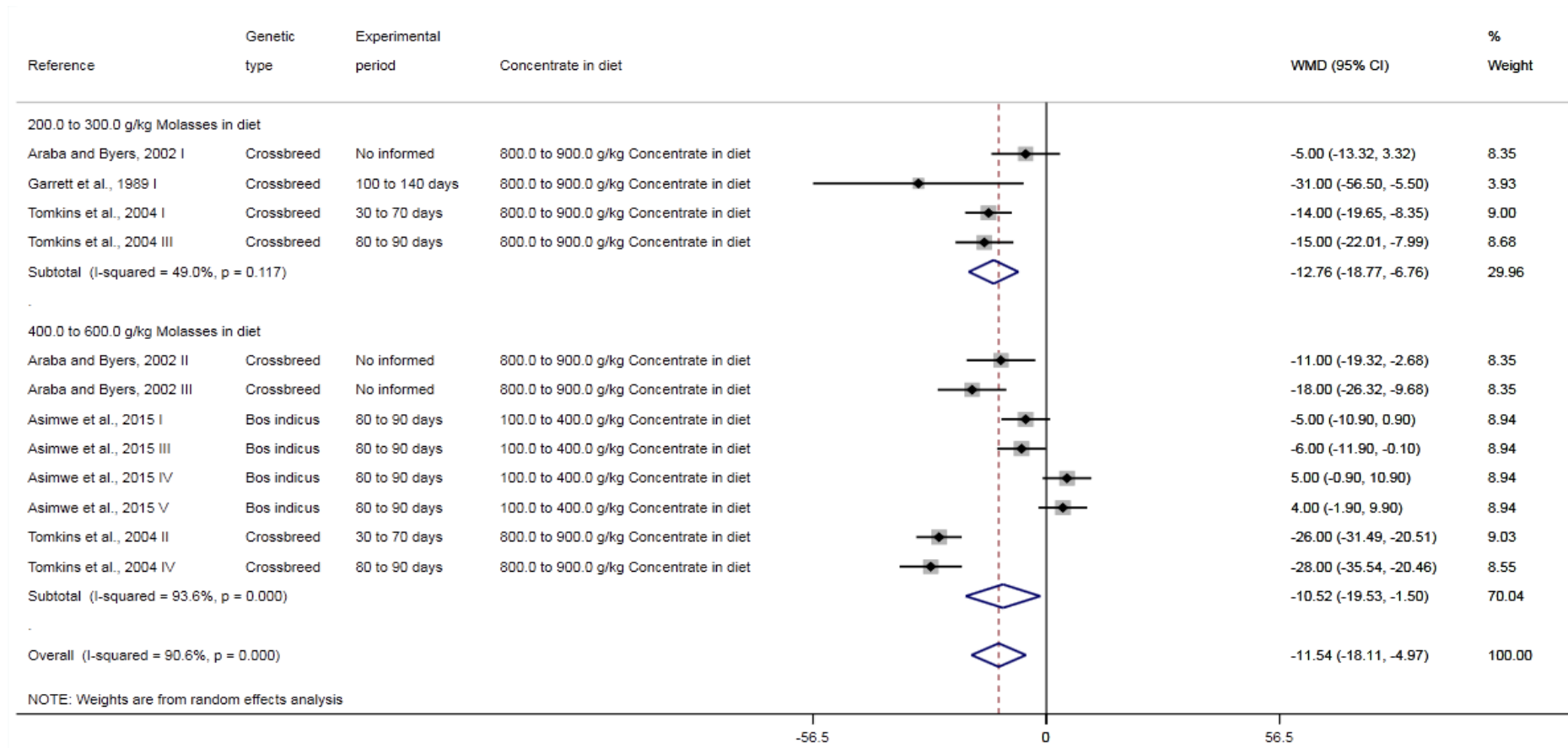


Figure 2. Forest plot (with subgroup analysis (covariate= Amount of molasses in diet (g/kg of DM)) showing the effect of the use of Molasses on the diet the beef cattle on carcass weight (kg). The x-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent a reduction 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 treatment 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.

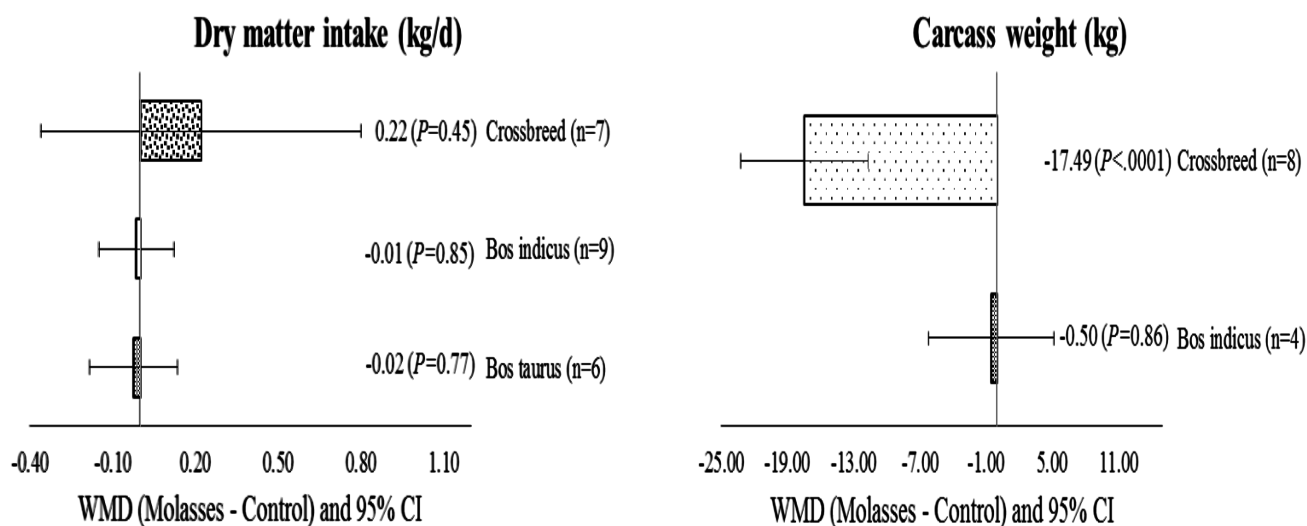


Figure 3. Subgroup analysis (subgroup=Genetic Type) of the effect of the use of molasses in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

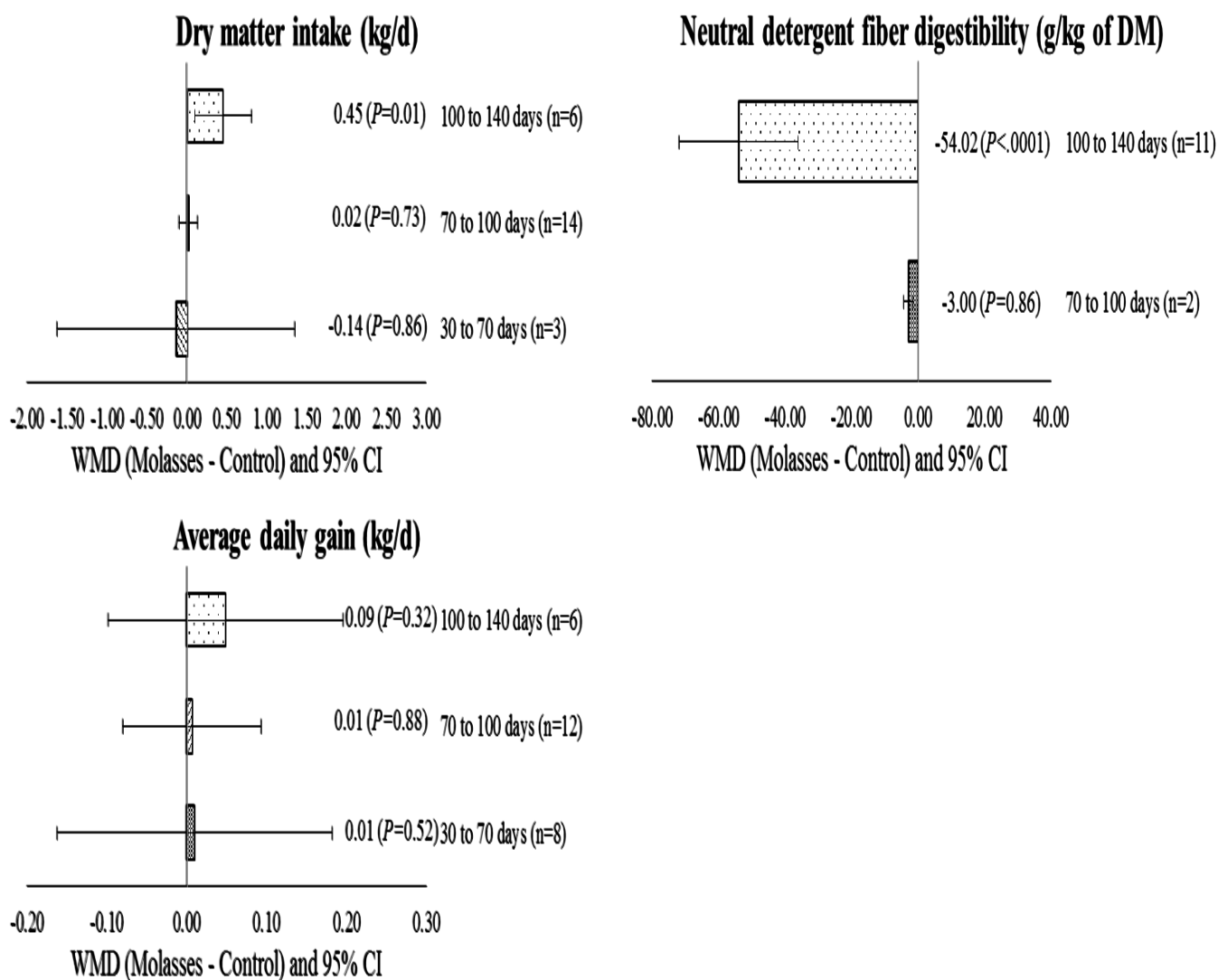


Figure 4. Subgroup analysis (subgroup= Experimental period) of the effect of the use of molasses in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

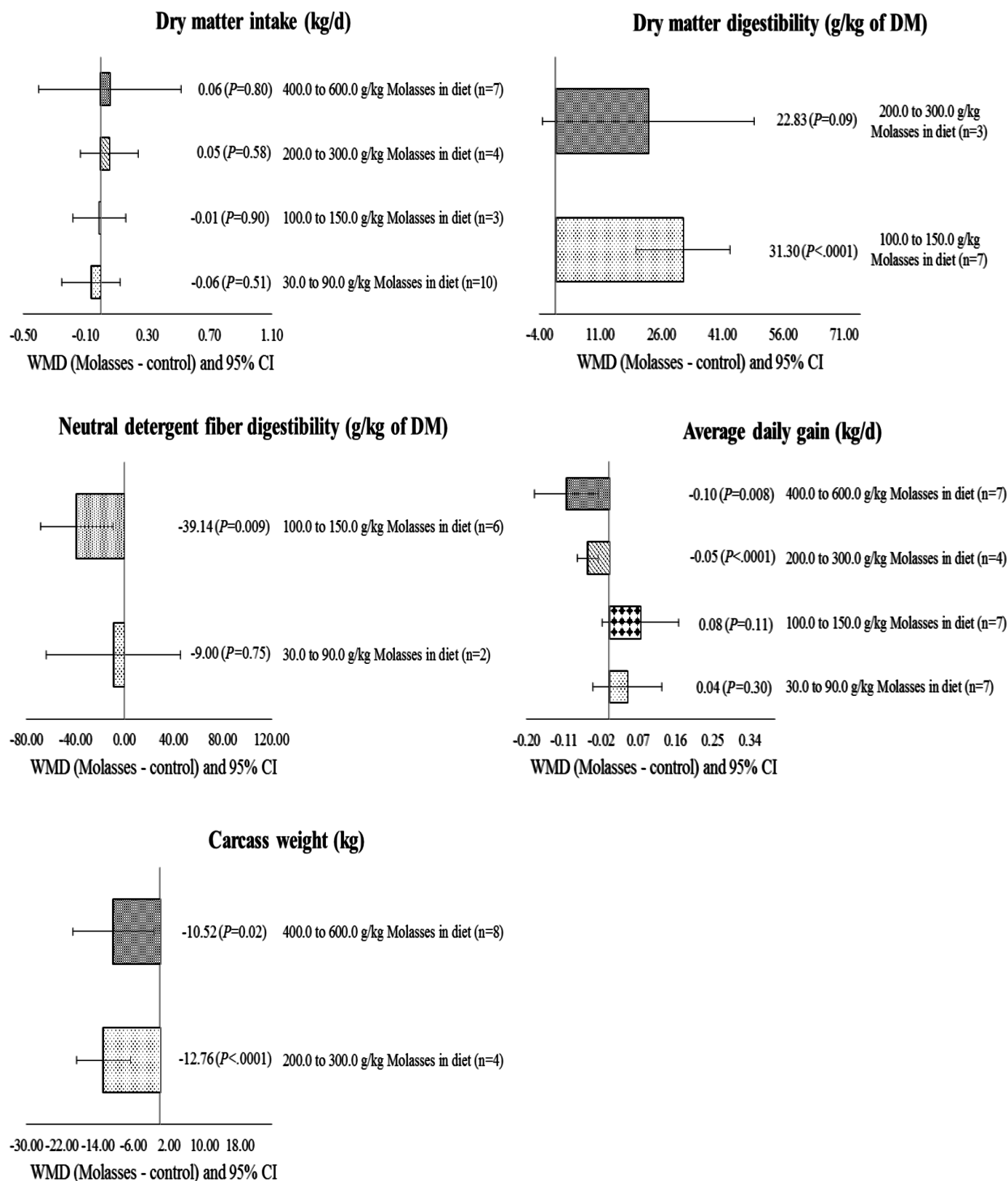


Figure 5. Subgroup analysis (subgroup=Amount of molasses in diet (g/kg of DM)) of the effect of the use of molasses in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

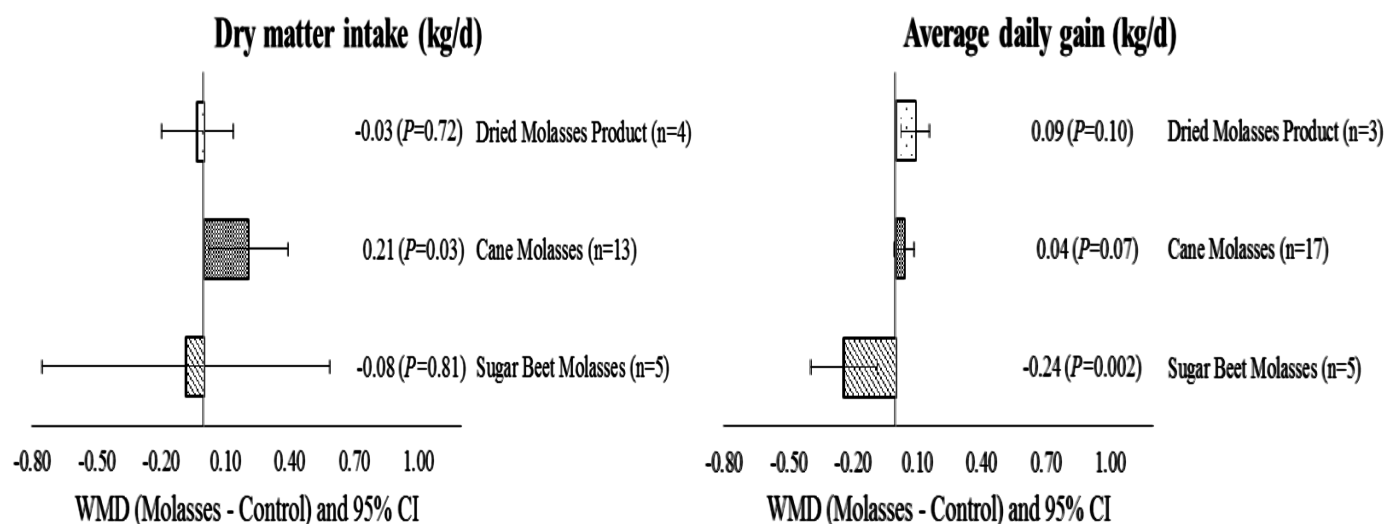


Figure 6 Subgroup analysis (subgroup=Molasses type) of the effect of the use of molasses in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

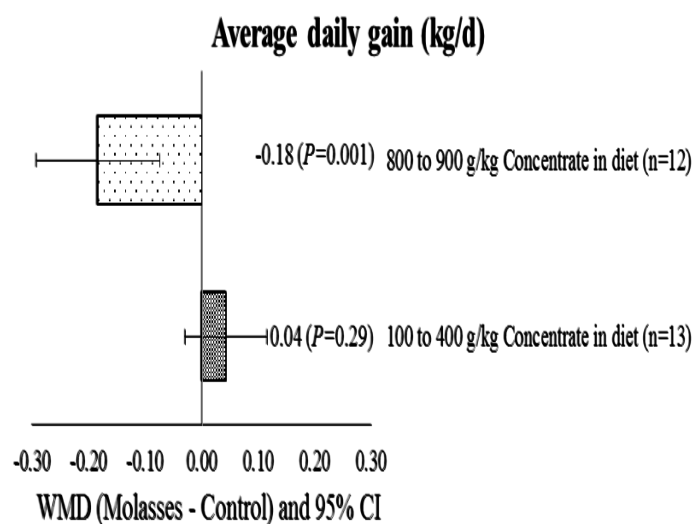


Figure 7 Subgroup analysis (subgroup=Amount of concentrate in diet (g/kg of DM)) of the effect of the use of molasses in diet of the beef cattle, WMD = weighted mean differences between treatment and control treatments.

CAPÍTULO 5 - Meta-analysis to evaluate the effect of including molasses in the diet for dairy cows on performance, milk fat synthesis and milk fatty acid

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista **“Livestock Science”** exceto a posição das tabelas. Publicado em maio de 2021 sob identificação DOI:

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Meta-analysis to evaluate the effect of including molasses in the diet for dairy cows on performance, milk fat synthesis and milk fatty acid

Abstract

Different types of molasses have been included in diets for ruminant animals, mainly as a substitute for energetic ingredients, such as corn and sorghum. However, the positive effect of this inclusion on the performance, fatty acid profile and composition of cows' milk is not yet clear. In this sense, this study aimed to evaluate by meta-analysis the effect of including molasses in diets for dairy cows on the milk fatty content and fatty acid in milk. The eleven peer-reviewed publications with 52 treatments means were included in data set. The effect of including molasses in the diet of dairy cows were evaluated using random effect models to examine the weighted mean differences (WMD) between molasses treatment (diets with molasses inclusion) and control treatment (without molasses inclusion in the diet). Heterogeneity was explored by meta-regression and subgroup analysis considering genetic group, days in milk, molasses in the diet (g/kg DM), type of molasses, concentrate in the diet (g/kg DM). The covariates genetic group and days in milk influenced most the variability of responses to the inclusion of molasses in the diet for dairy cows. Molasses supplementation to dairy cow diets negatively impacted animal performance and milk composition by reducing milk yield and milk protein, lactose yield and concentration (with inclusions levels of molasses greater than 60 g/kg of DM). However, inclusions of molasses up to 120 g/kg of DM in diet, did not affect the yield and concentration of fat in milk. The inclusions of molasses in the diet caused a reduction in the concentration of unsaturated fatty acids (Total UFA -10.67%); however, it increased the concentration of omega-3 fatty acids in milk (11.52%), without affecting total of polyunsaturated fatty acids and C18: 2 cis-9, trans-11 CLA.

Keywords: omega-3, milk solids, co-product, polyunsaturated fatty acid, soluble carbohydrates

1. Introduction

Milk and dairy-derived foods are important sources of many nutrients in the human diet (Hobbs et al., 2016). The possibility of increasing the concentration of monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids and conjugated linoleic acid (CLA) in milk fat, allows not only to increase its consumption, but also to increase human health (Robinson and Kowey, 2016; Flemming et al., 2016; Machado et al., 2017).

The concentration of fat in milk and its fatty acid profile is a multifactorial response, involving nutrition, ruminal kinetics, physiological and health status of the animal and environmental factors (Harfoot and Hazlewood, 1997). Among these factors, nutrition is the one with the greatest impact, as it has a direct effect on the ratio of saturated to unsaturated fatty acids consumed, as well as the rumen microbial activity that will influence the biohydrogenation process (Fuentes et al., 2011), as well as the production biohydrogenation intermediates, such as trans-10, cis-12 CLA, which has anti-lipogenic functions in the mammary gland (Martel et al., 2011), thus associated with the reduction of the fat content in milk.

The type of carbohydrates in the diet (i.e., starch, sugars and fiber) has a significant effect on rumen microbial activity and its fermentation (Hall et al., 2010). In this context, the inclusion of molasses allows the energetic densification of the diet with less negative impact on the production of fat in milk when compared to starchy foods, for example (Broderick and Radloff, 2004; Brito et al., 2017). The replacement of starch by sugar in dairy cow diets did not increase the risk of ruminal acidosis and/or induce milk fat depression (Martel et al., 2011), despite sugar fermentation being faster than starch in the rumen (Chamberlain et al., 1993), in addition to reducing the production of propionic acid. Thus, the inclusion of molasses may promote *de novo* fatty acid synthesis in the mammary gland of cows fed high-energy rations by regulating ruminal pH (Martel et al., 2011). In this context, the hypothesis of this study is that the inclusion of molasses in the diet increases the concentration of fat in milk, with the highest concentration of saturated fatty acids in milk.

However, variables such as level of inclusion, type of molasses, diet intake period, forage: concentrate ratio, and genetic group of animals can provide controversial results, as they present high heterogeneity due to the wide variation in experimental conditions, requiring the use the meta-analytical approach because it has greater detection power compared to a single experiment (Sauvant et al., 2008). In addition, this approach still allows greater capacity to explore sources of variation on a response (Glass, 1976; Higgins and Green, 2011). Thus, the

objective of this study was to critically review and summarize published research studies on the effects of molasses inclusion in diets for dairy cows on milk fat content and milk fatty acid.

2. Materials and Methods

2.1 Data set

A comprehensive literature search was conducted using three 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 (<https://www.journalofdairyscience.org>). Around 400 publications were retrieved using search terms including “molasses and dairy cow” and “ruminants and molasses”. Of the papers retrieved, only those that satisfied the predetermined inclusion criteria were included in the analysis. For inclusion into the meta-analysis, studies had to have the following (inclusion criteria): 1) The control treatment no present the addition of molasses in diet and 2) Experiments with dairy cows in lactation. A flowchart detailing the process of identifying and selecting studies for analysis is shown in Figure 1.

2.2 Data Extraction

Based on inclusion criteria, 11 peer-reviewed publications (15 studies with 52 treatment means) were rated by the first author, publication reference, genetic group, forage in the diet (g/kg of DM), amount of molasses fed (g molasses/kg of DM), molasses 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 adopted and measurements of mean dispersion (SEM and SD). The following variable responses were extracted for both, the control and the molasses treatments: nutrient intake, feed efficiency, total tract diet digestibility, milk yield, and milk composition and nitrogen metabolism.

2.3 Statistical analysis

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 molasses in lactating dairy cows diets were evaluated using random-effect models to examine the weighted mean difference (WMD) between molasses treatment (diets with molasses inclusion) and control treatment (without molasses). Treatments means were weighted by the inverse of the

variance, according to the method proposed by Der-Simonian and Laird (1986) for random effects model.

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 25 to 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 analysis was conducted to identify categorical covariate effects (described previously). The mixed model was applied to adjust the data to the meta-regression analysis using WMD as the dependent variable. The mixed-effect models were given by

$$\Theta_i = \beta + \beta_1 x_{ij} + \dots \beta_p x_{ip} + \mu_i$$

Where Θ_i = the true effect treatment in the i th explanatory variable; β = the overall true effect treatment; x_{ij} = the value of the j th covariate ($j = 1, 2, \dots, p$) for the i th explanatory variable; β_j = change in the true effect size for unit increase in the j th covariate; and $\mu_i \sim N(0, t^2)$. Here t^2 indicates the amount of heterogeneity not explained by the covariate (Viechtbauer, 2010).

Tests of null hypothesis for the covariate coefficients were obtained from the multiparameter Wald test (Harbord and Higgins, 2008). The adjusted R^2 for the model, which represents the proportion of between-study variance (heterogeneity) explained by the covariates (Harbord and Higgins, 2008; Viechtbauer, 2010), was calculated by comparing the estimated between-study variance when covariates were included in the model (σ^2) with corresponding values when they were excluded (σ_0^2); adjusted R^2 (%) = $(\sigma_0^2 - \sigma^2) / \sigma_0^2 \sigma$.

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 variation -2.5 to 2.5 (outliers). WMD was evaluated by subgroup analysis when the categorical covariates were significant at P-value ≤ 0.10 . The covariates were divided as following: genetic group (Holstein and Jersey), Days in milk (initial [≤ 100 days], middle [100 to 200 days] and final [> 200 days in milk]); Types of molasses (liquid molasses (sugarcane), dried molasses

(sugarcane) and sugar beet molasses); The level of molasses inclusion in diet (≤ 30 , 30 to 60, 60 to 90, and 90 to 120 g/kg of DM); Feeding system (Pasture and total mixed ration (TMR)); Concentrate in diet (≤ 400 , 400 to 600 and 600 to 800 g/kg of 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 by Roma-Garcia et al. (2016).

3. Results

The database was composed by 11 peer-reviewed publications (15 studies with 52 treatment means). Holstein was the predominant genetic group (82.05% of the studies), followed by Jersey (17.95%). The middle lactation stage was predominant (73.08% of the studies [100 to 200 days in milk]), followed by the beginning (≤ 100 days in milk) and end of lactation period (> 200 days in milk), and no informed, representing 19.23, 3.85 and 3.85% of the studies, respectively.

The types of molasses included were liquid molasses (sugarcane), dried molasses (sugarcane) and sugar beet molasses, representing 84.61, 9.62, and 5.77% of studies, respectively. The level of molasses inclusion observed in the studies was ≤ 30 (6.00%), 30 to 60 (42.00%), 60 to 90 (40.00%) and 90 to 120 g/kg, representing 12.00% of the studies. The predominant type of feeding system was TMR (98.07%), followed by pasture (1.93%). The level of inclusion of concentrate in the diets of the animals was ≤ 400 , and between 400 and 600, 600 to 800 g/kg and no informed, representing 16.67, 55.55, 18.52 and 9.26% studies, respectively.

3.1 Intake and digestibility of DM and nutrients

The inclusion of molasses in the diets increased the intake of dry matter (DMI; $P = 0.011$), the digestibility of the neutral detergent fiber ($P = 0.020$; Table 1) and reduced the crude protein intake ($P = 0.022$) and digestibility ($P < 0.0001$). However, it did not affect the digestibility of dry matter and organic matter ($P > 0.05$; Table 1).

3.2 Performance, composition and milk fatty acids

The inclusion of molasses in the diet reduced milk yield ($P=0.004$), milk lactose yield ($P=0.005$), milk lactose concentration ($P=0.054$), total unsaturated fatty acids (Total UFA; $P=0.045$) and total cis unsaturated fatty acids in milk (Total cis-18:1; $P=0.001$; Table 2; Figure 1). It also reduced the feed efficiency ($P=0.044$), and body weight change ($P=0.015$; Table 1).

of the animals. However, inclusion of the molasses in diet, increased solid non-fat in milk concentration (SNF; $P=0.053$), total omega-3 fatty acid in milk (Total omega-3; $P<.0001$; Table 2; Figure 1) and milk NEL (; $P<.0001$). There was no significant effect on milk fat and protein yield and concentration, milk urea nitrogen (MUN), total trans unsaturated fatty acids in milk (Total trans-18:1), total omega 6 fatty acids in milk (Total omega-6), cis-9, trans-11 conjugated linoleic acid (CLA C18:2 trans- 11, cis-9), and total polyunsaturated fatty acid (Total PUFA; Table 2; Figure 2).

3.3 Ruminal and blood parameters and nitrogen metabolism

The inclusion of molasses in the diet for dairy cows increased the ruminal pH ($P = 0.051$; Table 3), whereas, reduced the rumen acetate concentration ($P=0.005$), nitrogen intake ($P <.0001$), and blood urea nitrogen (BUN; $P<.0001$; Table 3). However, the inclusion of molasses had no effect ($P>0.05$) on ruminal concentrations of $\text{NH}_3\text{-N}$, butyrate, and propionate, nitrogen excretion in urine (N-urine), feces (N-fecal) and urinary urea-N.

3.4 Meta-Regression Analysis and Asymmetry of the funnel plot

There was no evidence of publication bias ($P> 0.05$) from the funnel plot asymmetry test (Funnel test) for any of the variables analyzed (Tables 1, 2 and 3). However, high heterogeneity (I^2 statistic $> 50\%$) was observed for 42.86% variables studied (Tables 1, 2 and 3). Based on the meta-regression analysis in Tables 4, among all the analyzed covariates (genetic group, experimental period (days), days in milk, types of molasses, molasses in diet and concentrate in diet), the covariates genetic group and days in milk, were the most influential factors for the variability of responses to the inclusion of molasses in the diet for dairy cows.

The covariates explained less than 50% heterogeneity (Adjusted R^2) for 56.25% response variables (Table 4), indicating that unknown factors not identified in this meta-analysis should affect the responses with the inclusion of molasses in the diet for dairy cows. This low representativeness (Adjusted R^2) may be associated with the low number of treatments means for the variables, mainly for the milk fatty acid (Total UFA, Total omega-6, CLA C18:2 t11 c9 and Total PUFA), which is a multifactorial response, as described by Harfoot and Hazlewood (1997), who associated the change in the milk fatty acid with factors such as diet characteristics, animal physiological status and ruminal kinetics.

3.5 Subgroup analysis

Holstein cows (Figure 4) showed an increase in DMI (WMD= 1.66kg/d; P=0.003) when receiving molasses in the diets. However, the inclusion provided a reduction in the milk protein yield (WMD= -0.02 kg/d; P=0.020) of the animals. As for the effect of including molasses in the diet for Jersey animals, only an increase in milk protein was observed (WMD=0.81g/kg; P=0.034).

Considering the effect of the covariate “days in milk” (Figure 5), it was observed that the inclusion of molasses in the diet for cows in the initial (≤ 100 days in milk) and middle stage of lactation (100 to 200 days in milk) increased DMI (WMD= 2.39 kg/d; $P < .0001$ and WMD=0.41 kg/d; P=0.021, respectively). Animals that received molasses during the initial lactation period showed an increase in the contents of SNF (WMD=0.54 g/kg; $P < .0001$). However, when cows were fed with molasses in the diet during the middle lactation period, there was a reduction in the concentration of MUN (WMD= -0.53 mg/dL; P=0.005).

For the covariate level of concentrate in the diet (Figure 6), molasses inclusions in diets with up to 600g/kg diet had no effect on DM digestibility ($P > 0.05$; Figure 6). However, the inclusion in diets with 400 - 600 g, and 600 - 800g/kg concentrate reduced the body weight change of the animals (WMD= -0.004kg; P=0.057, and WMD= -0.01 kg; P=0.022, respectively).

The type of molasses (Figure 7) used in the studies also affected the response to the inclusion of molasses in the diet for dairy cows. Liquid molasses (sugarcane) increased the solid non-fat in milk (WMD= 0.72g/kg; P=0.001) of the animals. When evaluating the effect of the covariate “molasses in diet” (Figure 8), inclusions ≤ 30 , and between 60 to 90, and 90 to 120 g molasses/kg in the diet increased the animals’ DMI (WMD= 1.61 kg/d; P=0.026, 2.03 kg/d; P=0.013, and 0.01 kg/d; P=0.011, respectively).

To test the hypothesis of the effect of including molasses in the diet on the concentration of fat in milk, a subgroup analysis of the covariates molasses in diet (g/kg DM) and concentrate in diet (g/kg DM) was performed. However, there was no significant effect of these covariates (Figure 8) on the concentration of fat in milk. For a better understanding of the negative effect of including molasses on milk yield, a subgroup analysis was run for the covariate “molasses in diet (g/kg DM)” for the variables milk yield, milk lactose yield and milk lactose concentration. Inclusions between 60 and 90, and 90 - 120 g molasses/kg reduced milk yield (WMD= -0.50 kg/d; P=0.020, and WMD=-1.11 kg/d; P=0.001, respectively). While inclusions of 90 - 120 g/kg molasses reduced the milk lactose yield (WMD= -0.06 kg/d; P=0.004; Figure

8). Inclusions between 60 and 90 g/kg molasses reduced the concentration of milk lactose (WMD= -0.32 g/kg; P=0.022; Figure 8).

4. Discussion

The increases of 4.02% in the DMI (Table 1) of the animals observed when molasses was included in the diets may be associated with greater acceptability of the diet by the animals. Molasses is an ingredient rich in soluble sugars and ruminants have shown a preference for sweet foods rich in sugars (sucrose). As observed by Nombekela et al. (1994), that evaluated different foods in the diet for dairy cows and observed an increase of 12.8% in DMI for diets with sucrose. The influence of the inclusion of molasses on the DMI of Holstein cows may be due to the greater genetic merit, due to the intensive selection pressure imposed over the years on these animals in relation to the Jersey group, which was not influenced, thus resulting in increases in their requirements to support greater milk yield. This because, according to Moraes et al. (2015), animals with greater genetic merit have a higher demand for metabolic energy in response to greater heat production under fasting conditions. Thus, these animals are more sensitive to the imbalance in the EM/PM ratio of the diets and therefore seek adjustment through increased intake that will be limited by physical factors (Forbes, 2007). This hypothesis is favored by reduction in 3.24% crude protein and 3.00 % in nitrogen intake and 1.50% in milk yield, which is associated with a 2.02% reduction in feed efficiency, combined with a 2.96% reduction in body weight, with the inclusion of molasses in the diet. The negative effect of molasses on body weight is related to the level of concentrate in the diet (covariate = concentrate in diet (g/kg)), in which a reduction in body weight was found with the inclusion of molasses in diets over 40% concentrate. According to Phuong et al. (2013), there is a decreasing linear relationship with the increase in concentrate in the diet on the efficiency of energy use in dairy cows.

Nevertheless, the negative effect of including molasses on milk yield, feed efficiency and change in body weight, should not only be related to a reduction in energy use efficiency, but possibly also to an imbalance in the metabolizable energy (ME)/metabolizable protein (MP) ratio in the diet due to the energy density caused by the inclusion of molasses, without adequate adjustment on solubility or rumen degradability of protein in the diet due to the increase in the concentration of sugar in the diet.

The hypothesis of the imbalance in the ME/MP ratio can be understood by the lack of effect of the inclusion of molasses on the NH₃-N rumen concentration, even with the reduction

in BUN, which may be related to greater recycling of nitrogen to the rumen. The BUN reduction can occur due to increase the recycling of urea to the rumen is/or increase excretion by urinary urea-N (Hristov and Jouany, 2005). However, the inclusion of molasses reduced the concentration of urinary urea-N (3.18%), demonstrating that the inclusion of molasses caused greater recycling of urea to the rumen. The positive effect of molasses on nitrogen recycling can be associated with reduced digestibility of dietary crude protein (2.70%) and in nitrogen intake (3.00%), indicating imbalance between nitrogen and energy to maximize microbial growth when NFC content of the diet is increased.

However, the need for a better fit in the relationship between the digestibility of protein fraction and carbohydrates with the inclusion of molasses in the diet, so that there are no negative effects on the performance and composition of milk, must occur when the inclusion of molasses in the diet is above 60 g/kg. It is necessary to adjust the RDP/RUP ratio in the diet when molasses is included, allowing maximum microbial growth, resulting in an increase in the passage of microbial protein for absorption by the animal. Hall et al. (2010), studying different sources of NFC (including molasses) in diet for dairy cows associated with a lower proportion of RDP, showing results that corroborate with our hypothesis, besides to observed an increase in the milk NEL and reductions in the milk protein concentration. The authors concluded that in these conditions, cows need to receive additional RUP in the diet. In the present study, the same behavior was observed, with an increase of 4.45% in milk NEL and a reduction in protein production (within covariates = genetic group (Figure 3)). These results demonstrate the need to adjust the RDP/RUP ratio, to assess accurately the effect of including molasses on the protein production in milk.

Inclusions of molasses up to 120 g/kg increased the digestibility of NDF, which may be related to an associative effect between sources of carbohydrates with rapid ruminal degradation, on the digestibility of fiber carbohydrates (Nussio et al., 2011). When evaluated the inclusion of sugars such as sucrose in the diet for dairy cows, an increase in fiber digestibility was observed (Hristov et al., 2005). The inclusion of rapidly fermentable carbohydrates in rumen, favors the growth of microorganisms that use ammonia (Hall et al., 2010). The fiber carbohydrate fermenting bacteria use ammonia as their only source of nitrogen (Russell and Wallace, 1997). When compared to the inclusion of dietary molasses in relation to the other sources of carbohydrates, the molasses still favors a shorter time of permanence of the ruminal pH below 5.8 (Razzaghi et al., 2020), further favoring the growth of fibrolytic microorganisms.

However, the inclusion of molasses allowed the stability in rumen pH, which may be associated with greater lipolytic activity and biohydrogenation of fatty acids in the diet (Fuentes et al., 2011), as well as the reduction in formation of trans-10, cis-12 conjugated linoleic acids (CLA t-10, c-12) in rumen, which has anti-lipogenic activity in the mammary gland without impairing the synthesis and incorporation of fat in milk (Harfoot and Hazlewood, 1997). This would explain the lack of effect of the inclusion of molasses on the concentration of fat in the milk. The value of $I^2 = 0.00\%$ (Heterogeneity) for this variable, represents that the whole variation is due to chance (Vesterinen et al., 2014), demonstrating the consolidation of the lack of effect of the inclusion of molasses on this response. In this context, the inclusion of molasses by up to 120 g/kg in the diet does not affect the concentration of fat in milk.

The lack of effect of including molasses on the concentration and synthesis of protein in milk may be associated with high heterogeneity ($I^2 = 97.18$ and 89.05% , respectively), for this variable. However, it was influenced by the genetic group and days in milk. The increase in milk protein concentration for Jersey cows fed with molasses can be associated with the lower nutritional requirement of this breed compared to Holstein animals as mentioned before. 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. When associated with greater efficiency in the use of nutrients with lower nutritional requirements, this breed is better adapted to diets with imbalance in the EM/PM ratio than Holstein.

The inclusion of molasses reduced the milk lactose concentration and production. It was observed that the heterogeneity for this variable is lower ($I^2 \leq 25\%$), and is associated only with the treatment effect (molasses). However, it was observed that inclusions of molasses above 60 g/kg reduced the concentration of lactose in milk. It is possible that the imbalance in the EM/PM ratio in the diet in response to the inclusion of molasses that led the animal to the negative energy balance (decreasing in body weight), activates mechanisms for the reduction in milk yield via reduction of lactose synthesis in the mammary gland.

The studies included in the dataset used in our meta-analysis presented the composition of milk fatty acids in the grouped form (i.e., omega-3, PUFA), therefore it was not possible to perform the WMD analysis to assess the effect of including molasses in the diet on the concentration of individual fatty acids in milk (i.e., oleic acid). Thus, it is necessary the quantifying separate fatty acids in the next studies making it possible to have a more accurate

assessment of the effects of molasses in the diet on the fatty acids in milk. The inclusion of molasses in the diet caused a reduction in the concentration of unsaturated fatty acids (Total UFA -10.67%); however, it increased the concentration of omega-3 fatty acids in milk (11.52%). These results partially confirm the hypothesis of this study, in which the inclusion of molasses would increase the concentration of milk fat in response to greater stability of the rumen, with the highest concentration of saturated fatty acids in milk. The reduction in the Total UFA in milk may be associated with better pH stability in the rumen as observed by Razzaghi et al. (2020) when replacing starch with sugars in diets for dairy cows. The authors observed an adequate biohydrogenation of fatty acid in the rumen, which would explain the absence of molasses effect on the Total trans-18:1 in milk. According to Shingfield et al. (2010), the increase in the production of Trans fatty acid isomers in milk increase with feeding a high-concentrate diet because of the incomplete biohydrogenation of fatty acid in the rumen.

The reduction in the concentration of total UFA in the milk with the inclusion of molasses in the diet should also be associated by the negative energy balance (body weight change -2.96%), resulting in the mobilization of body reserves causing greater incorporation of saturated fatty acids in milk. Cows kept in negative energy balance showed higher concentrations of saturated fatty acids in milk (Gross et al., 2011). During the period of negative energy balance, there was a greater mobilization of body reserves, as well as greater synthesis of C4:0 to C16:0 fatty acids by the mammary gland (Stoop et al., 2009; Brito et al., 2015). The increase in the incorporation of omega-3 in milk may be associated with changes in the microbial profile due to biohydrogenation with the inclusion of molasses in the diets. The inclusion of molasses increased the ruminal pH of the animals, and according to Fuentes et al. (2011), increases in pH from 5.6 to 6.4 increase the lipolysis of omega-6 (13.95%) and omega-3 (8.89%) fatty acids and the extension of biohydrogenation (90.48 and 51.92%), respectively. Thus, the increase in ruminal pH could respond only by reducing the Total SFA concentration, however, it does not allow full understanding of the increase in omega-3 and the absence of an effect of including molasses in the diet on cis -9, trans-11 CLA. In this context, to validate this hypothesis, further studies are needed evaluating the effect of molasses on the alteration of the microbiota with a consequent change in ruminal biohydrogenation, mainly to better understand the increase in the omega-3 concentration in milk.

5. Conclusion

Inclusions of molasses greater than 60 g/kg of DM in dairy cow diets affect negatively yield of milk and lactose. Moreover, concentration of protein and lactose in the milk was reduced. However, inclusions of molasses up to 120 g/kg of DM in diet, did not affect the yield and concentration of fat in milk.

The inclusions of molasses in the diet caused a reduction in the concentration of unsaturated fatty acids (Total UFA -10.67%); however, it increased the concentration of omega-3 fatty acids in milk (11.52%), without affecting total of polyunsaturated fatty acids and C18: 2 cis-9, trans-11 CLA. Our meta-analysis demonstrates no effectiveness of molasses in improving milk composition and fatty acid of importance to human health as the C18: 2 cis-9, trans-11 CLA.

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Table 1: Effect of the molasses in the diet of dairy cow on the intake, digestibility performance and body weight

Item ¹	Control ²	N ³	Molasse		Heterogeneity ⁵		Funnel test ⁶
	Mean (SD)		⁴ WMD _{Random effect} (95% CI)	<i>P</i> -value	<i>P</i> -value	I ² (%)	<i>P</i> -value
Intake, kg/d							
DM	22.3 (3.14)	42	0.90 (0.21, 1.58)	0.011	<.0001	88.80	0.361
CP	3.39 (0.82)	7	-0.11 (0.09, 0.49)	0.022	0.562	0.00	0.089
Digestibility, g/kg of DM							
DM	654 (53.6)	13	2.01 (-9.62, 13.63)	0.735	<.0001	83.81	0.571
OM	689 (63.8)	21	0.41 (-4.89, 5.71)	0.879	0.684	0.00	0.792
CP	658 (88.9)	20	-17.8 (-24.7, -10.8)	<.0001	0.862	0.00	0.403
NDF	504 (109)	22	7.89 (1.24, 14.5)	0.020	0.932	0.00	0.662
Feed Efficiency	1.24 (0.31)	42	-0.03 (-0.04, -0.00)	0.044	<.0001	96.21	0.470
Body Weight, kg							
Change	0.27 (0.16)	38	-0.008 (-0.01, -0.00)	0.015	<.0001	79.25	0.630

¹DM= dry matter; OM= organic matter; NDF= neutral detergent fiber; CP= crude protein.

²Control treatment (without molasses).

³N = number of comparisons of control and treatments (molasses) (complete data set is available in Supplementary File S1).

⁴WMD = weighted mean differences between control and with treatments.

⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test.

Table 2: Effect of the molasses in the diet of dairy cow on milk yield and milk composition

Item ¹	Control ² Mean (SD)	N ³	Molasse		Heterogeneity ⁵		Funnel test ⁶
			⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
Milk yield, kg/d	29.4 (8.48)	45	-0.44 (-0.75, -0.14)	0.004	0.592	0.00	0.285
Milk fat yield, kg/d	1.09 (0.32)	50	0.001 (-0.02, 0.03)	0.930	<.0001	95.34	0.745
Milk protein yield, kg/d	0.94 (0.22)	50	-0.01 (-0.02, 0.00)	0.158	<.0001	97.18	0.116
Milk lactose yield, kg/d	1.38 (0.57)	18	-0.04 (-0.07, -0.01)	0.005	0.841	0.00	0.134
Milk fat, g/kg	37.9 (6.52)	44	-0.05 (-0.44, 0.33)	0.791	0.678	0.00	0.854
Milk protein, g/kg	32.4 (2.41)	49	-0.22 (-0.55, 0.12)	0.206	<.0001	89.85	0.112
Milk lactose, g/kg	47.5 (1.13)	20	-0.17 (-0.33, 0.00)	0.054	0.306	11.91	0.854
Milk SNF, g/kg	88.4 (1.60)	11	0.40 (-0.00, 0.80)	0.053	0.008	58.06	0.741
MUN, mg/dL	13.6 (2.20)	25	-0.16 (-0.48, 0.16)	0.333	<.0001	65.01	0.198
Milk NE _L , Mcal/d	24.5 (1.26)	11	1.09 (0.63, 1.54)	<.0001	0.516	0.00	0.079
Fatty Acid in Milk, mg/g FA milk							
Total UFA	305 (11.0)	4	-32.5 (-64.3, -0.69)	0.045	<.0001	92.29	0.386
Total <i>cis</i> -18:1 ⁷	166 (40.6)	5	-6.59 (-10.57, -2.61)	0.001	0.419	0.00	0.085
Total <i>trans</i> -18:1 ⁸	34.8 (13.5)	5	-0.58 (-1.50, 0.34)	0.213	0.975	0.00	0.559
Total omega-3	8.68 (1.15)	4	1.00 (0.51, 1.49)	<.0001	0.249	27.03	0.368
Total omega-6	18.3 (2.85)	4	-3.36 (-7.72, 1.00)	0.132	<.0001	97.45	0.539
CLA C18:2 <i>cis</i> 9, <i>trans</i> 11	6.91 (1.91)	8	-0.23 (-0.77, 0.31)	0.412	<.0001	81.01	0.923
Total PUFA	44.3 (3.85)	4	-2.85 (-9.63, 2.94)	0.335	<.0001	88.98	0.627

¹ SNF= solid not fat; MUN= nitrogen ureic in milk; FA= fatty acid; CLA=acid linoleic conjugated; UFA= unsaturated fatty acid; PUFA= polyunsaturated fatty acid; NE_L= Energy net of lactation. ² Control treatment (without molasses); ³N = number of comparisons of control and with treatments (molasses) (complete data set is available in Supplementary File S1). ⁴WMD = weighted mean differences between control and with treatments. ⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity. ⁶Egger's regression asymmetry test. ⁷Total *cis*-18:1 = *cis*-9 18:1 + all *cis*-18:1 FA

⁸ Total *trans*-18:1 = *trans*-10 18:1 + *trans*-11 18:1 + all *trans*-18:1 FA

Table 3: Effect of the molasses in the diet of dairy cow on the ruminal parameters, nitrogen metabolism.

Item ¹	Control ²	N ³	Molasses		Heterogeneity ⁵		Funnel test ⁶
	Mean (SD)		⁴ WMD _{Random effect} (95% CI)	P-value	P-value	I ² (%)	P-value
pH	6.01 (0.40)	11	0.04 (-0.00, 0.08)	0.051	0.478	0.00	0.738
N-NH ₃ , mg/dL	12. 2 (4.97)	11	-0.37 (-1.24, 0.52)	0.417	0.139	32.46	0.392
SCFA, mol/100mol							
Acetate	68.4 (9.44)	7	-0.76 (-1.29, -0.23)	0.005	0.993	0.00	0.469
Propionate	23.9 (4.40)	15	0.05 (-0.50, 0.60)	0.866	0.985	0.00	0.519
Butyrate	12.9 (2.33)	15	0.15 (-0.18, 0.49)	0.380	0.937	0.00	0.967
Nitrogen Metabolism, g/d							
N-Intake	484 (102)	5	-14.53 (-18.1, -10.9)	<.0001	<.0001	87.71	0.195
N-Urine	212 (76.4)	12	-6.08 (-15.9, 3.72)	0.224	0.088	37.96	0.072
N-Fecal	311 (6.02)	6	15.1 (-3.19, 33.5)	0.106	0.223	28.29	0.088
Urinary ureia-N	139 (26.9)	9	-4.44 (-13.8, 4.91)	0.352	0.039	51.98	0.938
BUN, mg/dL	22.5 (3.60)	7	-1.74 (-2.35, -1.14)	<.0001	0.993	0.00	0.751

¹ SCFA= short- chain fatty acid; NUUE= nitrogen urea urine excretion; BUN= Nitrogen urea blood; N=nitrogen.

² Control treatment (without molasses)

³N = number of comparisons of control and with treatments (molasses) (complete data set is available in Supplementary File S1).

⁴WMD = weighted mean differences between control and with treatments

⁵I² = proportion of total variation of size effect estimates that is due to heterogeneity; P-value to χ^2 (Q) test of heterogeneity.

⁶Egger's regression asymmetry test.

Table 4. Meta-regression of the effect molasses in the diet of dairy cow on weighted mean differences (WMD) between treatment and control.

Dependent variable (Y, WMD)	Meta-regression parameters (<i>P</i> -value) ¹							Adjusted R ² (%)	N ²
	Intercept	Genetic Group	Days in milk	ED	Molasses Type	Molasses diet	CON in diet		
DMI, kg/d	-3.31 (0.641)	-1.21 (0.040)	5.04 (0.105)	--	2.43 (0.582)	1.39 (0.071)	2.36 (0.601)	100	42
DMD, g/kg of DM	15.3 (0.179)	19.4 (0.051)	-23.1 (0.223)	--	11.2 (0.384)	-35.7 (0.031)	-22.6 (0.001)	100	13
Feed Efficiency	0.18 (0.246)	-0.18 (0.200)	-0.15 (0.279)	--	-0.13 (0.136)	0.04 (0.612)	0.04 (0.791)	0.00	42
BW change, kg/d	-0.05 (0.791)	-0.14 (0.463)	-0.22 (0.245)	--	0.14 (0.216)	0.03 (0.820)	0.01 (0.101)	0.00	38
Milk Fat, kg/d	0.13 (0.572)	-0.23 (0.195)	0.07 (0.301)	--	-0.12 (0.377)	0.11 (0.322)	0.08 (0.619)	16.15	50
Milk Protein yield, kg/d	-0.17 (0.074)	-0.08 (0.011)	0.08 (0.001)	--	0.13 (0.010)	0.07 (0.192)	0.01 (0.811)	48.31	50
Milk Protein, g/kg	-5.53 (0.001)	2.07 (0.109)	2.32 (0.071)	--	2.95 (0.001)	0.71 (0.552)	-0.58 (0.729)	59.49	49
SNF, g/kg	-0.70 (0.113)	--	-1.61 (0.022)	--	2.20 (0.001)	0.03 (0.988)	--	90.87	11
MUN, mg/dL	0.06 (0.964)	1.80 (0.091)	-2.01 (0.078)	--	-0.59 (0.292)	0.09 (0.913)	--	54.03	25
CLA C18:2 t11c9	-0.32 (0.781)	0.96 (0.448)	0.92 (0.551)	--	--	-0.64 (0.635)	--	0.00	8
N-Intake, g/d	-14.2(0.011)	--	-28.8 (0.150)	--	--	20.0 (0.481)	--	0.63	5
Urinary urea-N, g/d	-38.5 (0.011)	11.2 (0.253)	--	--	24.8 (0.001)	12.5 (0.401)	--	100	9

¹ED =experiment in day; CON= concentrate in diet (g/kg); DMI=dry matter intake; CPI= crude protein intake; DMD= dry matter digestible; BW= body weight; SNF= solid non-fat in milk; MUN= nitrogen ureic milk; UFA= unsaturated fatty acid; CLA=acid linoleic conjugated; N= nitrogen. Adjusted R² = proportion of the between-study variance (heterogeneity) explained by the covariate. ³N = number of comparisons between treatment with (molasses) and control (without molasses) (complete data set is available in Supplementary file S1).

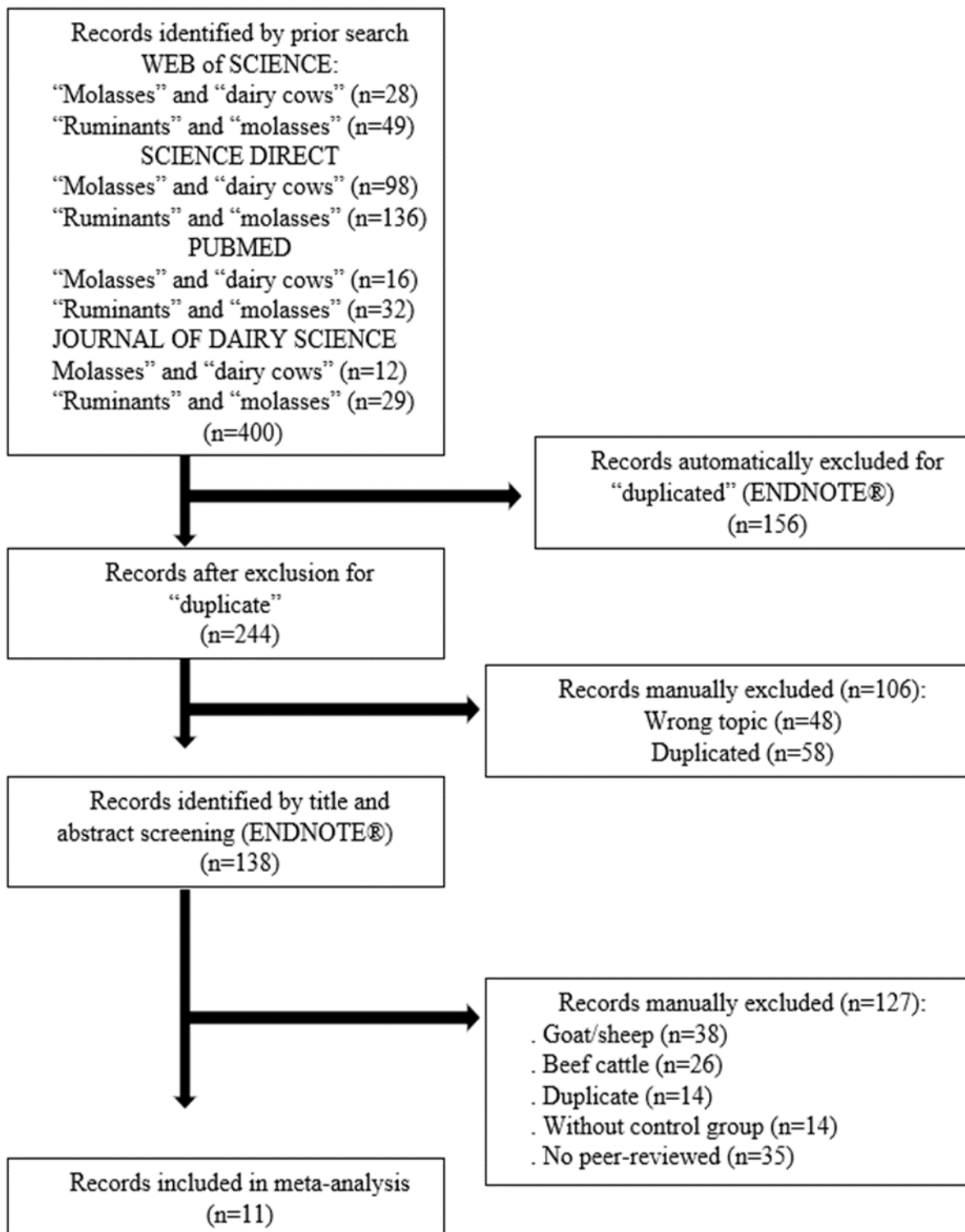


Figure 1. Flowchart showing inclusion criteria for selection of the studies used for conducting the meta-analysis of the effects of molasses in dairy cow diet

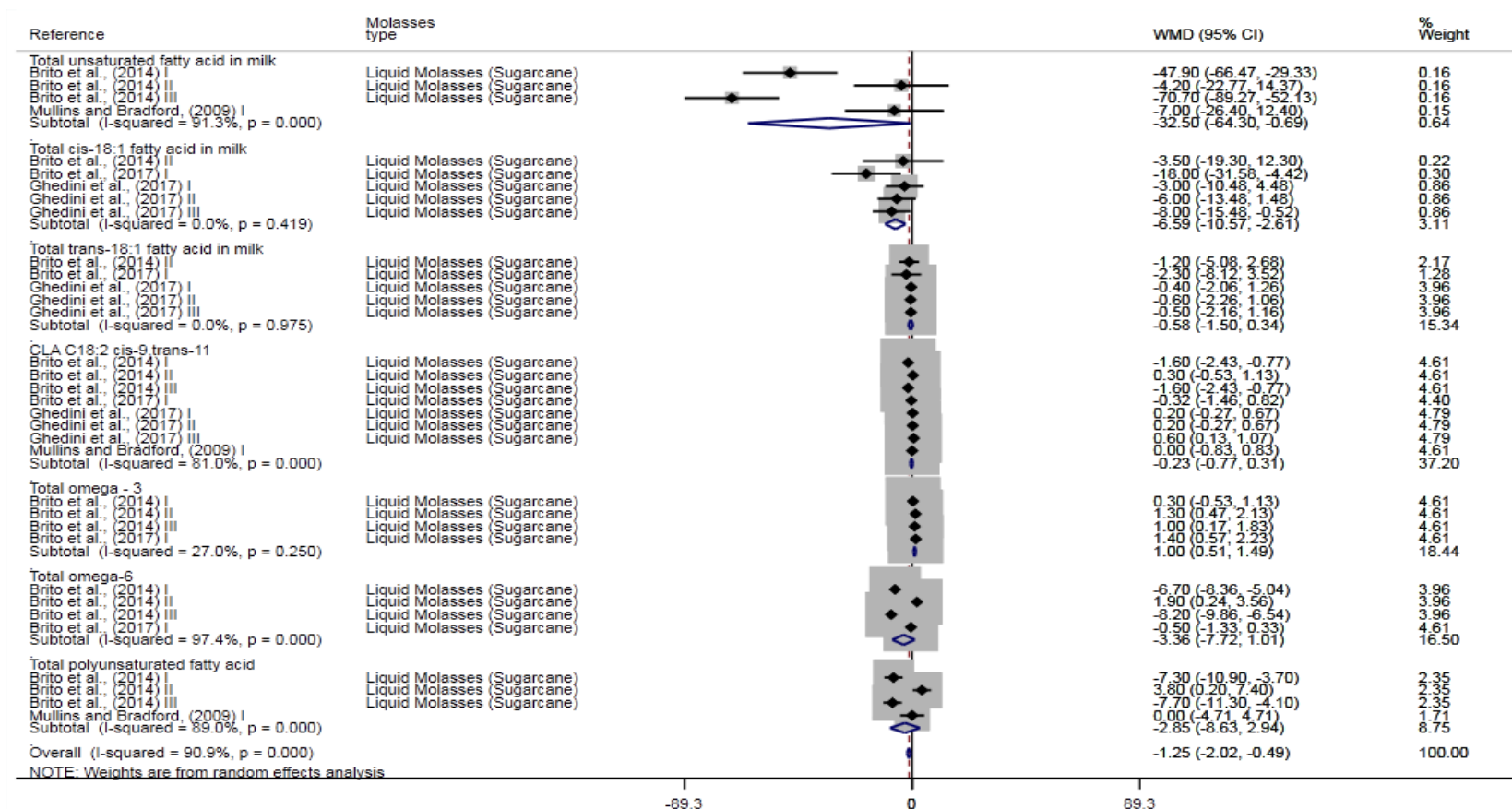


Figure 2. Forest plot showing the effect of the use of Molasses on the diet of dairy cows on milk fatty acid profile (mg/g of FA Milk). The x-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent a reduction in the measure, whereas diamonds to the right of the 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.

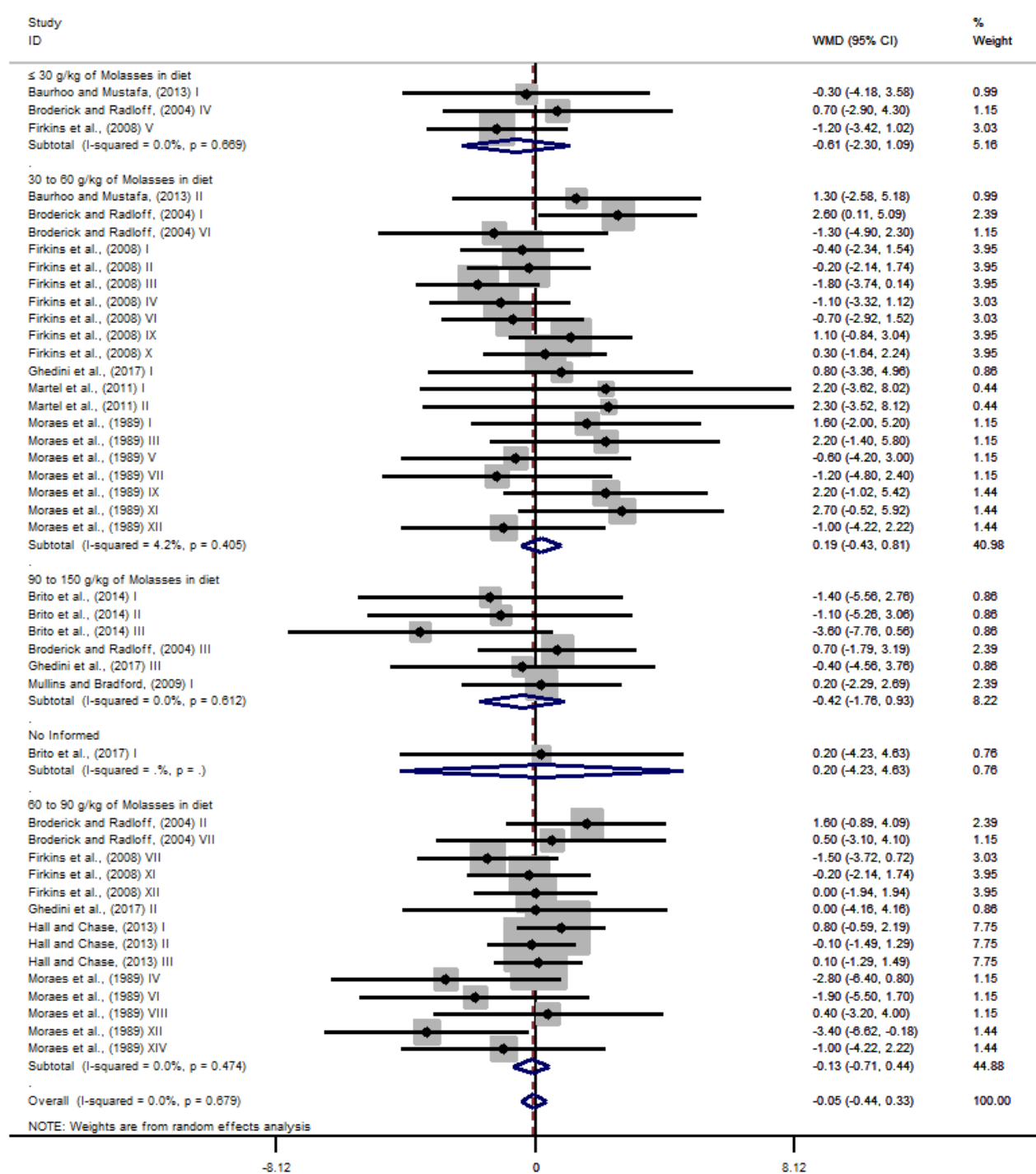


Figure 3. Forest plot (with Subgroup analysis (Covariate= Molasses in diet (g/kg)) showing the effect of the use of molasses on the diet of dairy cows on milk fat (g/kg). The x-axis shows the weighted mean difference (WMD); diamonds to the left of the solid line represent a reduction in the measure, 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.

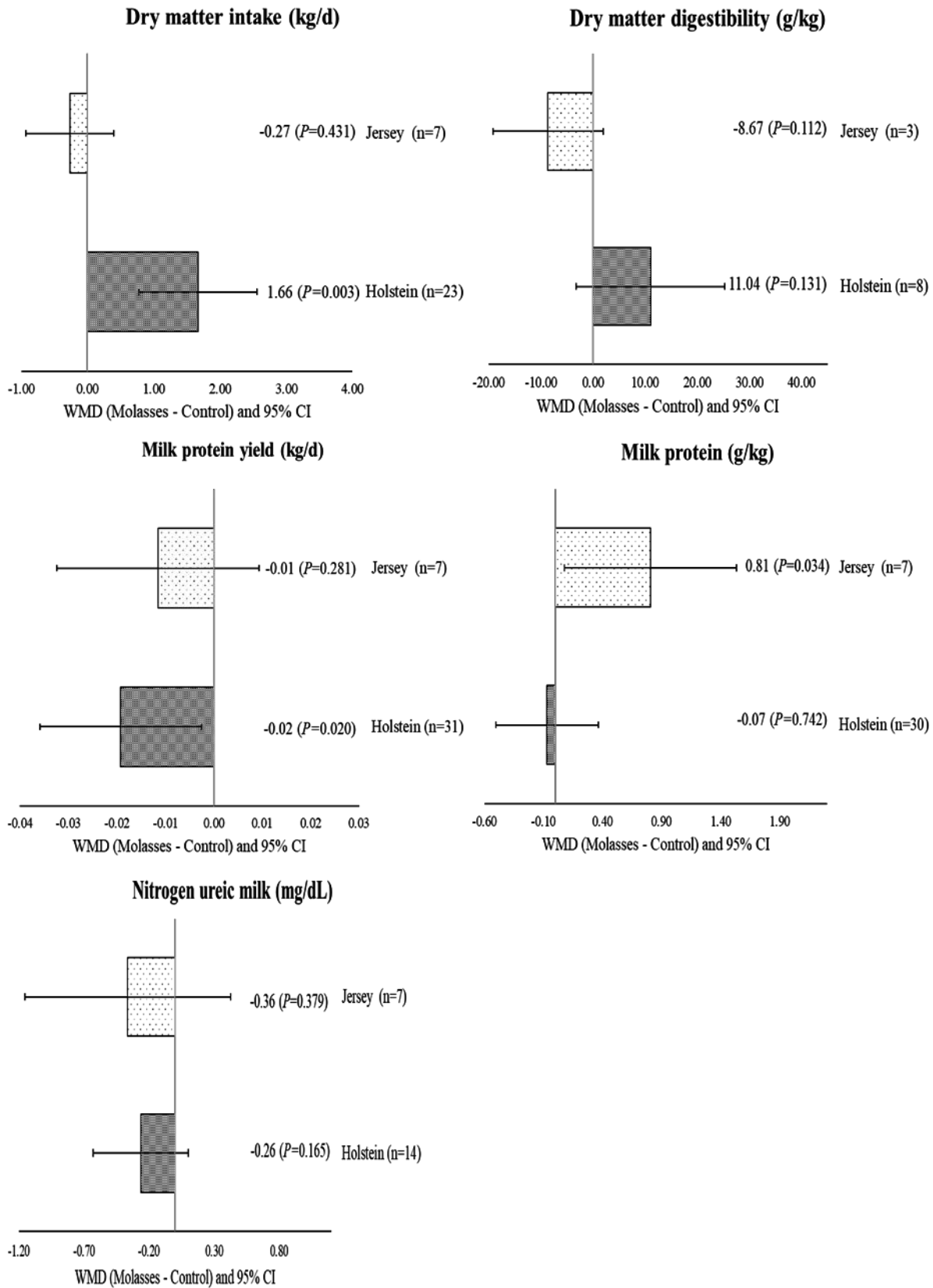


Figure 4. Subgroup analysis (Subgroup = Genetic group) of the effect of the use of molasses in the diet of dairy cows, WMD = weighted mean differences between molasses and control treatments.

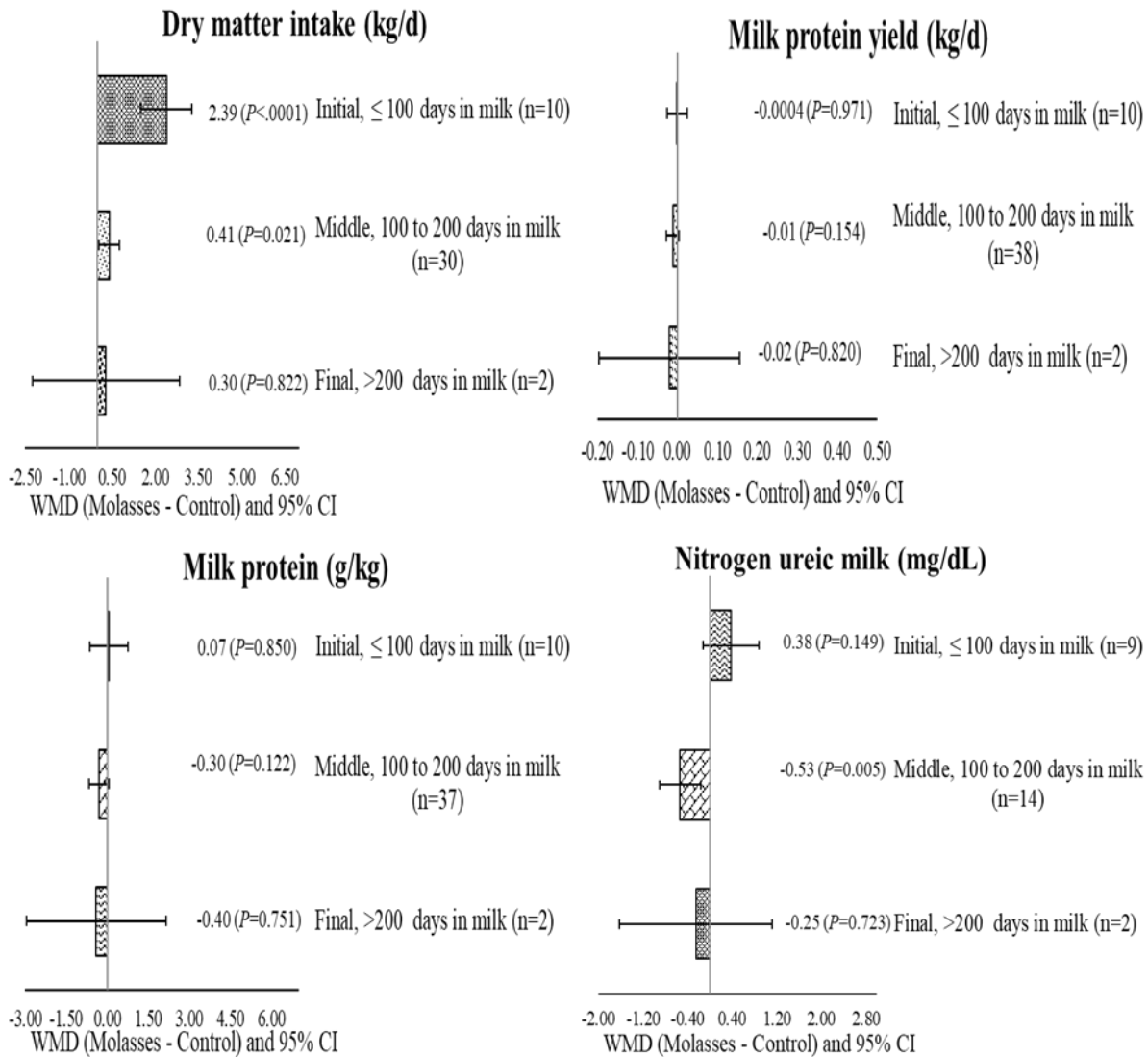
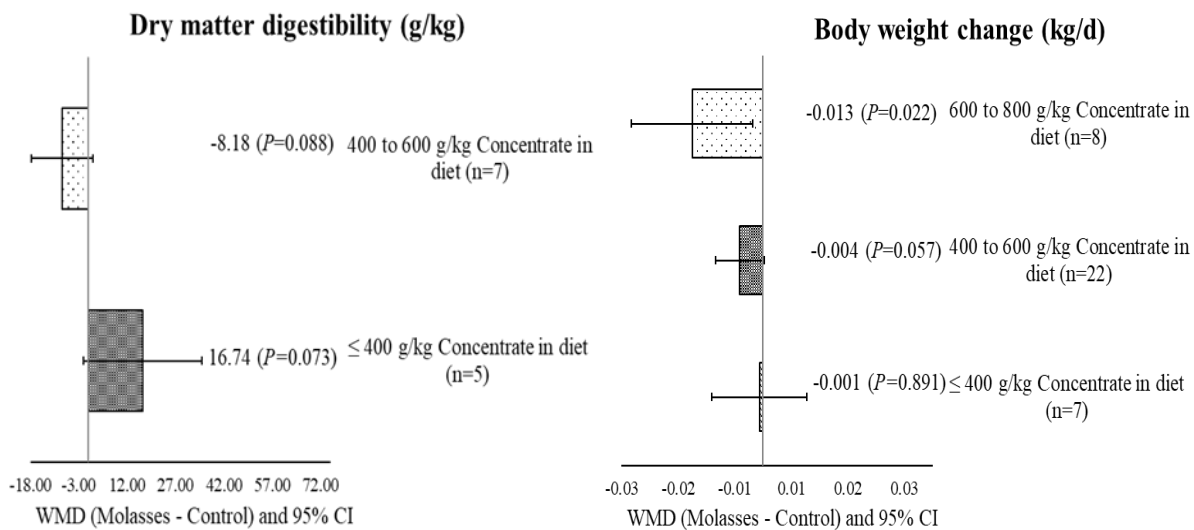


Figure 5. Subgroup analysis (Subgroup = Days in Milk) of the effect of the use of molasses in the diet of dairy cows, WMD = weighted mean differences between molasses and control treatments.



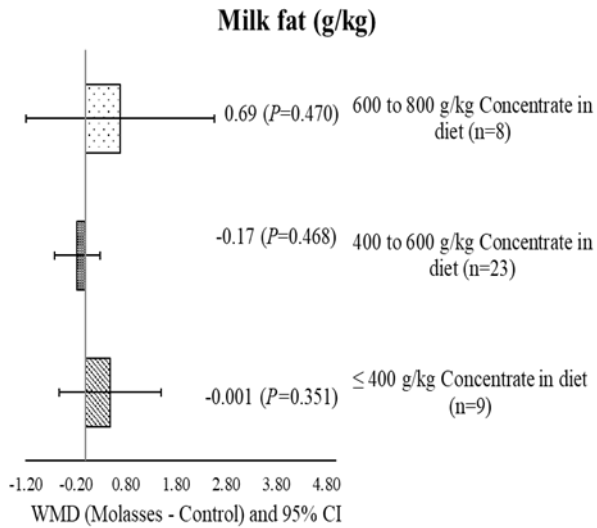


Figure 6. Subgroup analysis (Subgroup = Concentrate in diet (g/kg)) of the effect of the use of molasses in the diet of dairy cows, WMD = weighted mean differences between molasses and control treatments.

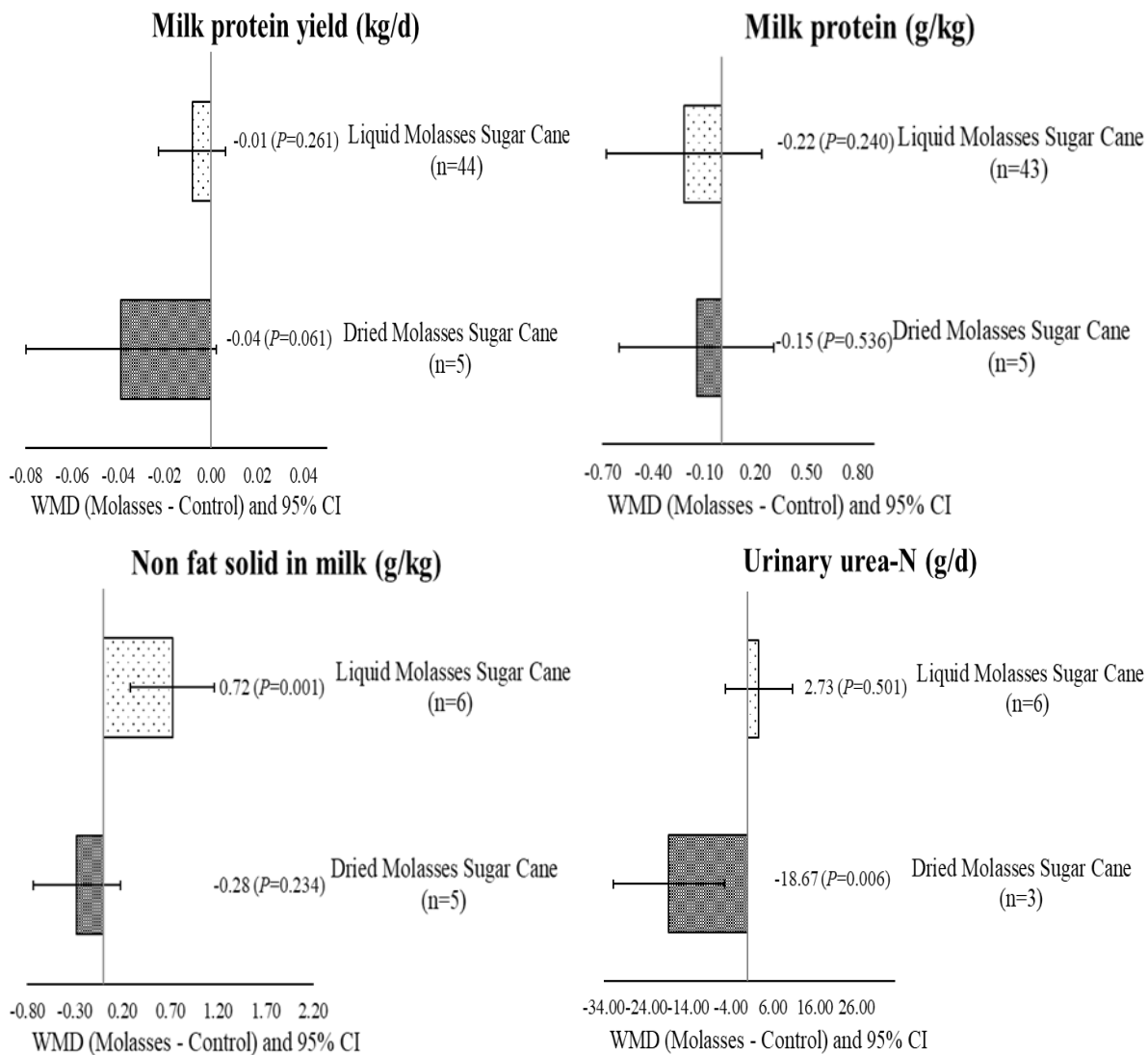
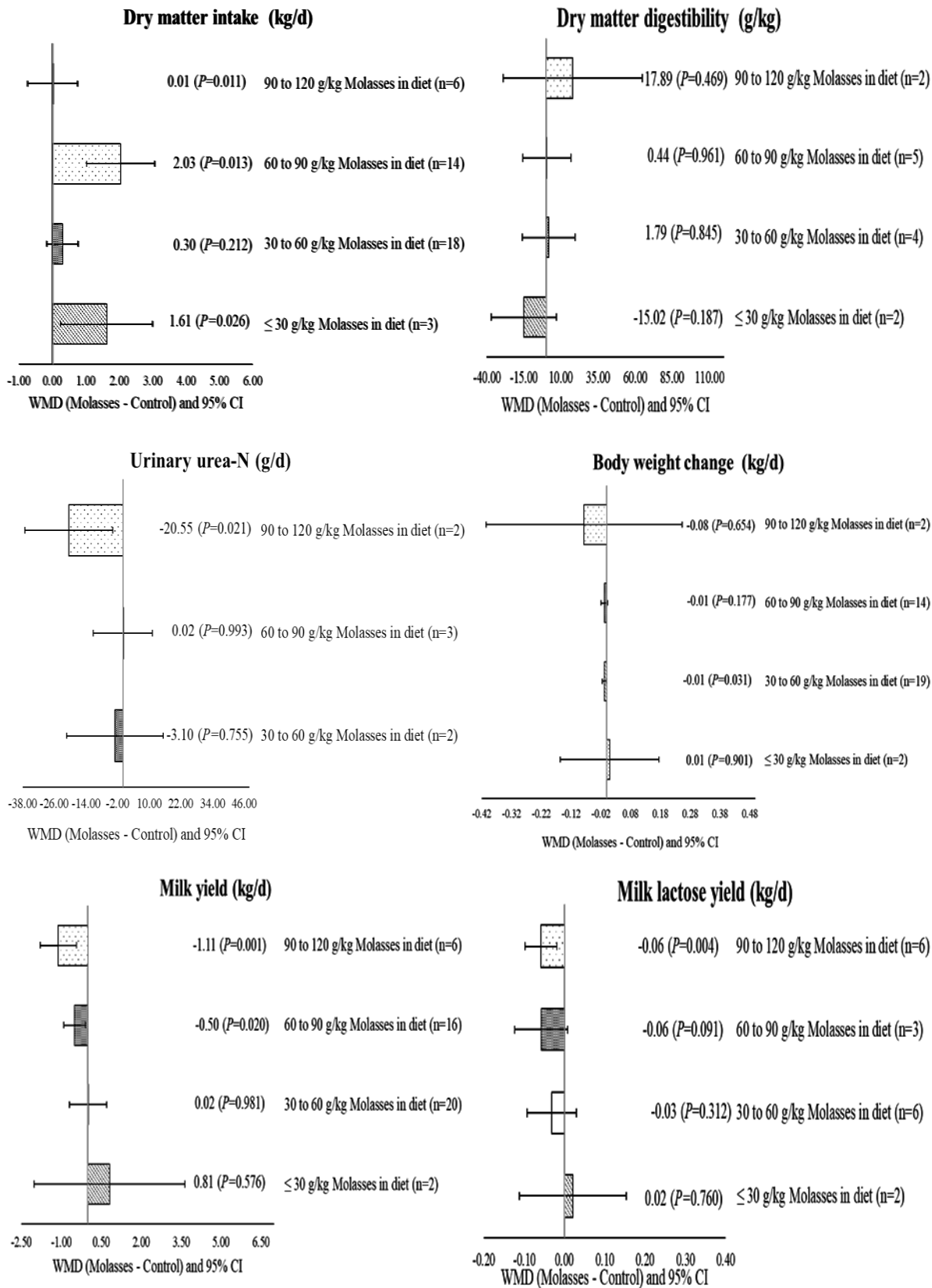


Figure 7 Subgroup analysis (Subgroup = Type of molasses) of the effect of the use of molasses in the diet of dairy cows , WMD = weighted mean differences between molasses and control treatments.



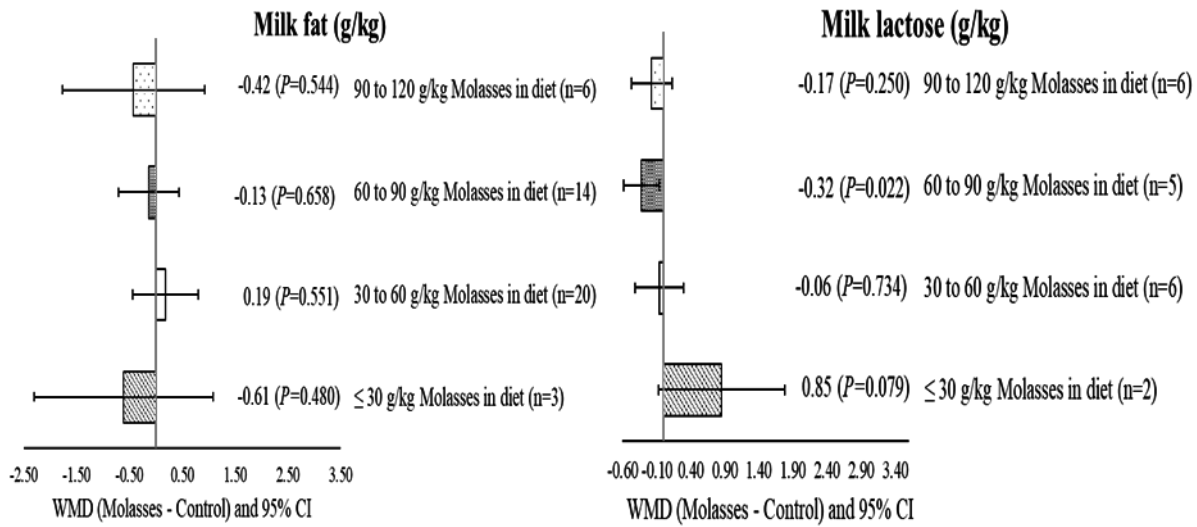


Figure 8. Subgroup analysis (Subgroup = Molasses in diet (g/kg)) of the effect of the use of molasses in the diet of dairy cows, WMD = weighted mean differences between molasses and control treatments.