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Research Article

Dietary Oil Source and Inclusion Level Determine Milk Fatty Acid Health Indices in Dairy Cows: A Meta-Analysis

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Abstract | This study aimed to identify the effective oil sources and inclusion levels for modulating milk fatty acid composition to improve milk fat–related health indices in dairy cows. A meta-analysis was conducted to synthesize evidence from studies investigating dietary oil supplementation and its effects on milk fatty acid profiles. From 270 initially identified publications, 32 studies met the inclusion criteria for quantitative analysis. Oil supplementation significantly increased the PUFA/SFA ratio (estimate = 0.23; CI = 0.118–0.343; P < 0.001) and reduced the atherogenicity index (estimate = −0.158; CI = -0.3–0.0015; P = 0.03) and thrombogenicity index (estimate = −0.119; CI = -0.229 to -0.009; P = 0.034), indicating a shift toward a more favorable milk lipid profile, reflecting an increase in beneficial unsaturated fatty acids and a reduction in atherogenic and thrombogenic lipid fractions. Soybean and linseed oils (approximately 2.3% DM), and sunflower oil at 2–4% DM, improved PUFA/SFA ratio, IA, IT, and the Unsaturation Index. Fish oil (approximately 0.23% DM) was most effective in increasing EPA+DHA (estimate = 1.171; CI = 0.605–1.737; P < 0.001) and reducing thrombogenicity. In contrast, HH, HPI, and LA/ALA showed numerical but non-significant changes. Trans fatty acids increased significantly (estimate = 1.822; CI = 1.458–2.185; P < 0.001), reflecting enhanced ruminal biohydrogenation, with a potential trade-off in lipid quality that does not offset the overall benefits observed. Overall, dietary oil supplementation effectively improves milk fatty acid health indices, with responses varying depending on oil source and inclusion level.

Keywords | Dairy cows, Meta-analysis, Milk fatty acid health indices, Oil, Supplementation

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INTRODUCTION

Fat is one of the main nutritional components of milk. With the increasing public awareness of healthy lifestyles, greater attention has been given to the quality of milk fat. Milk fat is often discussed in relation to its content of saturated fatty acids (SFA), which have traditionally been associated with adverse health effects, particularly

cardiovascular diseases, including caproic, caprylic, lauric, and palmitic acid (Despal *et al.*, 2021). These saturated fatty acids have been associated with various health disorders, particularly cardiovascular diseases. However, recent advances in nutritional science indicate that the health effects of SFA are not uniform and depend on their specific structure and biological context and their effects depend on chain length, structure, and metabolic context. Therefore, evaluating milk fat solely based on total SFA content may lead to oversimplified conclusions. Nevertheless, milk fat also contains unsaturated fatty acids that provide health benefits, including linoleic acid, conjugated linoleic acid (CLA), and palmitoleic acid, which are known to reduce the risk of cardiovascular disease and metabolic disorders (Despal *et al.*, 2021). Accordingly, a more comprehensive evaluation of milk fat quality is often conducted using fatty acid health indices, such as the index of atherogenicity (IA), index of thrombogenicity (IT), and related metrics, which integrate fatty acid composition into indicators of lipid nutritional quality. However, these indices should be interpreted as indirect proxies rather than direct measures of human health outcomes, as their implications depend on overall dietary patterns and physiological context. The use of these indices allows for a more objective comparison of milk fat quality, although they simplify the complexity of individual fatty acids and do not fully capture the heterogeneity of saturated fatty acids, whose health effects may vary depending on their structure and metabolic context.

Efforts to improve milk fat quality through nutritional manipulation have become a major focus in ruminant nutrition research. Feed is recognized as one of the primary factors influencing overall milk composition and quality (Apriliana *et al.*, 2025, 2026). Consequently, dietary strategies are considered a key approach to modulating specific milk components, including milk fat and its fatty acid composition. One of the most widely studied approaches is dietary oil supplementation, as oils are concentrated sources of fatty acids with highly variable compositions, particularly in terms of unsaturated fatty acid content. Numerous studies have examined the effects of oil supplementation on milk fatty acid profiles. The fatty acid profile data generated from these studies can subsequently be used to calculate milk fatty acid health indices. Oil supplementation represents a strategic approach because the fatty acid composition of oils, especially their unsaturated fatty acid content, can influence ruminal biohydrogenation processes and, consequently, the fatty acid composition of milk. A recent study demonstrated that supplementation with different types of oils resulted in variations in milk fatty acid profiles, leading to differences in milk fatty acid health indices (Benchaar *et al.*, 2025). Oil supplementation represents a strategic approach because the fatty acid composition of oils, particularly their high content of unsaturated fatty acids, can influence ruminal lipid metabolism. In ruminants, dietary unsaturated fatty acids undergo ruminal biohydrogenation, a microbial process in which unsaturated fatty acids are progressively hydrogenated into more saturated forms. This process also produces intermediate fatty acids that may subsequently be incorporated into milk fat (Dewanckele *et al.*, 2020). Therefore, differences in oil source and inclusion level can modify the extent of ruminal biohydrogenation and, consequently, alter the fatty acid profile and health-related indices of milk.

However, although previous meta-analyses have evaluated the effects of dietary lipid supplementation on milk fatty acid composition, these studies were generally limited to a narrower range of lipid sources and primarily focused on individual fatty acids. In addition, earlier studies mainly evaluated oilseeds in whole, ground, or processed forms, rather than oil-based or modified lipid supplements. In contrast, the present study incorporates a broader diversity of lipid sources, including sunflower, safflower, linseed, palm oil, rubber seed, crystallized fish oil, isomerized poppy seed oil, soybean oil, and other oil-based supplements. Furthermore, this study includes various forms of lipid supplementation, particularly extracted oils and modified lipid products, which are more representative of practical feeding strategies and may exert different effects on ruminal lipid metabolism. Moreover, rather than focusing solely on individual fatty acids, the present study integrates fatty acid composition into health-related indices (e.g., IA, IT, UI, HPI), allowing for a more comprehensive and functionally relevant evaluation of milk fat quality. Therefore, a systematic synthesis combining diverse lipid sources and evaluating their effects using fatty acid health indices remains limited. Therefore, this meta-analysis was conducted to integrate the available evidence and to determine the oil sources and supplementation levels that are most effective in producing the most favorable milk fatty acid health indices.

MATERIALS AND METHODS

Literature search and data collection

A database was constructed from scientific publications retrieved from the Scopus database through the IPB University institutional network. The literature search was conducted using the Scopus database due to its extensive coverage of peer-reviewed journals and accessibility through the institutional network, and was performed up to October 2025. The search strategy was structured using a PICO framework, including population (dairy cows), intervention (dietary oil supplementation), and outcomes

(milk fatty acid profile and derived lipid health indices), combined with Boolean operators and structured keywords.

The search string included combinations of terms such as: ("dairy cow" OR cattle OR bovine) AND ("oil supplementation" OR "dietary lipid" OR "fat supplementation") AND ("milk fatty acid" OR "lipid profile" OR "milk composition"). To minimize the risk of missing relevant studies, reference lists of selected articles and relevant reviews were also screened manually. Articles were included only if they were published in English, contained a control group, involved in vivo oil supplementation in dairy cows, and reported milk fatty acid measurements. Studies were excluded if they lacked sufficient quantitative data, were not conducted on dairy cows, or were review articles or conference proceedings.

Study selection and data extraction

Study selection and data extraction were performed independently by three reviewers, and any discrepancies were resolved through discussion and consensus to ensure consistency and accuracy of the extracted data. Although formal inter-rater agreement statistics (e.g., Cohen's kappa) were not calculated, consistency among reviewers was ensured through this process. The screening process followed a structured procedure including title/abstract screening and full-text evaluation, as summarized in Figure 1. Reasons for exclusion at the full-text stage included lack of quantitative fatty acid data, absence of a control group, and studies not involving dairy cows. Following abstract and full-text screening, 32 studies (comparisons) were retained for analysis, as summarized in Table 1. In studies reporting multiple treatments or experimental periods, each condition was extracted and analyzed as a separate comparison. Therefore, the number of comparisons exceeds the number of individual publications. When multiple experiments were reported within a single publication, each experiment was coded as an independent data unit. This approach was used to maximize data utilization; however, potential within-study dependency is acknowledged as a limitation. The resulting database comprised milk fatty acid composition data used to calculate PUFA/SFA, IA, IT, HH, HPI, EPA+DHA, FLQ, LA/ALA, and TFA according to the equations of Chen and Liu (2020), as presented in Table 2.

Data analysis

The data obtained were analyzed using a random-effects meta-analysis approach. A random-effects model was applied to account for substantial between-study heterogeneity, which is common in nutritional meta-analyses involving diverse experimental conditions such as differences in oil type, inclusion level, and experimental design. Effect sizes were calculated using Hedges' d as a standardized measure to allow comparison across studies with heterogeneous measurement scales and reporting units. Although fatty acid data are expressed as proportions (g/100 g of total fatty acids), values were generally not near the theoretical bounds, and thus the use of standardized effect sizes was considered appropriate for comparative analysis.

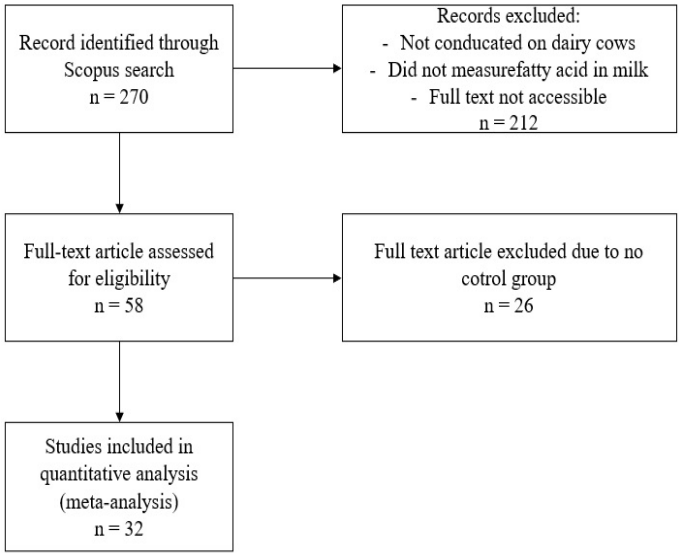

Figure 1: Flow diagram of literature search and study selection process for the meta-analysis.

Meta-analyses were conducted using OpenMEE software (build date: 2016-07-26). Model convergence and stability of estimates were checked to ensure consistency of the results. Heterogeneity among studies was assessed using the I^2 statistic to quantify the proportion of variance attributable to between-study differences. Values of approximately 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively.

A formal risk-of-bias assessment (e.g., SYRCLE) was not conducted. Although such tools can be applied to animal studies, substantial variability and incomplete methodological reporting across the included studies limited the feasibility of consistent assessment. This limitation is acknowledged and results were interpreted with caution.

RESULTS AND DISCUSSION

All health-related indices evaluated in this study reflect distinct layers of lipid remodeling along the rumen–mammary axis, illustrating how dietary oil supplementation reshapes milk fatty acid composition through coordinated ruminal and mammary metabolism. Indices such as PUFA/SFA, IA, and IT primarily capture shifts in dominant fatty acid classes driven by altered ruminal biohydrogenation and reduced mammary de novo synthesis, whereas composite metrics including HH, HPI, and LA/ALA

Table 1: Studies on milk fatty acid profiles in dairy cows receiving dietary fat supplementation.

No	References[1]	Fat Source	Feeding rate (%) DM Intake
1	(Benchaar *et al.*, 2025)	Sunflower; Safflower; Linseed	4; 4; 4
2	(Gunun *et al.*, 2025)	Palm Oil; Rubber Seed	2.1; 2.14
3	(Bodkowski *et al.*, 2024)	Crystalisation Fish oil (14 d; 30 d)	1; 1
4	(Bodkowski *et al.*, 2020)	Isomerized poppy seed oil (7 d; 14 d;30 d)	1; 1; 1
5	(Vargas-Bello-pérez *et al.*, 2019)	Soybean oil; Fish oil	3; 3
6	(Castro *et al.*, 2019)	Soybean oil; Linseed oil	2.3; 2.3
7	(Salles *et al.*, 2019)	Sunflower	4
8	(Kliem *et al.*, 2019)	Calcium salts of palm and linseed oil	4.4
9	(Prieto-Manrique *et al.*, 2018)	Sunflower oil	2; 4
10	(Halmemies-Beauchet-Filleau *et al.*, 2017)	Camelina oil	2; 4; 6
11	(Macedo *et al.*, 2016)	Soybean Oil (Low Concentrate; High Concentrate)	5.6; 2.8
12	(Suksombat *et al.*, 2016)	Palm oil+Linseed oil; Linseed oil	2.9; 2.9
13	(Yang & He, 2016)	Garlic essential oil; Juniper berry essential oil	0.02; 0.01
14	(Puppel *et al.*, 2016)	Fish Oil	0.23
15	(Boerman & Lock, 2014)	Soybean oil	2
16	(Dallaire *et al.*, 2014)	Sterculia foetida oil; Fish oil	0.028; 1.78
17	(Benchaar *et al.*, 2012)	Linseed oil	2; 3; 4
18	(Halmemies-Beauchet-Filleau *et al.*, 2011)	Rapeseed; sunflower-seed oil; Camelina-seed oil	2.9; 2.9; 2.9
19	(Kupczyński *et al.*, 2011)	Fish Oil (0 week; 4 week; 8 week)	2; 2; 2
20	(Caldari-Torres *et al.*, 2011)	Ca-salt of transoctadecenoic FA; Safflower oil FA	1.8; 1.8
21	(Glasser *et al.*, 2010)	Linseed oil (high concentrate; low concentrate)	3; 3
22	(Caroprese *et al.*, 2010)	Fish oil - microencapsulated	1.1
23	(van Vuuren *et al.*, 2010)	Linseed and soybean oil - emulsion gel (restricted grazing); Linseed and soybean oil - emulsion gel (Indoor feeding)	4.2; 2.5
24	(Medeiros *et al.*, 2010)	Ca-salt palm oil FA P1 (42-47 d); Ca-salt palm oil FA P1 (70-74 d)	0.7; 0.7
25	(Zachut *et al.*, 2010)	Flaxseed oil; Sunflower	3.8; 3.8
26	(Rego *et al.*, 2009)	Rapeseed oil; Sunflower oil; Linseed oil	10; 10; 10
27	(Flowers *et al.*, 2008)	Linseed oil	2.57; 5.12; 7.67
28	(Huang *et al.*, 2008)	Soy oil; Soy oil + CLA; Soy oil + Ca(CLA)2	5; 4; 4
29	(AbuGhazaleh & Holmes, 2007)	Fish oil+ sunflower oil	5
30	(Rego *et al.*, 2005)	Fish oil	4; 8
31	(Perfield II *et al.*, 2002)	Ca-salts mix CLA isomers + palm oil FA	0.39
32	(Donovan *et al.*, 2000)	Fish Oil	1; 2; 3

[1]Each row may include multiple independent comparisons derived from a single study (e.g., different durations, supplementation levels, or treatments), which were treated as separate data points in the meta-analysis.

Table 2: Summary of nutritional indices.

S.	Indeks	Full name	Calculation formula
1	PUFA/SFA	Polyunsaturated fatty acid/saturated fatty acid ratio	ΣPUFA/ΣSFA
2	IA	Index of atherogenicity	[C12:0 + (4 × C14:0) + C16:0]/ΣUFA
3	IT	Index of thrombogenicity	(C14:0 + C16:0 + C18:0)/[(0.5 ×ΣMUFA)+(0.5 ×Σn-6 PUFA) +(3 ×Σn-3 PUFA) +(n-3/n-6)]
4	HH	Hypocholesterolemic /hypercholesterolemic ratio	(cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 +C16:0)
5	HPI	Health-promoting index	ΣUFA/[C12:0+(4 × C14:0) + C16:0]
6	UI	Unsaturation index	1×(%monoenoics)+2×(% dienoics)+3×(% trienoics)+4×(% tetraenoics)+5×(% pentaenoics) + 6 × (% hexaenoics)
7	EPA+DHA	Sum of eicosapentaenoic acid and docosahexaenoic acid	C22:6 n-3 + C20:5 n-3
8	FLQ	Fish lipid quality	100 × (C22:6 n-3 + C20:5 n-3)/ΣFA
9	LA/ALA	Linoleic acid /α-linolenic acid ratio	C18:2 n-6/C18:3 n-3
10	TFA	Trans fatty acid	ΣTFA

All nutritional index equations presented in this table were calculated according to the methodology described by Chen and Liu (2020).

Table 3: Descriptive analysis of oil supplementation on PUFA/SFA ratio, IA, IT, HH, HPI, UI, EPA+DHA, FLQ, LA/ALA ratio, and TFA in dairy cow milk.

Indeks	Full Name	N[2]	Mean C	Mean T	SD[3] C	SD[3] T	Min C	Min T	Max C	Max T
PUFA/SFA	Polyunsaturated fatty acid/saturated fatty acid ratio	32	0.07	0.11	0.79	0.79	0.02	0.03	0.18	0.38
IA	Index of atherogenicity	32	2.30	1.73	5.71	5.87	0.86	0.60	4.03	3.20
IT	Index of thrombogenicity	32	2.83	3.89	4.09	4.09	1.13	0.75	7.90	18.02
HH	Hypocholesterolemic/hypercholesterolemic ratio	32	0.60	0.79	3.85	3.84	0.06	0.12	1.04	1.68
HPI	Health-promoting index	32	0.50	0.65	2.00	1.96	0.25	0.30	1.18	1.54
UI	Unsaturation index	23	37.46	43.25	10.41	10.43	0.49	0.51	59.76	73.43
EPA+DHA[1]	Sum of eicosapentaenoic acid and docosahexaenoic acid	16	0.11	0.36	0.22	0.22	0.00	0.00	0.40	3.06
FLQ	Fish lipid quality	18	0.99	1.01	5.18	5.73	0.00	0.00	3.07	3.62
LA/ALA	Linoleic acid /α-linolenic acid ratio	29	8.29	6.64	16.41	15.13	1.57	0.63	73.75	73.25
TFA[1]	Trans fatty acid	32	9.39	13.85	2.97	2.98	1.50	2.00	81.93	107.22

[1]Values for fatty acids (EPA+DHA and TFA) are expressed as g/100 g of total fatty acids. [2]Represents the number of studies/articles providing sufficient fatty acid composition data for calculation of each lipid health index. [3]The relatively large standard deviations observed for some indices reflect substantial between-study variability and should be interpreted with caution.

Table 4: Meta-analysis results of oil supplementation on PUFA/SFA ratio, IA, IT, HH, HPI, UI, EPA+DHA, FLQ, LA/ALA ratio, and TFA in dairy cow milk.

Variable	Estimate[1]	Lower bound	Upper bound	Std. error	P-value	I^2 (%)
PUFA/SFA	0.23	0.118	0.343	0.058	< 0.001	0
IA	-0.158	-0.3	-0.015	0.073	0.03	29.14
IT	-0.119	-0.229	-0.009	0.056	0.034	0
HH	0.049	-0.061	0.16	0.056	0.382	0
HPI	0.079	-0.03	0.189	0.056	0.154	0
UI	1.21	0.712	1.709	0.255	< 0.001	86.09
EPA+DHA	1.171	0.605	1.737	0.289	< 0.001	83.05
FLQ	0.228	-0.038	0.495	0.136	0.093	50.32
LA/ALA	-0.061	-0.177	0.055	0.059	0.3	0
TFA	1.822	1.458	2.185	0.186	< 0.001	84.31

[1]Estimate values represent standardized mean differences (Hedges' d).

represent broader systemic adjustments that require more extensive restructuring of the milk lipidome. Interpreting these indices within an integrated mechanistic framework is therefore essential to explain why some parameters respond markedly to lipid supplementation while others remain comparatively stable despite substantial changes in fatty acid supply.

PUFA/SFA RATIO

The PUFA/SFA ratio is widely used as an indicator of milk fat nutritional quality because it reflects the balance between hypocholesterolemic polyunsaturated fatty acids and cholesterol-raising saturated fatty acids. In the present study, oil supplementation increased the PUFA/SFA ratio from 0.07 in the control group to 0.11 in the supplemented group based on descriptive statistics (Table 3), and the meta-analysis confirmed a significant overall positive effect ($P < 0.001$; Table 4). These findings indicate that dietary lipid supplementation generally promoted a shift toward a more unsaturated milk fatty acid profile across studies.

For comparison, recommended PUFA/SFA ratios for human diets are generally above 0.45 (Kasapidou *et al.*, 2022), whereas values in ruminant milk commonly range from 0.02 to 0.15 due to extensive ruminal biohydrogenation (Chen and Liu, 2020). Therefore, although the values observed in the present study remained below recommended dietary thresholds, the increase observed following oil supplementation may still represent a biologically relevant improvement within the physiological constraints of ruminant lipid metabolism.

As illustrated in Figure 2, linseed oil and soybean oil (2%) tended to produce greater increases in PUFA/SFA compared with several other lipid sources. This pattern may be associated with the higher content of unsaturated fatty acids, particularly linoleic acid and α-linolenic acid, in these oils. Previous studies have suggested that dietary

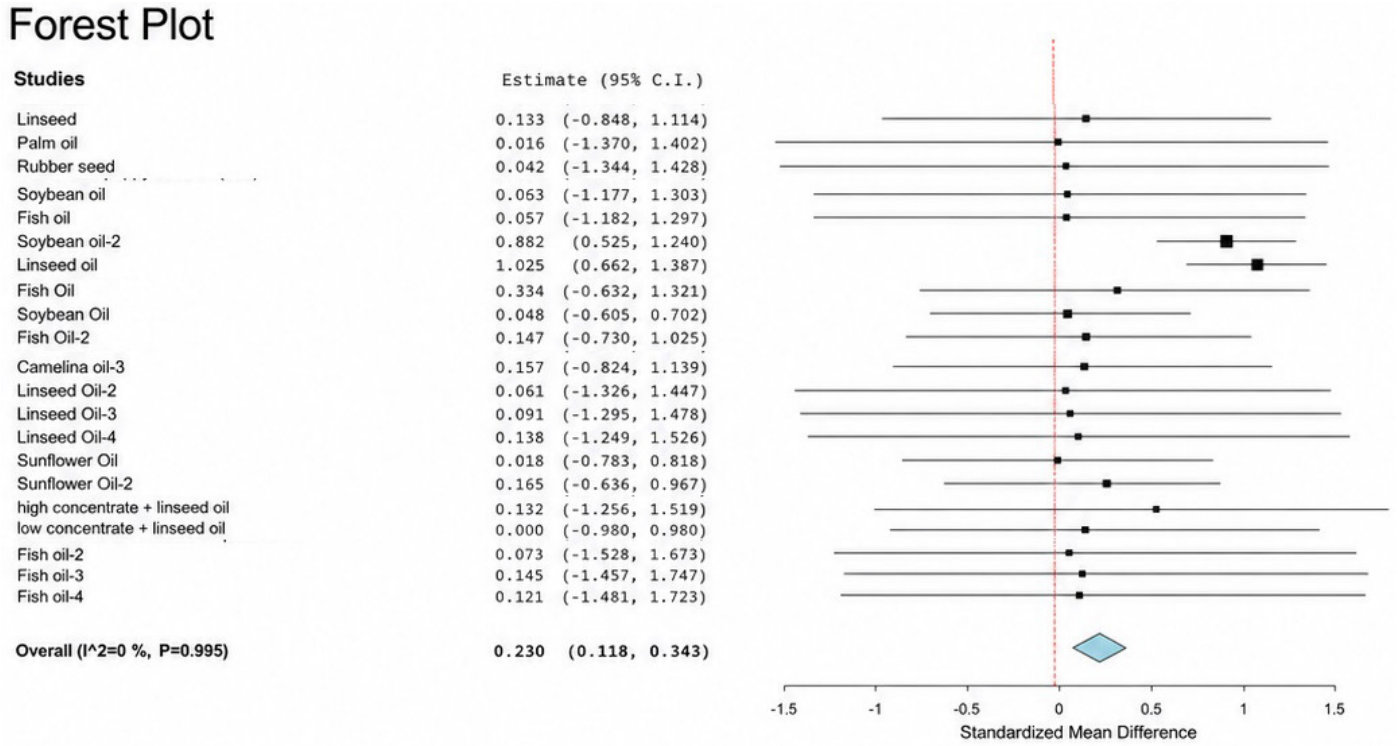

Figure 2: Forest plot of the effect of oil supplementation on PUFA/SFA ratio in dairy cow milk.

unsaturated fatty acids can partially escape complete ruminal biohydrogenation and subsequently contribute to greater incorporation of unsaturated fatty acids into milk fat (Benchaar *et al*., 2012; Oliveira *et al*., 2021). However, the mechanistic interpretations presented here are based on established literature and should be considered explanatory frameworks rather than direct evidence derived from this meta-analysis.

The persistence of relatively low PUFA/SFA values despite supplementation also suggests that ruminal metabolism and mammary lipid synthesis continue to favor saturated fatty acid production in dairy cows (Bionaz *et al*., 2020). Consequently, changes in PUFA/SFA should be interpreted as relative improvements in milk lipid quality rather than attainment of recommended dietary targets for human nutrition. These shifts may nevertheless contribute to downstream changes in functional lipid health indices such as IA and IT, which provide a more integrated assessment of the cardioprotective potential of milk fat composition.

Atherogenicity index (IA) and thrombogenicity index (IT)

Building upon the observed improvement in PUFA/SFA, the atherogenic (IA) and thrombogenic (IT) indices were evaluated to provide a more integrated interpretation of milk fat nutritional quality. Unlike individual fatty acid concentrations, these composite indices reflect the relative contribution of multiple fatty acids associated with cardiovascular health. Lower IA and IT values are generally associated with more favorable lipid profiles and reduced atherogenic and thrombogenic potential of dietary fat. However, because no official threshold values have been established for these indices, their interpretation remains comparative rather than absolute.

In the present study, oil supplementation was associated with reductions in IA and IT, indicating an overall shift toward a less atherogenic milk fatty acid profile. Descriptive data showed a decrease in IA from 2.30 in the control group to 1.73 in the supplemented group (Table 3), while meta-analysis confirmed significant reductions in both IA ($P = 0.03$) and IT ($P = 0.034$; Table 4). For comparison, IA values in bovine milk have previously been reported to range from 1.37 to 5.13 and IT from 0.39 to 4.65 (Chen and Liu, 2020), indicating that the values observed in the present study fall within the physiological range commonly reported for dairy products.

Considerable variability among studies was observed for both IA and IT, and therefore the pooled estimates should be interpreted cautiously. In particular, descriptive statistics and meta-analytic estimates were not always fully aligned, especially for IT, likely because descriptive values represent unweighted raw means whereas meta-analysis incorporates study-level variance and weighting. Consequently, interpretation was based primarily on the pooled meta-analytic estimates rather than descriptive statistics alone.

As illustrated in Figure 3, sunflower oil supplementation

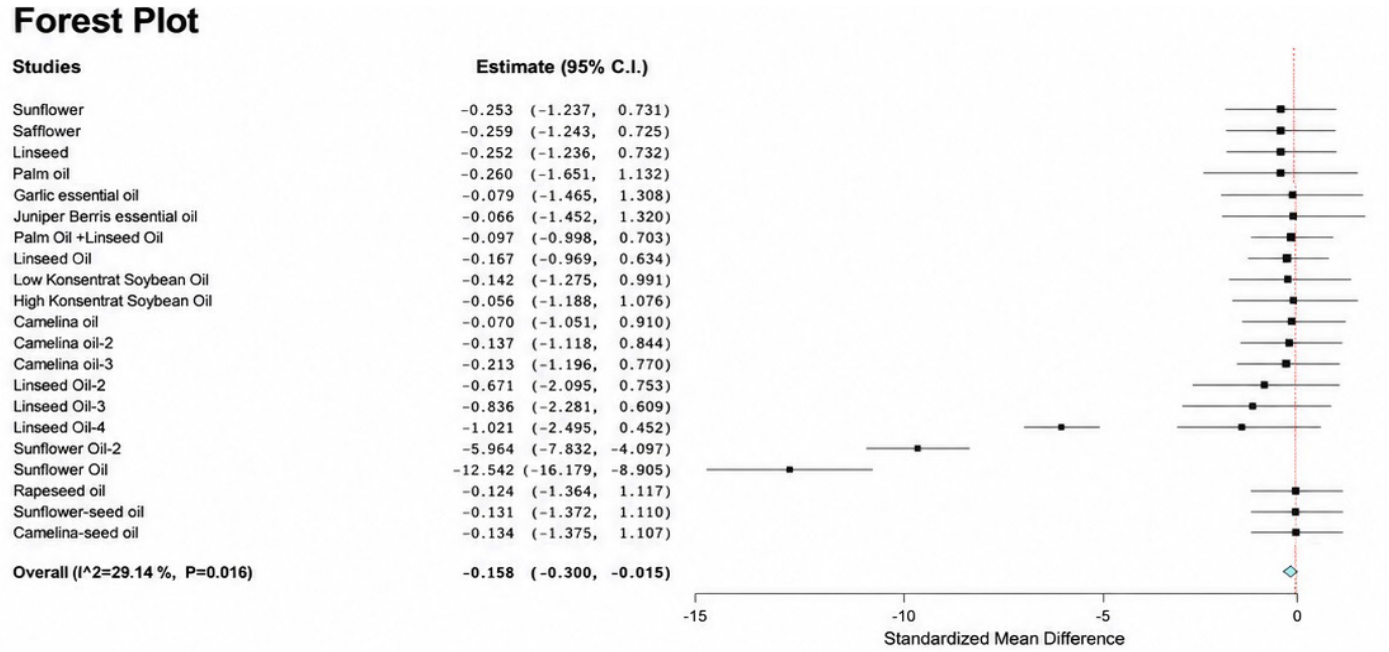

Figure 3: Forest plot of the effect of oil supplementation on IA in dairy cow milk.

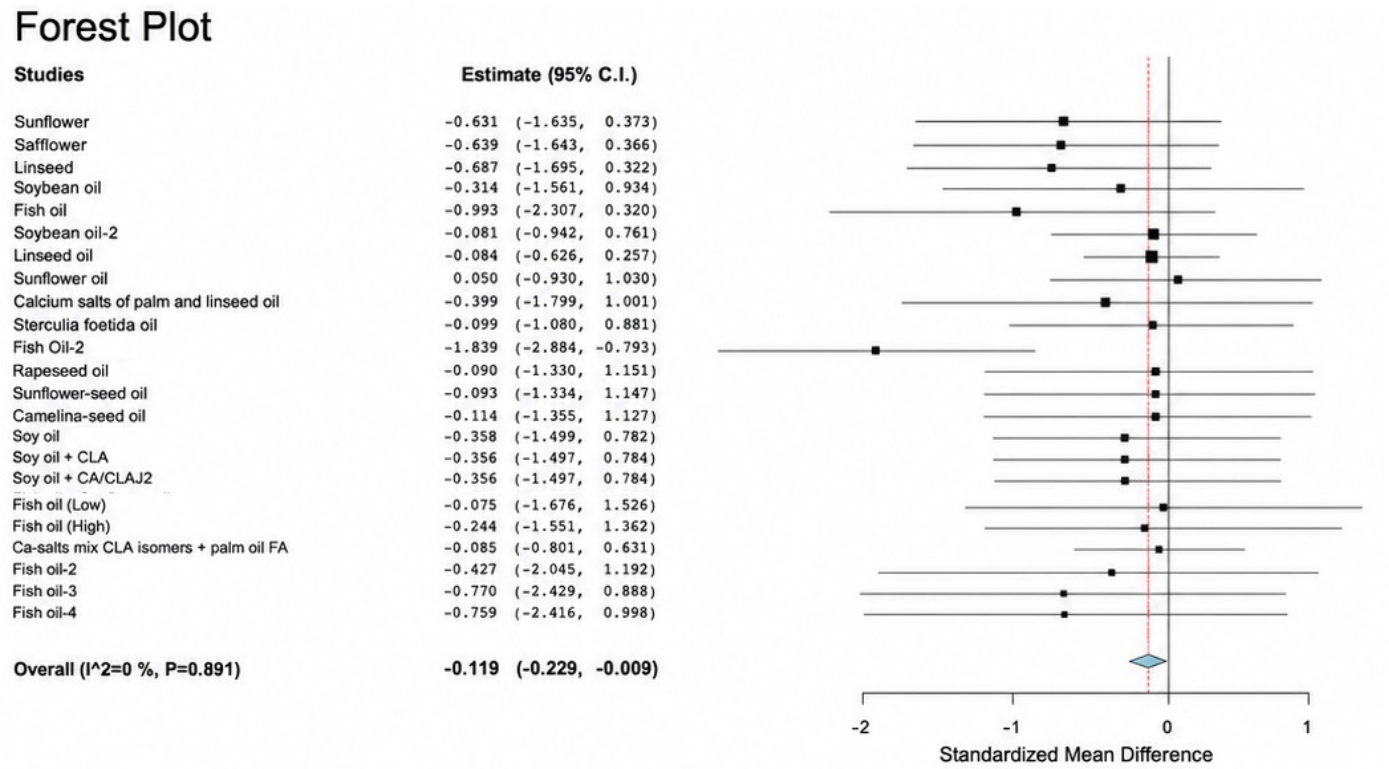

Figure 4: Forest plot of the effect of oil supplementation on IT in dairy cow milk.

tended to produce greater reductions in IA, particularly at inclusion levels of approximately 2–4% of dietary dry matter. Previous studies have similarly reported improvements in milk fatty acid composition following sunflower oil supplementation, including reductions in hypercholesterolemic saturated fatty acids (Rizzo *et al.*, 2023). Linseed oil also showed a tendency to reduce IA, although responses were less pronounced across studies.

A comparable pattern was observed for IT (Figure 4), where fish oil supplementation appeared to produce larger reductions than most plant-derived oils. Previous studies have suggested that marine lipids rich in long-chain n-3 fatty acids may alter ruminal lipid metabolism and contribute to lower thrombogenic lipid fractions in milk fat (Murphy *et al.*, 2008; Puppel *et al.*, 2012). However, these mechanistic interpretations are based on established literature and should be considered explanatory frameworks rather than direct evidence generated by the present meta-analysis.

Overall, the reductions observed in IA and IT support the interpretation that dietary oil supplementation can modify milk lipid composition toward a relatively more favorable nutritional profile within the biological constraints of ruminant lipid metabolism. These responses also provide a basis for evaluating broader lipid health indices such as HH and HPI, which reflect more integrated changes in milk fat quality.

HH and health-promoting index (HPI)

The hypocholesterolemic/hypercholesterolemic ratio (HH) and the Health-Promoting Index (HPI) were evaluated as complementary indicators of milk fat nutritional quality because they integrate the balance between beneficial unsaturated fatty acids and atherogenic saturated fatty acids. Higher HPI values are generally associated with more favorable lipid profiles in dairy products (Chen and Liu, 2020). In the present study, oil supplementation was associated with numerical increases in both HH and HPI; however, these responses were not statistically significant in the meta-analysis (HH: P = 0.382; HPI: P = 0.154; Table 4). Descriptive values increased from 0.60 to 0.79 for HH and from 0.50 to 0.65 for HPI (Table 3), indicating a tendency toward improved lipid quality that was not consistently observed across studies.

For comparison, HH values in bovine milk have previously been reported to range from 0.34 to 0.57, whereas HPI values in dairy products range from 0.16 to 0.68 (Chen and Liu, 2020). The values observed in the present analysis therefore remained within or slightly above these reported ranges. Nevertheless, because the pooled effects were not statistically significant, these responses should be interpreted as descriptive trends rather than confirmed improvements in milk fat nutritional quality.

The inconsistent responses observed for HH and HPI may reflect the integrative nature of these indices, which depend on coordinated changes across multiple fatty acid classes rather than shifts in individual fatty acids alone. In addition, variability in oil source, supplementation level, feeding duration, and degree of lipid protection among studies likely contributed to the absence of consistent pooled effects (Glasser *et al.*, 2008). The relatively small number of studies available for some comparisons may also have limited statistical power to detect modest changes in these indices.

As illustrated in Figures 5 and 6, sunflower oil, linseed oil, and rapeseed oil tended to produce higher HH and HPI values compared with several other lipid sources. Previous studies have associated these oils with increased unsaturated fatty acid content and reduced proportions of saturated fatty acids in milk fat (Chilliard *et al.*, 2007; Rego *et al.*, 2009). However, the mechanistic interpretations presented here are based on established literature and should be considered explanatory frameworks rather than direct evidence generated by the present meta-analysis.

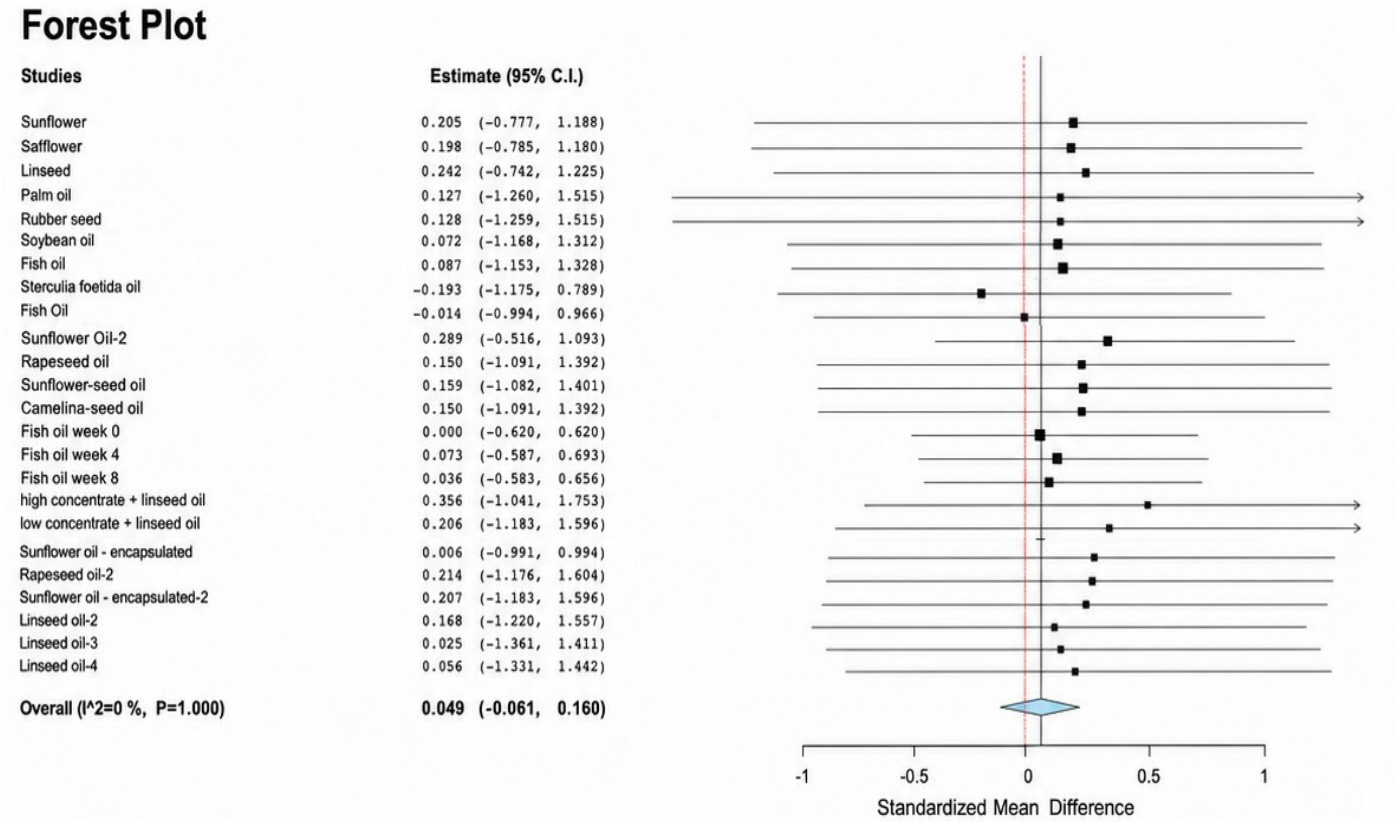

Figure 5: Forest plot of the effect of oil supplementation on HH ratio in dairy cow milk.

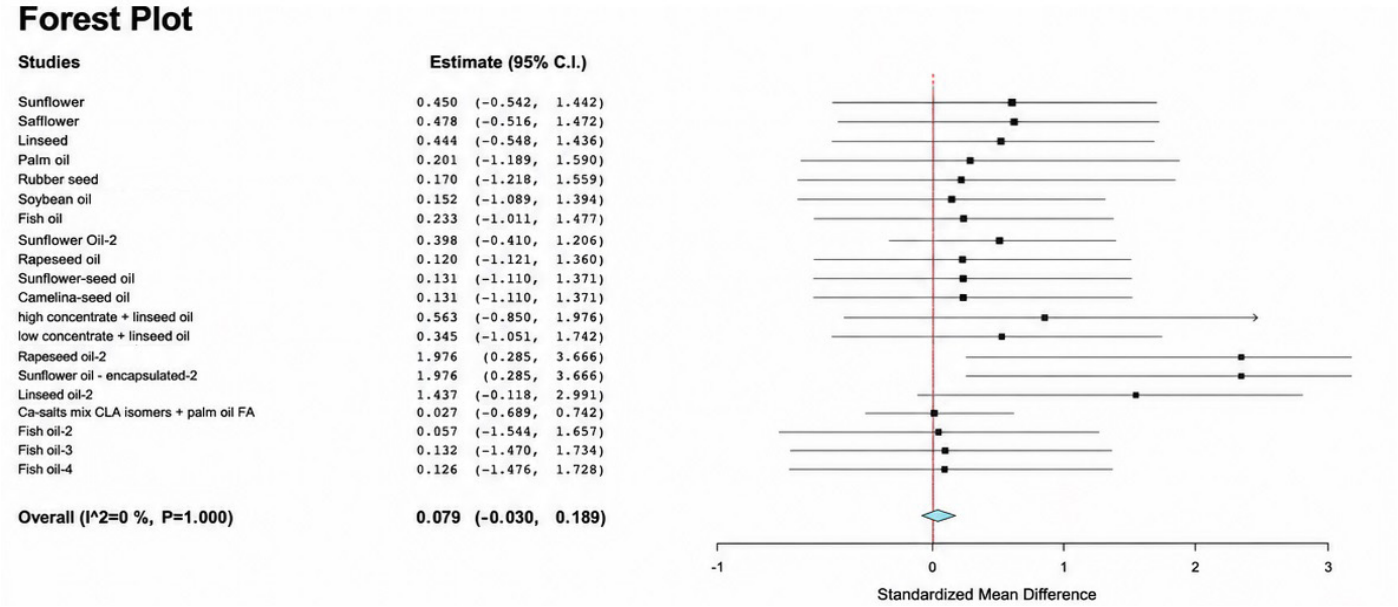

Figure 6: Forest plot of the effect of oil supplementation on HPI ratio in dairy cow milk.

Because HH and HPI represent integrated lipid balance, relatively modest changes in fatty acid composition may not be sufficiently reflected in these indices. Consequently, broader shifts toward milk fat unsaturation may be more sensitively detected by indices directly related to total double-bond abundance, such as the Unsaturation Index (UI), which is discussed in the following section.

Unsaturation index (UI)

The shift in HH and HPI toward more unsaturated lipid fractions was further reflected by the Unsaturation Index (UI), which represents the accumulation of double bonds within the milk fatty acid profile and is commonly used as an indicator of overall lipid unsaturation. The number of studies included in the UI analysis (N = 23) was lower than for several other indices because detailed fatty acid composition data required for UI calculation were not consistently reported across studies. Descriptive statistics indicated that oil supplementation increased UI from 37.46 in the control group to 43.25 in the supplemented group, while the maximum observed value increased from 59.76 to 73.43 (Table 3). These findings suggest an overall increase in milk fat unsaturation following dietary lipid supplementation. Because no universally established reference values exist for UI in bovine milk, the index is interpreted comparatively, with higher values reflecting relatively greater lipid unsaturation rather than absolute nutritional targets.

Meta-analysis confirmed a significant positive overall effect of oil supplementation on UI ($P < 0.001$; Table 4). However, substantial heterogeneity was observed among studies ($I^2 = 86\%$), indicating considerable variability in responses across experimental conditions. Therefore, although the pooled estimate suggests an overall tendency toward increased milk fat unsaturation, the magnitude of the effect should be interpreted cautiously and considered a general trend rather than a precise quantitative estimate. The observed heterogeneity likely reflects differences in oil type, supplementation level, feeding duration, basal diet composition, and experimental design among studies.

Compared with broader composite indices such as HH and HPI, UI appeared more responsive to dietary lipid supplementation because it directly reflects total double-bond abundance within the milk fatty acid profile. Previous studies have associated increases in UI with greater availability of dietary unsaturated fatty acids and subsequent modifications in ruminal biohydrogenation and mammary lipid metabolism (Shingfield *et al*., 2013; Dewanckele *et al*., 2020). However, the mechanistic interpretations presented here are based on established literature and should therefore be considered explanatory frameworks rather than direct evidence generated by the present meta-analysis.

As illustrated in Figure 7, plant-derived oils, particularly linseed, sunflower, and rapeseed oil, tended to produce greater increases in UI than animal-derived lipid sources. This pattern may be associated with the higher concentration of C18 unsaturated fatty acids in plant oils, which has previously been linked to increased MUFA and PUFA proportions in milk fat. In contrast, fish oil supplementation appeared to contribute more specifically to enrichment of long-chain omega-3 fatty acids such as EPA and DHA rather than to overall unsaturation. Consequently, the increase in UI observed in the present study may reflect broader changes in milk fatty acid unsaturation that are further explored through subsequent indices such as EPA+DHA and Fish Lipid Quality (FLQ).

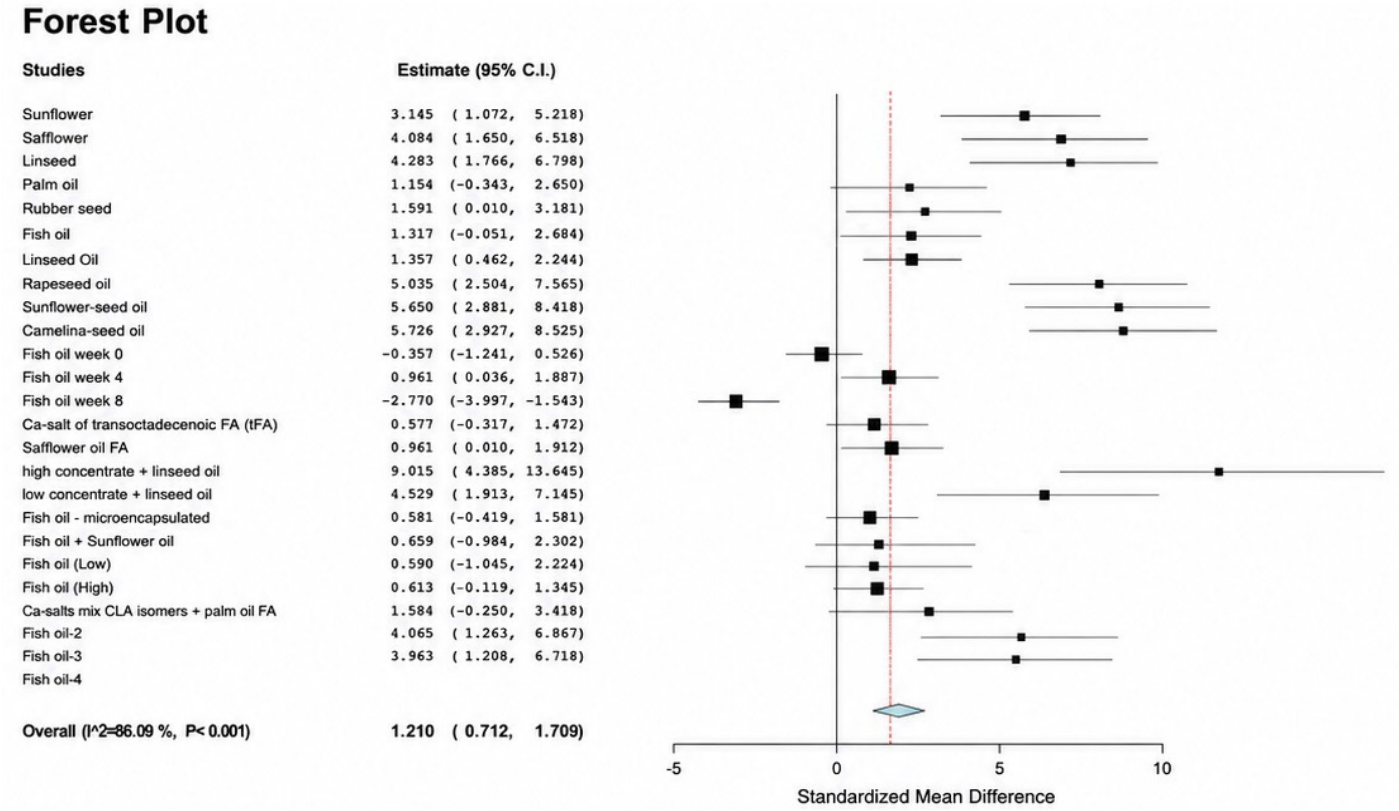

Figure 7: Forest plot of the effect of oil supplementation on UI ratio in dairy cow milk.

EPA + DHA AND FLQ

The increase in Unsaturation Index (UI) was accompanied by changes in long-chain omega-3 fatty acids, particularly EPA and DHA, which represent functionally important components of milk fat nutritional quality. EPA+DHA reflects the combined concentration of long-chain omega-3 fatty acids, whereas Fish Lipid Quality (FLQ) represents their proportion relative to total fatty acids. In the present study, descriptive statistics indicated an increase in EPA+DHA from 0.11 to 0.36 g/100 g of total fatty acids following oil supplementation (Table 3), and the meta-analysis confirmed a significant positive overall effect ($P < 0.001$; Table 4). These findings suggest that dietary lipid supplementation, particularly with omega-3–rich oils, generally enhanced enrichment of long-chain omega-3 fatty acids in milk fat across studies.

In bovine milk, EPA and DHA are normally present at relatively low concentrations, typically ranging from approximately 0.03 to 0.10 g/100 g of total fatty acids under conventional feeding conditions, but may increase to approximately 0.30–0.40 g/100 g following dietary lipid supplementation (Bodkowski *et al*., 2024). Therefore, because no standardized reference thresholds have been established for EPA+DHA enrichment in dairy products, the increases observed in the present study should be interpreted as relative improvements within the biological constraints of ruminant lipid metabolism rather than attainment of specific nutritional targets.

Although enrichment of EPA and DHA has previously been associated with potential anti-inflammatory and cardioprotective properties in human nutrition, the present meta-analysis did not evaluate direct health outcomes. Consequently, the interpretation of improved nutritional quality is based on established literature regarding long-chain omega-3 fatty acids rather than direct evidence generated by this study.

In contrast to EPA+DHA, FLQ showed only a numerical increase and did not reach statistical significance in the meta-analysis ($P = 0.093$; Table 4). Descriptive values increased only slightly from 0.99 to 1.01 (Table 3), indicating limited and inconsistent responses across studies. Because FLQ was originally developed for marine products, no established reference values currently exist for dairy systems; therefore, interpretation remains comparative rather than absolute (Chen and Liu, 2020). The absence of a significant pooled effect suggests that improvements in omega-3–based lipid quality were not consistently expressed across experimental conditions.

Variability among studies likely reflected differences in oil source, supplementation level, feeding duration, and ruminal biohydrogenation dynamics, which can influence transfer efficiency of long-chain omega-3 fatty acids into milk fat (Huang *et al*., 2020). As illustrated in Figures 8 and 9, marine-derived lipid sources, particularly fish oil, tended to produce greater increases in EPA+DHA and FLQ

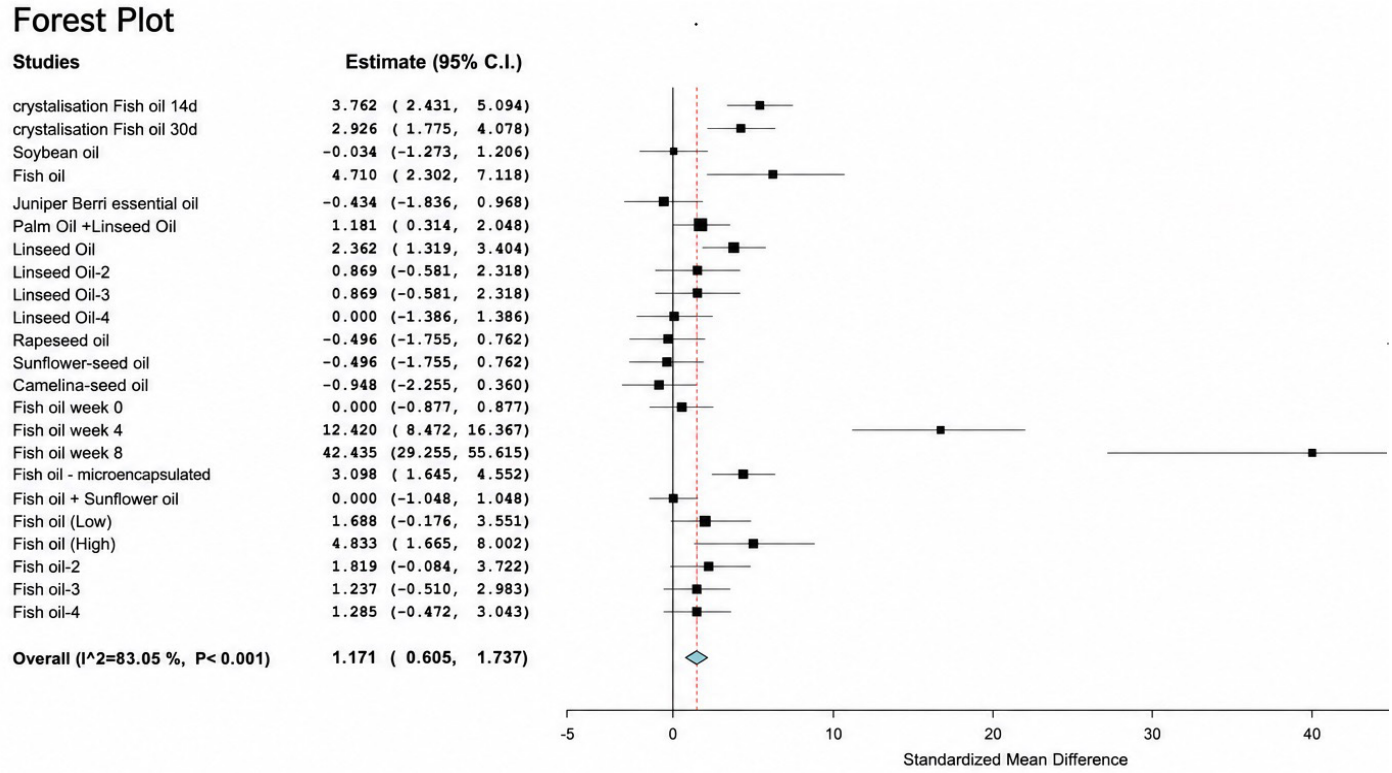

Figure 8: Forest plot of the effect of oil supplementation on EPA+DHA in dairy cow milk.

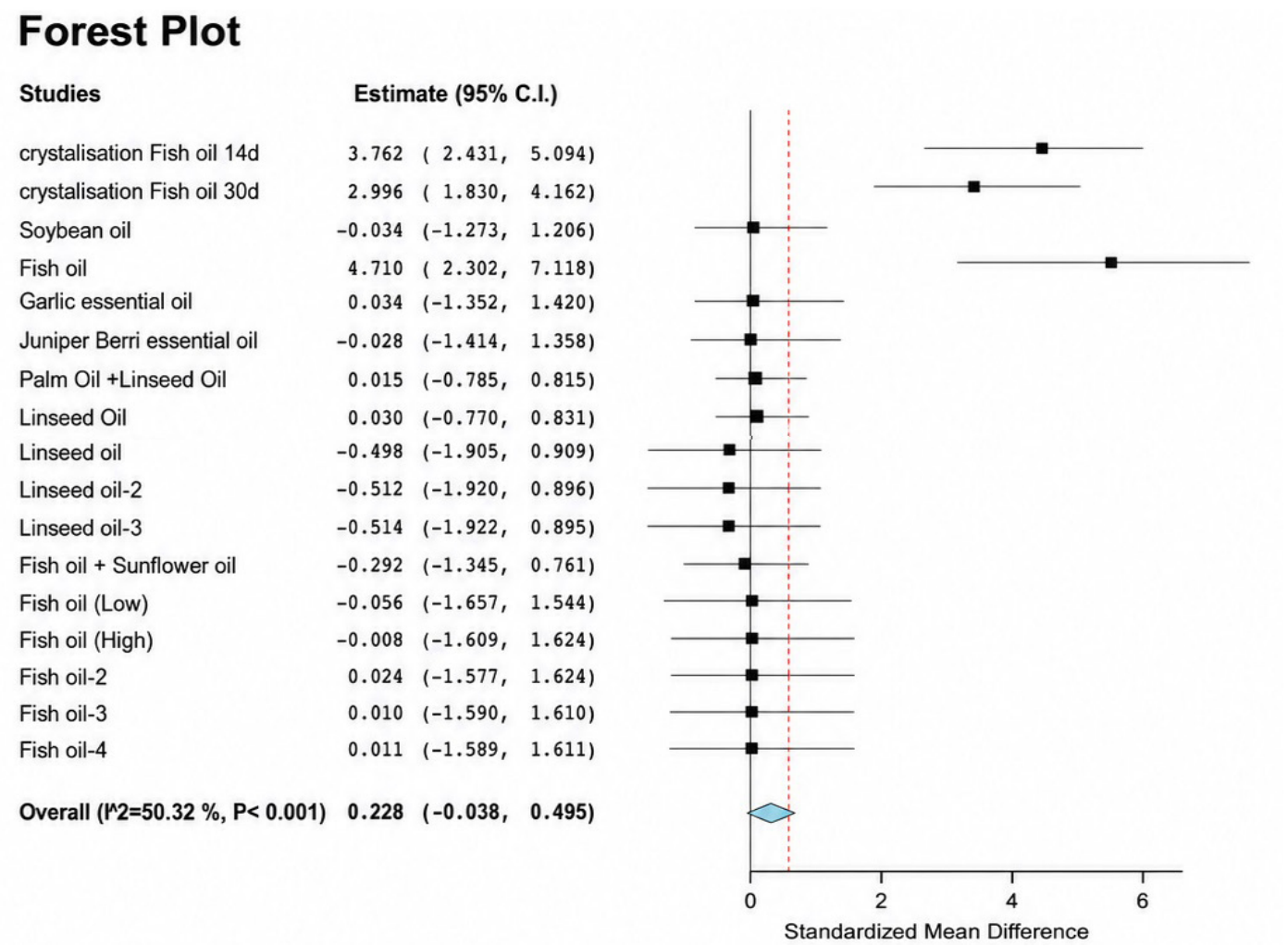

Figure 9: Forest plot of the effect of oil supplementation on FLQ in dairy cow milk.

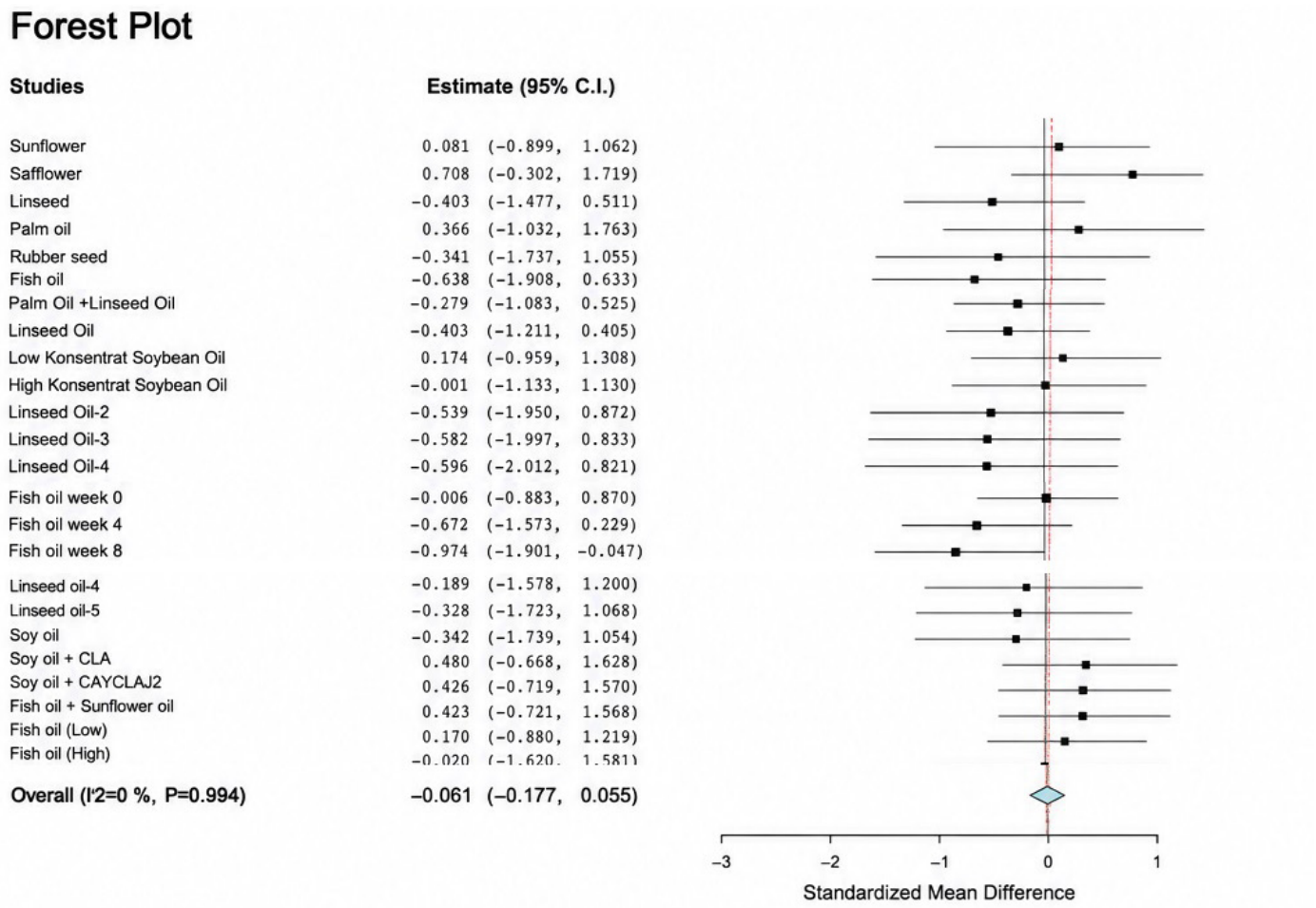

Figure 10: Forest plot of the effect of oil supplementation on LA/ALA ratio in dairy cow milk.

compared with conventional vegetable oils. Previous studies have similarly associated marine lipids with greater enrichment of long-chain omega-3 fatty acids in dairy products (Moallem, 2018). However, these mechanistic interpretations are based on prior literature and should be considered explanatory frameworks rather than direct mechanistic evidence from the present meta-analysis.

The increase in EPA+DHA observed in the present study also did not necessarily correspond to proportional changes in precursor-based indices such as LA/ALA, indicating that downstream enrichment of long-chain omega-3 fatty acids may respond differently from precursor fatty acid ratios. Collectively, these findings suggest that dietary lipid supplementation can modify milk fat composition toward greater omega-3 enrichment, although responses remain strongly influenced by ruminal metabolism and variability among experimental conditions.

LA/ALA RATIO

The LA/ALA ratio reflects the balance between omega-6 (linoleic acid) and omega-3 (α-linolenic acid) fatty acids and is commonly used as an indicator of milk fat nutritional quality. In the present meta-analysis, oil supplementation resulted in a numerical reduction in the LA/ALA ratio; however, the pooled effect was not statistically significant (P = 0.3; Table 4). These findings suggest that dietary lipid supplementation alone did not consistently modify the balance between omega-6 and omega-3 precursor fatty acids across studies.

For comparison, LA/ALA ratios in bovine milk have previously been reported to range from approximately 2.5 to 3.4 (Chen and Liu, 2020), and the values observed in the present study remained within this physiological range. In contrast, nutritional guidelines for infant formula, such as those from Food Standards Australia New Zealand, recommend broader LA/ALA ratios of approximately 5:1 to 15:1 because essential fatty acid balance is considered more critical during early development. Therefore, the nutritional interpretation of LA/ALA differs between infant nutrition and dairy fat evaluation in adult diets, where endogenous conversion of precursor fatty acids to long-chain omega-3 PUFA is relatively limited.

As illustrated in Figure 10, most effect estimates were distributed close to the null line with wide and overlapping confidence intervals, indicating weak and heterogeneous responses among studies. Although some treatments, particularly linseed oil and specific lipid combinations,

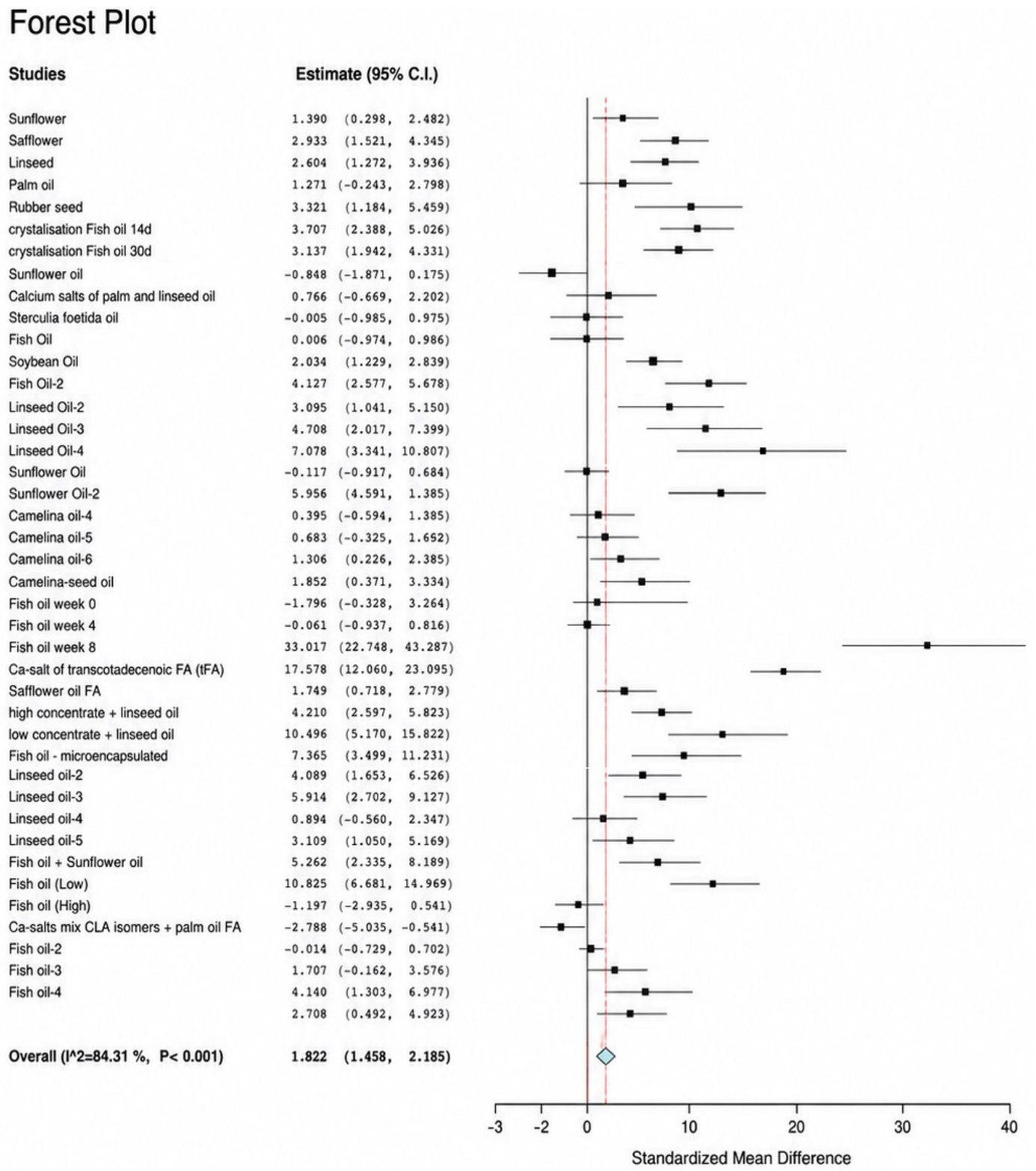

Figure 11: Forest plot of the effect of oil supplementation on TFA in dairy cow milk.

tended to reduce LA/ALA values, many responses remained neutral. The relatively small number of studies available for this outcome, together with substantial variability among dietary treatments, may also have limited statistical power to detect modest differences in LA/ALA responses.

Previous studies have suggested that extensive ruminal biohydrogenation may limit transfer of dietary α-linolenic acid to milk fat because a substantial proportion of dietary ALA is converted into more saturated intermediates before reaching the mammary gland (Santin *et al*., 2019). Consequently, dietary strategies based solely on precursor fatty acid supplementation may not consistently induce measurable changes in LA/ALA ratios in dairy products. In the present study, changes associated with dietary lipid

supplementation appeared to be more clearly reflected in downstream indices such as EPA+DHA enrichment and trans fatty acid (TFA) accumulation, which may provide more sensitive indicators of altered ruminal lipid metabolism than precursor fatty acid ratios alone.

Trans fatty acids (TFA)

Trans fatty acids (TFA) are major intermediates formed during ruminal biohydrogenation of unsaturated fatty acids and are commonly used as indicators of shifts in ruminal lipid metabolism following dietary lipid supplementation. In the present study, descriptive TFA values increased from 9.39 to 13.85 (Table 3), and meta-analysis confirmed a significant positive pooled effect ($P < 0.001$; Table 4). The distribution of effect sizes in Figure 11 showed that most responses shifted toward positive values, indicating that increased TFA formation was a relatively consistent outcome across studies supplemented with unsaturated lipid sources.

From a nutritional perspective, increased TFA concentrations in milk are generally considered undesirable because high trans fatty acid intake has been associated with adverse cardiovascular effects in humans. However, interpretation of these findings requires caution because the present meta-analysis evaluated total TFA only, and most included studies did not report individual trans fatty acid isomers. Consequently, the present study cannot determine whether the observed increases were predominantly associated with potentially less harmful ruminant-derived isomers, such as vaccenic acid, or with other trans fatty acid fractions. Therefore, no direct conclusions can be drawn regarding the specific health implications of the increased TFA observed in this analysis.

Previous studies have reported that ruminant-derived TFA differ structurally and metabolically from industrially produced trans fatty acids, and certain ruminant TFA isomers, particularly vaccenic acid (trans-11 C18:1), may serve as precursors for conjugated linoleic acid (CLA) synthesis in the mammary gland (Santin *et al*., 2019). In addition, controlled dietary studies have suggested that moderate intake of ruminant-derived TFA may not exert the same adverse vascular effects commonly associated with industrial TFA (Raff *et al*., 2006). However, these findings originate from previous literature and were not directly evaluated in the present meta-analysis.

The increase in TFA observed following oil supplementation may reflect altered ruminal biohydrogenation pathways associated with greater dietary unsaturated fatty acid supply. As illustrated in Figure 11, fish oil supplementation tended to produce larger TFA responses than most plant-derived oils. Previous studies have similarly associated marine lipids rich in EPA and DHA with accumulation of biohydrogenation intermediates through partial inhibition of the final hydrogenation steps in the rumen (Huang *et al*., 2020; Kupczyński *et al*., 2011). Nevertheless, these mechanistic interpretations should be considered explanatory frameworks derived from prior literature rather than direct mechanistic evidence generated by the present study.

Overall, the observed increase in TFA indicates that dietary oil supplementation modifies ruminal lipid metabolism and biohydrogenation dynamics. However, because individual TFA isomers were not consistently reported across studies, interpretation of the nutritional implications of increased TFA should remain cautious.

CONCLUSION

Dietary oil supplementation was associated with consistent modifications in milk fatty acid composition and several lipid health indices in dairy cows, indicating that nutritional manipulation can influence milk fat quality within the physiological constraints of ruminant lipid metabolism. Among the evaluated lipid sources, linseed oil, soybean oil and sunflower oil at supplementation levels of approximately 2-4% of dietary dry matter generally showed more favorable responses in PUFA/SFA, IA, IT, and Unsaturation Index (UI), whereas fish oil supplementation at approximately 0.2–0.3% of dietary dry matter was more strongly associated with enrichment of EPA+DHA. These findings suggest that different lipid sources and inclusion levels may influence distinct aspects of milk fat remodeling, including overall unsaturation and long-chain omega-3 fatty acid enrichment.

However, responses varied substantially among studies, and some indices, particularly HH, HPI, FLQ, and LA/ALA, did not show significant pooled effects. In addition, high heterogeneity was observed for several outcomes, including UI, indicating considerable variability related to oil type, supplementation level, feeding duration, and experimental conditions. Therefore, pooled estimates should be interpreted cautiously as general trends rather than precise quantitative predictions. The absence of formal risk-of-bias assessment and the inclusion of multiple comparisons from some studies also represent important limitations of the present meta-analysis.

The observed increase in TFA further suggests that dietary lipid supplementation modifies ruminal biohydrogenation pathways; however, because individual trans fatty acid isomers were not consistently reported, the nutritional implications of increased TFA could not be fully evaluated. Consequently, interpretation of milk fat nutritional quality should consider both beneficial changes in unsaturation and the complexity of ruminal lipid metabolism.

From a practical perspective, moderate supplementation with plant-derived oils such as linseed or sunflower oil at approximately 2–4% of dietary dry matter may represent a feasible nutritional strategy to improve milk fatty acid composition, whereas lower inclusion levels of marine oils may be more suitable when the objective is enrichment of long-chain omega-3 fatty acids. Nevertheless, optimal supplementation strategies likely depend on production goals, dietary context, and acceptable trade-offs between improvements in unsaturation and increases in biohydrogenation intermediates. Further studies using standardized experimental designs and detailed reporting of individual fatty acid isomers are needed to improve the robustness and biological interpretation of future meta-analyses.

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NOVELTY STATEMENT

Unlike previous meta-analyses that primarily focused on individual milk fatty acids or specific dietary lipid supplements, this study comprehensively integrates multiple milk fatty acid health indices (PUFA/SFA, IA, IT, HH, HPI, UI, EPA+DHA, FLQ, LA/ALA, and TFA) to evaluate the effects of diverse dietary oil sources and supplementation levels on milk fat nutritional quality in dairy cows. This integrative approach provides a broader and more functionally relevant assessment of dietary lipid supplementation strategies than evaluations based solely on individual fatty acids.

AUTHOR'S CONTRIBUTION

Afsitin Joan Tatra, Tri Wahyu Apriliana, and Fandini Meilia Anjani were responsible for formal analysis, investigation, visualization, data curation, methodology, and writing-original draft. Despal, Rika Zahera, Idat Galih Permana, and Wulansih Dwi Astuti contributed to the conceptualization, resources, supervision, writing-review & editing, software, and validation.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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