



The Impact of Organic Substrate Fatty Acids on the Fatty Acid Composition of *Hermetia Illucens* Larvae: A Meta-Analysis

MOCHAMAD DZAKY ALIFIAN¹, ANURAGA JAYANEGARA², NAHROWI², SUMIATI², RAKHMAD PERKASA HARAHAP^{3*}, EKO LELA FITRIANA¹, IRWAN SUSANTO¹, QORRY NURUL HASANAH¹

¹Graduate School of Nutrition and Feed Science, IPB University, Bogor, Indonesia; ²Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia; ³Study Program of Animal Science, Faculty of Agriculture, Tanjungpura University, Jl. Prof. Hadari Nawawi, Pontianak, Kalimantan Barat, Indonesia.

Abstract | This study aimed to exploring the dynamic changes fatty acid composition of organic waste through *Hermetia illucens* larvae using a meta-analysis method. A database was created using studies that have documented different compositions of fatty acid profile substrates in *Hermetia illucens*. Data tabulation only involved credible international journals as indicated by Scopus indexed. A total of 11 articles were integrated into the database. The statistical analysis used the mixed model methodology. Random effects were considered in the studies, and the fatty acid substrate dose was treated as a fixed effect. The results showed that the increase C_{16:0}, C_{18:0}, C_{16:1}, C_{18:1}, C_{18:2}, omega-3, omega-6, omega-3: omega-6 SFA, MUFA, and PUFA in substrate increased C_{16:0}, C_{18:0}, C_{16:1}, C_{18:1}, C_{18:2}, omega-3, omega-6, omega-3:omega-6, SFA, MUFA and PUFA content on *Hermetia illucens* larvae (P<0.05). This study concludes that there are several fatty acids of *Hermetia illucens* larvae influenced by fatty acids in the substrate.

Keywords | Bioconversion, *Hermetia illucens*, Lipid profile, Meta-analysis, Nutrient dynamics, Organic substrate

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***Correspondence** | Rakhmad Perkasa Harahap, Study Program of Animal Science, Faculty of Agriculture, Tanjungpura University, Jl. Prof. Hadari Nawawi, Pontianak, Kalimantan Barat, Indonesia; **Email:** rakhmad@faperta.untan.ac.id

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INTRODUCTION

Black Soldier Fly Larvae (BSFL), or *Hermetia illucens*, have substantial weight increase during their developmental phases, particularly during the larval phase. Beginning at around 200-300 mg in the first week (Zulkifli *et al.*, 2023), the quantity taken could rise to 500-600 mg by the second week (Li *et al.*, 2011) and 1-1.5 grams by the third

week (Kinasih *et al.*, 2018). The maximum weight, around 2 grams or greater, is generally reached by the end of the fourth week (El-Kaiaty *et al.*, 2022) prior to the prepupal stage. The nutritional profile of BSFL is important, with elevated protein levels (40-44%) and fat content (30-35%), which fluctuate according to their age and diet (Moula *et al.*, 2018; Xiao *et al.*, 2018). The quick growth and substantial nutritional value of BSFL make it a valuable resource

for animal feed, especially in poultry and aquaculture, in addition to its use in waste management applications (Abd El-Hack *et al.*, 2020; Putri *et al.*, 2024).

Organic waste is a significant environmental problem in various sectors, including the agricultural, livestock and food industries. Organic waste contains various types of organic compounds, including fatty acids, which can have a negative impact on the environment if not managed properly. *Hermetia illucens* larvae are bioconversion commonly used to accelerate composting organic matter. *Hermetia illucens* larvae can efficiently utilize organic resources, such as fruit, vegetable, food waste, and manure (Čičková *et al.*, 2015). *Hermetia illucens* larvae can reduce organic waste by as much as 84.5% under managed environments, including temperatures hitting 34°C and a pH of 8, with a larval density of 200 larvae per reactor (Uswatun *et al.*, 2024). In another research, BSFL reported a 64.3% efficiency in organic waste degradation, followed by a notable rise in larval biomass, showing effective waste conversion (Huaripata *et al.*, 2023).

The fatty acid component of *Hermetia illucens* larvae is influenced by the substrate and larval weight (Spranghers *et al.*, 2017). The fatty acid synthase (FAS) is responsible for producing essential saturated fatty acids, including lauric acid, which is comparable to coconut oil in content. This synthesis is crucial for cellular processes and is influenced by genetic factors (Jiang *et al.*, 2024). The moisture content of the substrate is a critical factor affecting larval growth and survival, which in turn influences lipid accumulation. Optimal moisture levels enhance larval performance, thereby potentially affecting the fatty acid profile (Frooninckx *et al.*, 2024).

Research has shown that Black Soldier Fly Larvae (BSFL) can assimilate and modify dietary fatty acids, leading to variations in their own fatty acid content. BSFL are capable of converting organic waste into nutrient-rich biomass, which includes the assimilation and modification of dietary fatty acids (Danieli *et al.*, 2019; Fitriana *et al.*, 2022a). Quantitative fatty acid signature analysis (QFA-SA) faces challenges in accurately determining diet composition due to variations in fatty acid assimilation and metabolism, which can affect the calibration coefficients used in these analyses (Marco *et al.*, 2021). Modification of fatty acids can be done by knowing which fatty acids are sensitive to changes in fatty acids in the substrate. Lauric fatty acid (C12:0) as the highest medium chain fatty acid (MCFA) in the larvae had varied in several studies, about 43.0-60.5% (Spranghers *et al.*, 2017) and 17.0-44.0% (Li *et al.*, 2022). The variation also was shown in the omega 3 fatty acids in *Hermetia illucens* larvae is about 2.3% with food residue as a substrate (Ewald *et al.*, 2020). However, the enrichment of omega-3 fatty acids in the substrate can increase the amount omega-3 fatty acids in the larvae. The

enrichment of omega 3 fatty acids increases it to 11.8% with 50% substrate of *Schizochytrium* micro algae industrial waste (El-Dakar *et al.*, 2020).

The novelty of this research lies in its use of a meta-analysis approach to synthesize data from multiple studies and explore the overall trends and variations in fatty acid composition across a wider range of organic waste substrates. This comprehensive analysis will provide valuable insights into the factors influencing fatty acid modification in BSFL and contribute to optimizing their use in various applications, including animal feed and biodiesel production. According to the variation results in several studies, the study aimed to exploring the dynamic changes fatty acid composition of organic waste through *Hermetia illucens* larvae using a meta-analysis method.

MATERIALS AND METHODS

DATA DEVELOPMENT AND INCLUSION CRITERIA

A database was developed based on scientific publications available online at the Scopus database, accessed through the institutional IPB University network (<https://www.scopus.com/search/>, accessed on 27 February 2023). The articles used in the database had several criteria, including that the articles must be published in English, the studies must involve treatment of *Hermetia illucens* larvae with measured fatty acid content in organic substrate and *Hermetia illucens* larvae, and the experiments must be conducted *in vivo* on *Hermetia illucens* larvae. All relevant titles of publication from the respective websites were further imported into the reference manager for selection purposes. Using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol (Liberati *et al.*, 2009), a database was created.

A total of 278 articles were initially identified through the keyword search. The process of literature search and selection is illustrated in Figure 1. These articles were then assessed based on their abstracts and full texts, resulting in the inclusion of 70 studies (comparisons) in this study, as listed in Table 1. If an article reported on multiple experiments, each experiment was coded separately.

The database encompassed the fatty acid composition of *Hermetia illucens*, including C_{10:0}, C_{12:0}, C_{14:0}, C_{14:1}, C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, C_{18:2}, total omega-6, total omega-3, omega-3:6, saturated fatty acids (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA). The fatty acid proportions used in this research were based on dry matter (DM) g/kg substrate. To facilitate further data analysis, the data for the same parameters were converted into the same units of measurement before being tabulated into the database. Table 2 presents the descriptive statistics of the compiled database.

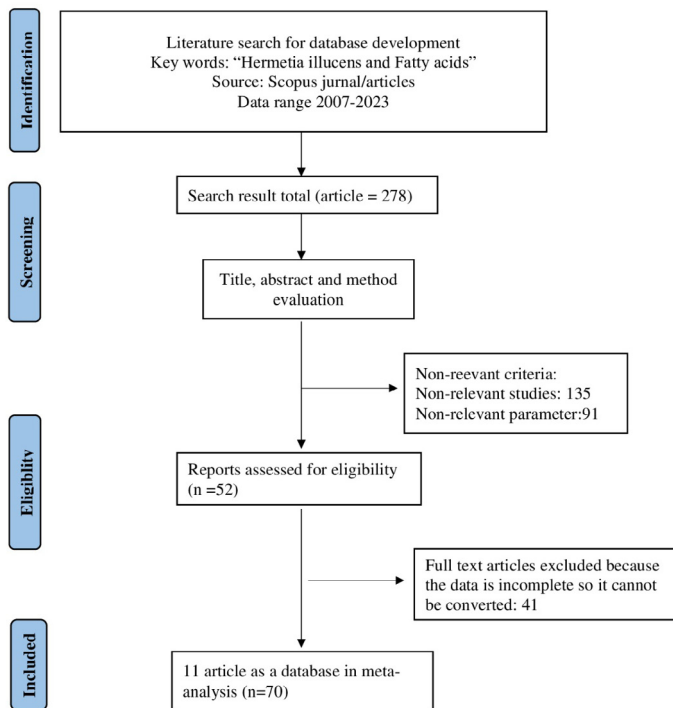


Figure 1: A PRISMA flow diagram of the literature search strategy and selection for meta-analysis.

MODELLING AND ANALYSIS OF DATA

The database was statistically analyzed using a mixed model methodology where the model has been widely used in meta-analyses related to animal nutrition (Sauvant *et al.*, 2008). The different studies considered a random effect, and the fatty acid level was treated as a fixed effect. The following statistical models were used:

$$Y_{ij} = B_0 + B_1 X_{ij} + B_2 X_{ij}^2 + s_i + b_i X_{ij} + e_{ij}$$

Where;

Y_j = dependent variable.

B_0 = overall intercept across all studies (fixed effect).

B_1 = linear regression coefficient Y on X (fixed effect).

B_2 = Y squared regression coefficient on X (fixed effect).

X_{ij} = predictor value of continuous variable (nutrition level).

s_i = random effect of study i.

b_i = random effect of study i on the regression coefficient Y on X in study i.

e_{ij} = unexplained residual error.

When the respective quadratic regression models were not significant at $P < 0.05$, an appropriate mixed linear regression model was applied. Research variables are stated in class statements because they do not contain quantitative information (St-Pierre, 2001). The statistical model used is p-values. The significance of an effect is stated when the P-value < 0.05 and tends when the P-value between 0.1 and 0.05. All statistical analyzes were performed using SAS software, version (9.4).

Table 1: Studies included in the meta-analysis.

No	Refer-ence	Country	Year	Substrate	Fase
1	Ooninx <i>et al.</i> , 2015	Nether-lands	2015	High protein high fat, high protein low fat, low protein high fat, low protein low fat	larvae
2	Sprang-herers <i>et al.</i> , 2017	Belgium	2017	Chicken feed, digeste, vegetable waste, restau-rant waste	larvae
4	Barosso <i>et al.</i> , 2017	Spain	2017	Chicken feed, fish	larvae
3	Me-neguz <i>et al.</i> , 2018	Iltaly	2018	Vegetable fruit, fruit, Winery by-product, brewery by-product	larvae
5	Barroso <i>et al.</i> , 2019	Spain	2019	Chicken feed, fish discard	larvae
6	Danieli <i>et al.</i> , 2019	Iltaly	2019	Corn, alfalfa, barley, wheat middlings,	larvae
7	Gianetto <i>et al.</i> , 2020	Italy	2020	Fruit	larvae
8	Ewald <i>et al.</i> , 2020	Sweden	2020	Fish, food waste, fress mussels, ensiled mussels, rotten mussels, bread	larvae
9	El-Da-kar <i>et al.</i> , 2020	China	2020	Schizochytrium waste	larvae
10	Marco <i>et al.</i> , 2021	China	2021	Wheat brand, quail manure	larvae
11	Li <i>et al.</i> , 2022	China	2022	Soybean meal, linseed oil, peanut oil, coconut oil, soybean oil, lard oil, fish oil	larvae

RESULTS AND DISCUSSION

Table 3 indicated that the levels of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{14:1}$ (DM g/kg) in the substrate (independent variable) did not influence the fatty acid profile content of *Hermetia illucens* (dependent variable) for the same fatty acids ($P > 0.05$). This fits with the results reported of Sprangherers *et al.* (2017), indicating that although substrate composition affects the fatty acid profile, it is not the exclusive factor. This suggests that *Hermetia illucens* larvae may have intrinsic metabolic processes that regulate the production and storage of fatty acids, irrespective of the fatty acid level in the substrate. The research conducted by Boukid *et al.* (2021) support this perspective, demonstrating that the fatty acid profiles of *Hermetia illucens* remain stable across diverse meals, suggesting a degree of metabolic independence from the

substrate's composition. This metabolic independence may elucidate why fluctuations in the concentrations of C_{10:0}, C_{12:0}, C_{14:0}, and C_{14:1} in the substrate do not lead to comparable modifications in the fatty acid profile of the larvae.

Table 2: Descriptive statistics of the database used in the meta-analysis.

Item	N	Min	Max	Mean	SD
Fatty acids composition of substrate g/kg DM					
C _{10:0} g/kg DM	70	0.00	3.43	0.18	0.55
C _{12:0} g/kg DM	70	0.00	21.53	1.55	3.64
C _{14:0} g/kg DM	70	0.00	15.25	2.64	4.71
C _{14:1} g/kg DM	70	0.00	0.09	0.00	0.02
C _{16:0} g/kg DM	70	1.46	32.14	11.44	8.34
C _{16:1} g/kg DM	70	0.00	13.58	1.85	4.02
C _{18:0} g/kg DM	70	0.05	10.97	3.00	3.41
C _{18:1} g/kg DM	70	0.53	43.87	13.21	11.24
C18:2 g/kg DM	70	0.53	49.80	13.84	10.68
Total omega 6 g/kg DM	70	0.08	49.80	14.03	10.71
Total omega 3 g/kg DM	70	0.00	67.15	10.24	19.50
Omega 3:6 g/kg DM	70	0.00	41.97	4.39	12.62
SFA g/kg DM	70	3.66	75.13	19.89	18.65
MUFA g/kg DM	70	1.01	51.52	18.00	16.74
PUFA g/kg DM	70	0.67	77.19	26.11	20.16
Fatty acids composition of larvae g/kg DM					
C _{10:0} g/kg DM	70	0.00	11.34	2.67	2.72
C _{12:0} g/kg DM	70	11.59	356.08	147.45	61.37
C _{14:0} g/kg DM	70	4.54	282.59	33.99	32.63
C _{14:1} g/kg DM	70	0.00	5.24	0.65	1.16
C _{16:0} g/kg DM	70	12.10	142.63	61.78	27.86
C _{16:1} g/kg DM	70	0.00	64.09	17.33	15.03
C _{18:0} g/kg DM	70	2.04	46.44	11.70	7.95
C _{18:1} g/kg DM	70	9.97	158.67	57.23	32.07
C _{18:2} g/kg DM	70	1.40	124.70	45.70	24.69
Total omega 6 g/kg DM	70	1.40	124.70	46.20	24.96
Total omega 3 g/kg DM	70	0.72	85.51	15.90	20.74
Omega 3:6 g/kg DM	70	0.09	38.65	3.09	7.21
SFA g/kg DM	70	31.47	554.90	259.26	91.88
MUFA g/kg DM	70	16.18	196.79	79.26	40.86
PUFA g/kg DM	70	4.29	139.32	62.74	34.93

Descriptions: N: number of data; DM: Dry Matter; CP: Crude Protein; CF: Crude Fat; C_{10:0}: capric acid; C_{12:0}: lauric acid; C_{14:0}: myristic acid; C_{14:1}: myristoleic acid; C_{16:0}: palmitic acid; C_{16:1}: palmitoleic acid; C_{18:0}: stearic acid; C_{18:1}: oleic acid; C_{18:2}: linoleic acid; SFA: Saturated Fatty Acid; MUFA: Mono-Unsaturated Fatty Acid; PUFA: Poly-Unsaturated Fatty Acid.

Li *et al.* (2021) performed a comprehensive analysis of the lipid profile in *Hermetia illucens* larvae, identifying 19 en-

riched metabolic pathways, of which nine are directly related to lipid metabolism, including pathways involved in glycerolipid (GL) and glycerophospholipid (GP) metabolism. These pathways are essential for regulating triglycerides (TG) and phospholipids, which are fundamental components of the larvae's lipid composition. The findings indicate that the metabolic pathways involved in lipid synthesis and storage are significantly active during the growth and development of *Hermetia illucens*. This underscores the larvae's capacity to adjust their lipid profile in response to metabolic requirements, rather than depending exclusively on the fatty acid composition of their substrate. The study indicated that these fatty acids may be partially synthesized from carbohydrates, with concentrations potentially increasing after the decomposition of organic substrates (Downer and Matthews 1976; Oonincx *et al.*, 2015; Ushakova *et al.*, 2016; Liland *et al.*, 2017; Spranghers *et al.*, 2017; Rabani *et al.*, 2019; Ewald *et al.*, 2020).

The meta-analysis revealed that the levels of C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, C_{18:2}, total omega-6, total omega-3, omega-3:6 ratio, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in the substrate significantly increased the fatty acid profile content of *Hermetia illucens* (P<0.05). These findings suggest that *Hermetia illucens* possesses the capacity to accumulate both C_{16:0} and C_{18:0} fatty acids, with a notably higher ability to synthesize C_{16:0} compared to C_{18:0}.

The conversion of C_{16:0} to C_{16:1} is facilitated by the presence of isomerases, specifically the 9-desaturase, which catalyzes this isomerization process. The variation in C_{16:1} levels observed in *Hermetia illucens* larvae is likely due to several factors, including the C_{16:1} content in the substrate, the non-fiber carbohydrate (NFC) content in the substrate, and the presence of key enzymes involved in fatty acid synthesis, such as thioesterase II, -9-desaturase, 12 fat2 desaturase, and 6 fat3 desaturase (Hoc *et al.*, 2021). These enzymatic processes collectively contribute to the modulation of the larvae's fatty acid profile. In addition, Boukid *et al.* (2021) demonstrated that the fatty acid profiles of *Hermetia illucens* larvae are significantly influenced by their dietary composition, particularly the abundance of C_{16:0} and C_{18:2}.

Carbohydrates, fats, and proteins undergo glycolysis, producing pyruvic acid, which is subsequently converted into acetyl-CoA. Acetyl-CoA serves as the precursor for fatty acid synthesis in *Hermetia illucens* (Hoc *et al.*, 2021). This metabolic pathway enables the larvae to accumulate and synthesize C_{18:1} fatty acids, which are essential for regulating membrane integrity. C_{18:1} fatty acids, particularly oleic acid, play a vital role in maintaining optimal membrane function, especially for critical membrane proteins like ATPases, which require oleic acid for optimal function (Starling *et al.*, 1993).

Table 3: Regression equations of the content level fatty acid profile substrate (independent variable) on content fatty acid profile *Hermetia illucens* larvae (response variable) from the mixed model analysis.

Response variable g/kg/ DM	Independent Variable g/kg/ DM	Model	N	Intercept	Standart Error Intercept	Slope	Standart Error Slope	P-Value	AIC
C _{10:0}	C _{10:0}	L	70	2.62	0.34	0.30	0.60	0.615	280.3
C _{12:0}	C _{12:0}	L	70	141.43	7.83	3.88	1.99	0.056	747.1
C _{14:0}	C _{14:0}	L	70	30.79	4.44	1.21	0.83	0.148	699.3
C _{14:1}	C _{14:1}	L	70	0.6	0.14	11.91	7.88	0.135	153.8
C _{16:0}	C _{16:0}	L	70	43.4	5.02	1.61	0.36	<0.001	632.4
C _{16:1}	C _{16:1}	L	70	13.5	1.66	2.07	0.38	<0.001	530.8
C _{18:0}	C _{18:0}	L	70	7.34	1	1.45	0.22	<0.001	449.8
C _{18:1}	C _{18:1}	L	70	40.44	5.36	1.27	0.31	<0.001	647
C _{18:2}	C _{18:2}	L	70	32.26	4.44	0.97	0.25	<0.001	637.6
Σ omega-6	Σ omega-6	L	70	31.94	4.48	1.02	0.25	<0.001	637.8
Σ omega-3	Σ omega-3	L	70	8.13	1.98	0.76	0.09	<0.001	584.3
Omega 3:6	Omega 3:6	L	70	1.11	0.57	0.45	0.04	<0.001	415.6
SFA	SFA	L	70	217.33	14.67	2.11	0.54	<0.001	803.2
MUFA	MUFA	L	70	51.54	5.63	1.54	0.23	<0.001	674.6
PUFA	PUFA	L	70	42.95	6.22	0.76	0.19	<0.001	680.6

Descriptions: L: Linear; N: number of data; DM: Dry Matter; C_{10:0}: capric acid; C_{12:0}: lauric acid; C_{14:0}: myristic acid; C_{14:1}: myristoleic acid; C_{16:0}: palmitic acid; C_{16:1}: palmitoleic acid; C_{18:0}: stearic acid; C_{18:1}: oleic acid; C_{18:2}: linoleic acid; SFA, Saturated Fatty Acid; MUFA: Mono-Unsaturated Fatty Acid; PUFA: Poly-Unsaturated Fatty Acid.

The enzymes present in *Hermetia illucens*, such as 9-desaturase, 9 and 12 fat2 desaturase, and 6 fat3 desaturase (Δ 12, 9 and 12 isomerases), facilitate the conversion of C_{18:0} to C_{18:1} and subsequently to C_{18:2n6} and C_{18:3n3} (Hoc *et al.*, 2021). These findings highlight the significant influence of the substrate's C_{18:0} content on the larvae's C_{18:0} and C_{18:1} levels. This relationship mirrors observations in feed formulation studies, where the omega-3 content in the larvae correlates with the omega-3 content of the feed ingredients (Finke 2013; Oonincx *et al.*, 2015; Meneguz *et al.*, 2018; Barroso *et al.*, 2019; Gao *et al.*, 2019).

Furthermore, the study by Schiavone *et al.* (2017) demonstrated that when *Hermetia illucens* larvae were fed substrates rich in polyunsaturated fatty acids (PUFAs), particularly linoleic acid (C_{18:2n-6}), there was a corresponding increase in the PUFA content of the larvae. This direct relationship between substrate composition and the fatty acid profile of the larvae underscores the larvae's ability to effectively utilize dietary fatty acids to enhance their own lipid profiles. Additionally, the findings of Anankware *et al.* (2021) emphasized the importance of the omega-3 to omega-6 ratio in the dietary fatty acid profile of *Hermetia illucens*, suggesting that a balanced intake of these fatty acids could improve the health outcomes of animals consuming these larvae. This further highlights the significance of substrate composition, not only in enhancing the fatty acid profile of *Hermetia illucens* but also in promoting better nutritional outcomes for the animals that consume them.

Moreover, *Hermetia illucens* larvae are unable to synthesize polyunsaturated fatty acids (PUFAs) de novo, suggesting that PUFA levels in the larvae are strongly determined by the PUFA content of the substrate (Liland *et al.*, 2017; Giannetto *et al.*, 2020; Fitriana *et al.*, 2022b). The positive correlations observed between substrate and larvae fatty acid profiles indicate that higher fatty acid concentrations in the substrate are mirrored by higher fatty acid levels in the larvae. However, it is important to consider that factors such as oxidation, microbial activity (both external and gut microbiota), digestive enzymes, and the age of the larvae can also influence fatty acid absorption and metabolism, potentially modifying the overall fatty acid profile in the larvae.

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that although substrate composition affects the fatty acid profile of larvae, it does not directly effect the concentrations of specific fatty acids, including C10:0, C12:0, C14:0, and C14:1, in the larvae. This indicates that the larvae have inherent metabolic mechanisms that govern fatty acid production and storage, irrespective of the fatty acid composition of the substrate. In any case, substrate composition can substantially increase the concentrations of C16:0, C16:1, C18:0, C18:1, and polyunsaturated fatty acids (PUFAs) in the larvae, especially when the substrates are abundant in these fatty acids.

I would like to express my sincere gratitude to IPB University for providing the research facilities and support. I also wish to thank my research colleagues for their valuable collaboration and contributions throughout this study.

NOVELTY STATEMENTS

This research is novel due to its application of a meta-analysis method to integrate data from several studies and examine the general trends and variations in fatty acid content over a broader spectrum of organic waste substrates. This extensive research will yield significant insights into the determinants affecting fatty acid alteration in BSFL and contribute in maximizing their application in diverse fields, such as animal feed.

AUTHOR'S CONTRIBUTIONS

Mochamad Dzaky Alifian as the main author. Nahrowi, Anuraga Jayanegara and Sumiati as mentors. Eko Lela Fitriana, Irwan Susanto, and Rakhmad Perkasa Harahap as the authors of the method and database selection, Qorry Nurul Hasanah as the journal format adjustment.

CONFLICT OF INTEREST

We confirm that there are no conflicts of interest related to the discussed material, including financial, personal, or other relationships with individuals or organizations.

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