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**Research Article** 

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# Production Performance and Egg Quality of Laying Hens Fed with Diet Containing Black Soldier Fly (*Hermetia illucens*) Larvae: A Meta-analysis

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#### **ABSTRACT**

Black Soldier Fly larvae (BSFL) are larvae of the *Hermetia illucens* beetle, which can serve as a protein source in poultry feed; however, their impact on production and quality remains inconsistent. Therefore, this study aimed to assess the utilization of BSFL as a protein source for laying hens in terms of production performance and egg quality. Study selection followed the PRISMA protocol. This meta-analysis retrieved 44 studies from 17 articles, utilizing Hedges'd as the effect size metric. The results indicated that the production performance of laying hens fed diets containing BSFL did not differ significantly from control diets regarding egg weight, egg mass, hen day production, feed conversion ratio (FCR), and feed intake. However, significant differences (P<0.05) were observed in all egg quality parameters except for shell thickness and cholesterol content. The inclusion of BSFL in laying hen diets significantly increased (P<0.05) Haugh Unit (HU), egg yolk color score, and level of lauric, myristic, and palmitic acids. Sub-group analysis of larvae forms revealed that non-defatted BSFL exhibited the best FCR. Meta-regression analysis identified the optimal BSFL inclusion level for laying hens as 12%.

Key words: BSF larvae, Egg, Laying hen, Meta-analysis, Production performance

# INTRODUCTION

One of the sources of animal protein in the poultry diet that is often used by the poultry industry is meat and bone meal (MBM). The advantage of MBM is that its protein content is quite high, approximately 44.6-62.8% (Garcia et al., 2016), and does not cause a rancid odor in chicken meat. Apart from its advantages, MBM has disadvantages, including the fact that it is an imported product. The Association of Animal Food Companies reported that MBM imports to Indonesia increased from 287×10<sup>3</sup> tons in 2018, to  $309 \times 10^3$  tons in 2019. The import of MBM is likely to continue to increase as the number of poultry in Indonesia increases. Apart from imports, one of the raw materials for MBM is organ tissue originating from sick or euthanized animals (animals killed intentionally due to certain diseases), animal carcasses originating from zoos, and pig organ tissue (Garcia et al. 2006)Thus, alternative ingredients are required to substitute for MBM, and one of them is the Black Soldier Fly larvae (BSFL).

BSFL (Hermetia illucens) is an insect larva often found in fruit and organic waste. BSFL is easy to obtain, grows quickly and abundantly (within 2 months, one adult insect can produce 400-500 eggs), its diet is not complicated, does not carry viruses and bacteria, and requires low input in its breeding (Smets et al. 2020). In addition, BSFL is an excellent organic waste decomposition agent, so it can be used as an alternative solution for processing organic waste, which, until now almost all developing countries have had problems managing it (Dortmans 2015; Grau et al. 2022), including Indonesia. The Ministry of Environment and Forestry of the Republic of Indonesia stated that the amount of waste piled up in Indonesia has reached 175,000 tons/day with 69% being transported and stockpiled in landfills, 10% buried, 7% composted and recycled, 5% burned, and the

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rest is not managed 7% (Ministry of Environment and Forestry 2013; Dhewanto et al. 2018; Ministry of National Development Planning/Bappenas 2021). All of these require quite a certain portion of waste transportation cost. The Ministry of Environment and Forestry further described that organic waste makes up the majority of waste in Indonesia, accounting for about 57% of the overall waste pile. Non-organic waste can be recycled, but organic waste causes odor problems due to decay and environmental pollution. Such organic waste has the potential to be used as the BSF cultivation medium (Fitriana et al. 2022; Zulkifli et al. 2023).

One of the most important factors is the nutritional content of BSFL. Jayanegara et al. (2017) BSFL reported good nutrient profiles, i.e., 44.9% crude protein, 29.1% crude fat, 16.4% crude fiber, and 8.1% ash content. BSFL also contains a balanced composition of essential amino acids (Smets et al. 2020). Proteins, which consist of various amino acids, are responsible for growth, including muscle formation, replacement of dead tissue, and antibody formation. Therefore, BSFL is expected to replace MBM without compromising laying hens' immunity, performance, or egg quality.

Researchers in various countries have begun to research BSFL and its use in feed. For instance, Choi et al. (2021) reported that the use of BSFL flour in broiler chicken feed at a level of 0.5-1.5% affected color, TBARS, DPPH, and free radicals in meat. In quail, at a level of 10% in the form of flour, it affected protein content, cholesterol levels, amino acid profile, fatty acid profile, and organoleptic properties (Cullere et al. 2018). Kurnia Citra et al. (2019) reported that the use of BSFL defatted flour has the ability to act as a natural antimicrobial, which can reduce the number of Escherichia coli colonies in quail intestines by 99.99% along with increasing defatted larvae flour to 6.18% in the ration. In Muscovy duck, according to (Gariglio et al. 2021), the use of BSFL flour in the feed of 3-9% affected the fatty acid profile and amino acid profile. In laying hens, according to (Bejaei & Cheng 2020), the substitution of soybean meal with dry BSFL at 10-18% resulted in egg production with egg quality comparable to control eggs. In the study of Al-Qazzaz et al. (2016)The addition of 0, 50, and 10g kg<sup>-1</sup> BSFL to laying hen feed significantly increased egg productivity and quality.

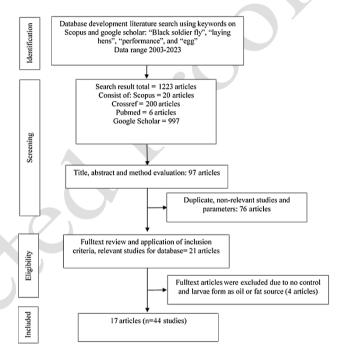
Based on the various information presented, the results of BSFL feeding on poultry varied, including those for the laying hens. Furthermore, there is no standard dose or level of BSFL for laying hens. Thus, it is necessary to integrate various data from previous studies to generalize the effects of BSFL feeding on laying hens. Therefore, this study aimed to evaluate the influence of BSFL feeding on production performance and egg quality of laying hens by integrating and synthesizing data from previously published studies using the meta-analysis approach.

#### MATERIALS AND METHODS

#### **Database development**

A database was developed using a variety of articles reporting the production performance and quality of chicken eggs fed with BSFL. Several keywords were used in the article search, i.e., "black soldier fly", "laying hen",

"performance", and "egg" on Scopus, Crossref, Pubmed, and Google Scholar platforms by using the Publish or Perish software. The literature selection process is presented in Fig. 1. The articles included in this study were published between 2016 and 2023. Seventeen papers met the inclusion criteria (Table 1), and the methodology was based on the PRISMA protocol, also known as the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (Liberati et al. 2009). The requirements for publications to be admitted to the database were as follows: 1) publication manuscripts are presented in English, 2) included BSFL as a feed protein source on laying hens, 3) there are control and treatment groups in one article, and 4) articles presenting the production performance and quality of chicken eggs fed with BSFL.



**Fig. 1:** The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) procedure in the selection process of literature.

BSFL was reported in the publications at varying percentages, from 1 to 24% of the dry matter content, when utilized as a feed protein source for laying hens. Parameters included in the database were production performance and egg quality. Production performance-related parameters included egg weight, egg mass, hen day production (HDP), feed conversion ratio (FCR), and feed intake. In contrast, egg quality parameters included Haugh unit (HU), egg yolk color score, shell thickness, cholesterol content, lauric acid (LAU), myristic acid (MYR), palmitic acid (PAL), and stearic acid (STE). The BSFL data were categorized into two groups, namely defatted and non-defatted. Defatted is larvae or larvae flour that has had its fat removed. Non-defatted refers to larvae or larvae flour that has no fat removed when used as a feed ingredient.

## Data analysis

The data were assessed by meta-analysis of random effects, with the effect size (d) determined using Hedges'd (Liberati et al. 2009). This approach was chosen because it may calculate the effect size regardless of sample size

heterogeneity or statistical test outcomes (Palupi et al. 2012):

$$[d = \frac{(\bar{X}^E - \bar{X}^C)}{S}J] \tag{1}$$

Where  $\overline{XE}$  is the mean of the experimental group,  $\overline{XC}$  is the mean of the control group, S is the pooled standard deviation, i.e.:

$$[S = \sqrt{\frac{(N^E - 1)(s^E)^2 + (N^C - 1)(s^C)^2}{(N^E + N^C - 2)}}]$$
(2)

and J is the correction factor for the small sample size, explained as

$$[J = 1 - \frac{3}{(4(N^C + N^E - 2) - 1)}] \tag{3}$$

where the sample size of the experimental group is symbolized in NE, the sample size of the control group is symbolized in NC, the standard deviation of the experimental group is symbolized in sE, and the standard deviation of the control group is symbolized in sC. This study used the random effect method to estimate the effect size with a 95% confidence interval according to the following formula:

$$[y_i = \beta_F + v_i] \tag{4}$$

where the effect size of the i-th observation is symbolized in *yi*, and the sampling in the i-th observation is symbolized

in vi. Using DerSimonian and Laird method (DerSimonian & Laird 1986), the estimated variation between studies ( $\tau^2$ ) was calculated with the following formula:

$$[\tau^2 = O - df C] \tag{5}$$

where the weighted sum square is symbolized in Q, df is degrees of freedom, and C for the value. The OpenMEE software, which is used for meta-analysis in this investigation, was developed based on work of Wallace et al. (2017). A cumulative forest plot of the tested parameters with a 95% confidence interval. Additionally, OpenMEE and JASP software were used to perform the Egger's test and funnel plot, which were used to visually and statistically detect publication bias.

#### **RESULTS**

#### Characteristics of articles

The meta-database of 44 observations was drawn from 17 papers (Table 1). Statistical descriptions of various parameters related to production performance and egg quality are reported in Table 2. Table 3 presents the meta-analysis results, which compare the experiment and

Table 1: Studies used in the database for meta-analysis of production performance and egg quality of laying hens fed with BSFL

No	Study	Larvae form	Sub-group	Level (%)
1	Al-Qazzaz et al. (2016)	Meal	Non defatted	1-5
2	Park et al. (2017)	Pupa Meal	Non defatted	3.5- 6.5
3	Mwaniki et al. (2018)	Defatted meal	Defatted	5- 7.5
4	Secci et al. (2018)	Meal	Non defatted	17
5	Irawan et al. (2019)	Fresh, Dried and Defatted meal	Non defatted and Defatted	8
6	Mwaniki et al. (2020)	Defatted meal	Defatted	10-15
7	Bejaei & Cheng (2020)	Dried	Non defatted	10-18
8	Liu et al. (2020)	Meal	Non defatted	1-5
9	Star et al. (2020)	Fresh	Non defatted	10
10	Heuel et al. (2021a)	Defatted meal +Oil	Non defatted	15
11	Heuel et al. (2021b)	Defatted meal +Oil	Non defatted	15
12	Park et al. 2021)	Defatted meal	Defatted	2-4
13	Patterson et al. (2021)	Meal	Non defatted	8-24
14	Heuel et al. (2022)	Defatted meal +Oil	Non defatted	15
15	Zhao et al. (2022)	Defatted meal	Defatted	1.5-3
16	Lokaewmanee et al. (2023)	Live/ Fresh	Non defatted	1-3
17	Nassar et al. (2023)	Meal	Non defatted	3-12

Table 2: Descriptive statistics on the effects of BSFL supplementation on production performance and egg quality of laying hens

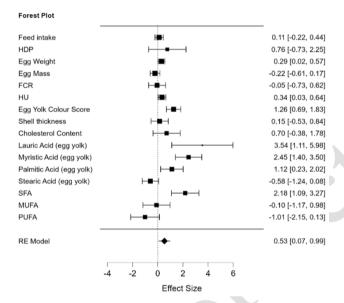
Variable	Unit	NC	1	Mean SD		Min		Max		
			Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Production Performance										
Feed intake	g/d	20	108.08	107.69	3.05	3.05	78.90	79.40	141.00	141.00
HDP	%	8	80.55	80.89	6.93	6.93	54.34	49.61	89.40	92.86
Egg weight	g	34	60.81	60.90	3.69	3.71	47.66	46.41	67	69.20
Egg mass	g/d	14	54.43	54.10	4.4	4.4	28.59	25.98	64.10	68.60
FCR	g/g	17	1.95	1.97	0.08	0.08	1.65	1.66	2.53	2.43
Egg Quality										
HU	%	28	85.72	86.99	4.21	4.14	75.6	77	90.83	93.00
Egg yolk color score		23	6.48	7.15	0.5	0.51	3.05	2.61	9.12	12.87
Shell thickness	mm	24	0.39	0.42	0.03	0.03	0.04	0.03	0.44	0.45
Cholesterol content	mg/dL	13	119.84	120.78	6.94	6.93	5.2	5.4	368.33	365.77
Lauric acid (egg yolk)	%	5	0.26	0.51	0.2	0.2	0.03	0.19	1.19	1.23
Myristic acid (egg yolk)	%	13	0.47	1.22	0.23	0.23	0.29	0.33	1.70	2.96
Palmitic acid (egg yolk)	%	13	24.75	25.64	1.25	1.25	20.82	19.96	26.68	27.40
Stearic acid (egg yolk)	%	13	8.27	7.96	0.41	0.41	7.07	6.57	9.88	9.36
SFA	%	13	34.50	35.90	0.78	0.78	33.42	33.67	36.05	38.90
MUFA	%	13	44.23	45.00	1.33	1.33	41.6	40.88	47.39	47.10
PUFA	%	13	21.05	18.88	1.32	1.32	16.94	15.8	24.50	22.27

NC: number of comparisons, SD: Standard deviation, Min: Minimum value, Max: Maximum value, HDP: Hen Day Production, HU: Haugh Unit.

Table 3: Meta-analysis results on the effects of BSFL supplementation on production performance and egg quality of laying hens

Variable	Unit	NC	Estimate	Lower	Upper	SD	P value	Tau <sup>2</sup>	Q	Het. p-value	$I^2$
Production Performance											
Feed intake	g/d	20	0.109	-0.222	0.439	0.169	0.519	0.231	32.483	0.028	41.51
HDP	%	8	0.758	-0.732	2.247	0.760	0.319	4.107	68.417	< 0.001	89.77
Egg weight	g	34	0.294	0.020	0.568	0.140	0.035	0.394	87.061	< 0.001	58.65
Egg mass	g/d	14	-0.220	-0.611	0.170	0.199	0.269	0.267	25.926	0.017	49.86
FCR	g/g	17	-0.052	-0.726	0.622	0.344	0.880	1.279	60.669	< 0.001	78.57
Egg Quality											
HU	%	28	0.338	0.032	0.643	0.156	0.030	0.352	58.293	< 0.001	53.68
Egg yolk color score		23	1.261	0.689	1.834	0.292	< 0.001	1.668	113.951	< 0.001	78.06
Shell thickness	mm	24	0.151	-0.533	0.834	0.349	0.666	2.851	295.758	< 0.001	91.55
Cholesterol content	mg/dL	13	0.703	-0.378	1.784	0.551	0.202	3.371	118.425	< 0.001	89.87
Lauric acid (egg yolk)	%	5	3.545	1.112	5.978	1.241	0.004	7.095	71.572	< 0.001	94.41
Myristic acid (egg yolk)	%	13	2.452	1.401	3.503	0.536	< 0.001	3.124	132.185	< 0.001	90.92
Palmitic acid (egg yolk)	%	13	1.124	0.231	2.017	0.456	0.014	2.332	114.145	< 0.001	89.49
Stearic acid (egg yolk)	%	13	-0.581	-1.237	0.076	0.335	0.083	1.180	70.851	< 0.001	83.06
SFA	%	13	2.182	1.093	3.271	0.556	< 0.001	3.524	139.215	< 0.001	91.38
MUFA	%	13	-0.096	-1.171	0.978	0.548	0.860	3.558	157.477	< 0.001	92.38
PUFA	%	13	-1.006	-2.147	0.136	0.582	0.084	3.943	166.980	< 0.001	92.81

NC: number of comparisons,  $\tau$  2: estimate of variance between studies in a random-effects meta-analysis, Q: study homogeneity, I2: percentage of variation across studies due to heterogeneity, HDP: Hen Day Production, HU: Haugh Unit.



**Fig. 2:** Cumulative forest plot on the effects of BSFL supplementation on production performance and egg quality of laying hens.

controls. The effect of BSFL supplementation on production performance and egg quality is shown by the cumulative forest plot which presented in Fig. 2.

The analysis results indicated a statistically significant increase (P<0.05) in the value of egg weight, HU, egg yolk color score, LAU, MYR, PAL, and SFA compared to the control. However, the results for feed intake, HDP, egg mass, FCR, shell thickness, cholesterol content, stearic acid, MUFA, and PUFA were not statistically significant (P>0.05).

# **Sub-Group and Meta-Regression Analysis**

The results of the subgroup analysis are presented in Fig. 3. This data is presented based on larvae form in the FCR parameter which is the main parameter in identifying the effect of BSFL supplementation on production performance. This FCR parameter is a reflection of the efficiency of feed use which ultimately has a subsequent

impact on economic value. The sub-group analysis findings based on larvae form revealed that the use of BSFL in non-defatted form contributed to (P<0.05) reducing the FCR value. The results of meta-regression in Fig. 4 show that on FCR parameter, BSFL supplementation is negatively correlated with increasing levels of BSFL administration using the aquation: Y=0.233- 0.035x. These findings indicate that when BSFL usage rises, FCR value decreases. Meta regression also shows that the most optimal level to decrease FCR value of BSFL use as a protein source for laying hens is at the level of 12%.

# **Publication bias**

Fig. 5 presents the publication bias results using the performance production and egg quality parameters' funnel plot test. The statistical analysis of publication bias using Egger's test produced significant results (P=0.027), and the funnel plot clearly displayed an asymmetrical image. This evidence substantiates the presence of publication bias in this study, which can be attributed to the differing sources of BSFL level and form utilized in each article. In order to determine their impact, this research employed the random effect approach and carried out a sub-grub analysis depending on the BSFL level, as shown in Fig. 3.

# DISCUSSION

# Production performance of chickens fed with feed containing BSFL

The production performance of laying hens fed feed containing BSFL showed no significant differences in Egg Mass, HDP, FCR, and Feed intake (P>0.05). This indicated that BSFL can be used as an origin of protein in laying hens feed up to a level of 24%. It was reported in previous research that soybean meal can be substituted with BSFL meal in laying hen feed without adverse effects on production performance and health (Al-Qazzaz et al. 2016; Park et al. 2017; Mwaniki et al. 2018; Mwaniki et al. 2020; Zhao et al. 2022; Alfian et al. 2023). The inclusion of BSFL in laying hen feed resulted in a significant increase in egg weight (P<0.05) compared to the control. Among the

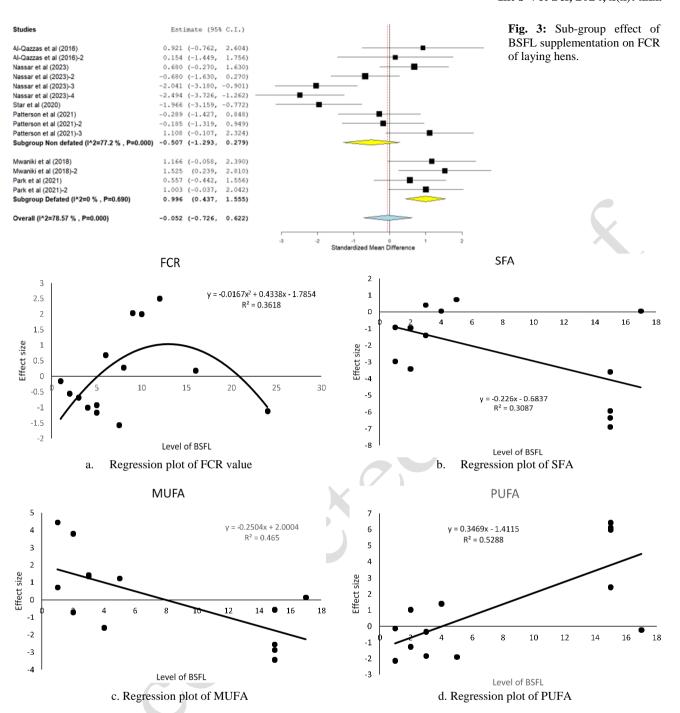


Fig. 4: Meta-regression of BSFL supplementation level on FCR, SFA, MUFA and PUFA.

factors influencing egg weight, besides genetics and parent age, nutritional content of the feed-especially protein, total amino acids, and minerals (Zhao et al. 2022).

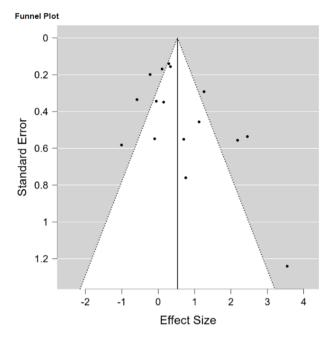
Overall, the crude protein content in BSFL meal was comparable to protein sources used in the control diets, namely fish meal (Zhao et al. 2022), soybean meal (SBM) (Mwaniki 2019; Secci et al., 2018, 2019) and MBM (Kurnia Citra et al. 2019). This suggests that BSFL meal can effectively replace fish meal and MBM in poultry feed as a protein source. Achieving a balanced and adequate amino acids profile is crucial for enhancing performance.

Previous studies have demonstrated that even the absence of a single amino acid from the diet can impact the performance of laying hens (Johnson et al. 1956). In this study, the amino acid composition of the BSFL diet was similar to the reported compositions in the literature for

most amino acids (Mwaniki 2019). Several studies have indicated that BSFL feed exhibits a well-balanced amino acid profile, comparable to fish meal (Zhao et al. 2022), SBM (Secci et al. 2018; Mwaniki 2019) and MBM (Kurnia Citra et al. 2019).

# Quality of chicken eggs that are fed containing BSFL

Based on the results of the meta-analysis (Table 3), all egg quality parameters of chickens fed feed containing BSFL showed significant differences compared to the control, except for shell thickness, cholesterol content, stearic acid, MUFA, and PUFA. The inclusion of BSFL in laying hen feed has been shown to increase the values of HU, egg yolk color score, Lauric Acid (in egg yolk), Myristic Acid (in egg yolk), Palmitic Acid (in egg yolk), and SFA.



**Fig. 5:** Funnel plot effect of BSFL inclusion on production performance and egg quality.

The HU value is an indicator of egg freshness, in this meta-analysis, it ranges from 77-95.29, which is higher than the control range of 75.6 to 92.44. These HU values fall within the United States Department of Agriculture Standards for egg quality (USDA 2000): AA quality eggs have an HU value above 72, A quality eggs range from 60 to 72, B quality eggs range from 31 to 60, and C quality eggs value below 31. A higher HU value indicates better egg quality due to increased albumen quality (Selim et al. 2018), and freshness (Maurer et al. 2016).

The egg yolk color score of chickens fed BSFL was also higher compared to the control, attributed to the chitin content in BSFL. Larval chitin is 33.7% according to (Maurer et al. 2016). Chitin and chitosan derived from BSFL, are widely used in various applications such as preservatives, heavy metal waste adsorbents, and dye adsorbents (Nafisah et al. 2019). It has been reported that chitin can bind pigments like Beta-carotene, a carotenoid responsible for egg coloration (Yunitasari et al. 2023). Beta-carotene is commonly sourced from yellow corn in poultry feed.

In previous research, it was reported that eggshell thickness increased with increasing levels of BSFL in feed (Maurer et al. 2016). Similar results have also been reported in Beski et al. (2015) and Mawaddah et al. (2018) research on quail eggs during the laying period. This effect is attributed to chitin's ability BSFL to absorb heavy metals, including mineral phosphorus and calcium, which play an important role in eggshell thickness formation. It was further explained by Beski et al. (2015), dan Wardhana (2017) and Mawaddah et al. (2018). BSFL also contains 0.6-0.63% phosphorus and 4.18-5.1% calcium.

The lipid profile analysis from this meta-analysis indicates that egg yolks from hens fed BSFL-based feed are richer in SFA, including lauric (LAU), myristic (MYR), and palmitic (PAL) acids compared to those from the control group. This highlights the influence of BSFL lipid composition on egg lipid profile. This is in-line with the research by Bejaei & Cheng (2020), who substituted soybean meal with non-defatted BSFL meal in laying hen

diets, resulting in increased proportion of MUFA and SFA compared to controls.

BSFL are known to contain higher total fat content compared to MBM. Several references state that fat collaborates with chitin and protein to increase LAU, MYR, and PAL which are classified as SFA. Numerous investigations have revealed that the growing medium's composition affects the lipid content of BSF larvae (Liberati et al. 2009; Al-Qazzaz et al. 2016; Ardiansyah et al. 2021; Wahyuni & Fadhlil 2022; Suryati et al. 2023), but lauric acid will always be dominated on lipid content (Wardhana 2017; Ewald et al. 2020; Kim et al. 2021).

Several other references also reveal that BSF oil has a high LAU content and its quality is equal to palm kernel oil and coconut oil. A lot of coconut oil's medical advantages are linked to LAU (Sprangers et al. 2017; Muller et al. 2019; Alfian et al. 2023). LAU has been reported to have antibacterial (Kabara et al. 1972), antiviral (Thormar et al. 1987), antifungal (Park et al. 2014; Akula et al., 2021), and anticancer activities (Lappano et al. 2017). Further explained by Ghorbannezhad et al. (2022) and Alfian et al. (2023) stated that among its many health advantages is that lauric acid acts as an antiviral, antiprotozoal, and antibacterial. It can lyse the viral membranes, impairing immunity and rendering viruses inactive. LAU in eggs may have a positive impact on the health of humans who consume them (Suryati et al. 2023).

#### **Conclusions**

Using BSFL as a protein source in laying hen feed does not negatively impact production performance; rather, it either maintains performance or demonstrates positive effects. Furthermore, BSFL supplementation positively influences the physicochemical quality of eggs. Sub-group analysis based on larvae form indicated that non-defatted BSFL resulted in the best FCR. Meta-regression analysis identified 12% as the optimal level of BSFL inclusion in laying hen diets for maximizing production outcomes.

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#### **Conflict of interest**

All authors declare that there is no conflict of interest.

# Author's contribution

Conceptualization: W, NU, AJ, and IIA; data collection: W; Data analysis, investigation, methodology, validation, data curation: W and MR; writing-original draft, review, and editing: W, AJ, NU.

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